Anticonvulsive effect of *Centella asiatica* in rat muscles with particular reference to carbohydrate metabolism

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ABSTRACT

This study evaluated the anticonvulsant effect of ethanol extract of *Centella asiatica* (CA) with particular reference to carbohydrate metabolism in different types of rat muscles. The rats were randomly divided into 4 groups having 6 in each group: i.e. Control group received Saline, PTZ-induced epileptic group (60 mg/kg b.w op/ 1 day), Epileptic group pretreated with ethanol extract (EE) and Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/ip/ day). The CA extract is administered at the dose of 200 mg/kg body weight orally for one week. The experimental results were observed that the decreased content of Total carbohydrates in the muscles i.e. White Vastus (WV), Red Vastus (RV), Soleus (Sol) and Gastrocnemius (GN); increased the glycogen and glucose levels during PTZ-induced epilepsy in all the muscles. The reversal changes were observed on pre-treatment with the ethanolic extract of CA and diazepam. Hence, it is evident that the different bioactive factors of *Centella* offered protection against PTZ-induced epilepsy.

Keywords: **Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, carbohydrate, rat muscle.**

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INTRODUCTION

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures, is a major health problem that affects 1-2% population world-wide. Despite the progress made in understanding the pathophysiology of epilepsy over the decades, the cellular basis of epilepsy in humans remains a mystery[1]. A number of mechanisms of epilepsy have been postulated such as: Imbalance between excitatory and inhibitory neurotransmission, Alterations in neurotransmitter expression and function, Development of epileptic ion-channels, Functional alterations in neurons, Aberrant neuronal synchronization and Genetic causes. It has been well established that impairment of ion channel function and GABA mediated transmission and reciprocal increase in excitation due to glutamatergic and cholinergic influences are implicated in the manifestation of epileptogenesis[2]. Considering the multi-factorial neurochemical and neurophysiological malfunctions in epileptic seizures, several attempts have been made to design anti-epileptic drugs. However, multiple antiepileptic drug treatment employed in ameliorating seizures generally met with partial success and suffers from substantial problems such as pharmaco-resistance and neurotoxic effects[3]. The importance of Herbal drugs, in general, is gaining momentum in recent times as an alternative and complementary therapy. Hence, the present study selected biochemical parameters in carbohydrate metabolism in different muscles of rat during Pentylenetetrazole induced epilepsy and antiepileptic effect of ethanolic extract of medicinal plant, *Centella asiatica.*

MATERIALS AND METHODS

Procurement and Maintenance of Experimental Animals

Male adult Wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±2^{0}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*.

Ethical guidelines:

The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 1538/01/A/CPCSEA/ dt:14.08.2015 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2016.

Selection of Drug:

Pentylenetetrazole (PTZ), an anticonvulsant drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Collection of the plant material:

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688).

Preparation of Plant Extract

The active principles of the leaves of plant were extracted into ethanol, since this solvent was predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants [4],[5]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with Ethnol.

Induction of Epilepsy:

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) in saline [6], [7].

Administration of Test substance:

Ethnolic extract of CA (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ [8]. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA [9]. The volume of administration was kept at 1ml/kg/ animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Drugs, Chemicals and apparatus:

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma, Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India). Pentylenetetrazole and diazepam were obtained from Sigma Aldrich (St. Louis, MO, USA). In the present investigation Barnstead Thermoline water purification plant for nanopure water, Kubota KR centrifuge and Hitachi U-2000 Spectrophotometer and other standard equipment's were used for biochemical analyses.

Isolation of Tissues:

The animals were sacrificed after the treatment by cervical dislocation. The muscle was isolated immediately and placed on a chilled glass plate. Functionally different muscles such as white vastus (WV), red vastus (RV), soleus (SOL) and gastrocnemius (GM) muscles were separated and frozen in liquid nitrogen (-180^{0}C) and stored at -40^{0}C until further use. At the time of analyses the tissues were thawed and used. Selected parameters were estimated by employing standard methods.

Experimental design for screening of plant extracts for anticonvulsant activity

The rats were randomly divided into 4 groups having 6 in each group: i.e. Control group received Saline, PTZ-induced epileptic group (60 mg/kg b.w./ i.p/ 1 day), Epileptic group pretreated with Ethanolic extract (EE) and Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/i.p). The plant extract was administered at the dose of 200 mg/kg body weight orally for one week.

Biochemical analysis

The total carbohydrate content was estimated by the method of Carroll *et al*[10]. The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue.

Glycogen was estimated by the method of Kemp and Van Hejnigen [11] . The glycogen content was expressed as mg glucose equivalents/gram wet weight of the tissue. Glucose was estimated by the method of Mendal *et al* [12]. The glucose content was expressed as mg glucose/gram wet weight of the tissue.

Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software (11.5 ver.) for different parameters. Difference between control and experimental assays were considered as significant at $P<0.05$.

RESULTS & DISCUSSION

Total Carbohydrates

The total carbohydrates were estimated in different muscles of the rat with reference to the administration of convulsant drug pentylenetetrazole (PTZ) and the treatment with anticonvulsant ethanolic extraction of *Centella asiatica* (CA). The decrease in total carbohydrate levels in the white vastus, red vastus, soleus and gastrocnemius muscles of PTZ treated rats indicates utilization of carbohydrates to meet energy demands during PTZ induced epileptic conditions[13]. The results are presented in Figure (1).

On treatment with CA extracts the total carbohydrate levels were increased which might be due to the synthesis of carbohydrates through glycogenesis and gluconeogenesis. Although not to the same extent, the total carbohydrates were increased in combination treatment (PTZ+EE extract) suggesting that CA extracts help to replenish the loss of carbohydrates that occur during epileptic seizures[14]. Figure (1).

GLYCOGEN & GLUCOSE:

Glycogen is a major storage form of carbohydrate in animals for biological function and the maintenance of the glycogen reserves is an important feature of the normal metabolism[15] . The amount of glycogen present in tissues varied widely with diet and physiological status[16]. Many cells store glycogen for the purpose of having glucose available for further use. Muscle glycogen is present to serve as a fuel reserve for the synthesis of ATP within that tissue for increased muscular activity.

The glycogen and glucose levels were increased in all the muscles during PTZ-induced epilepsy. Whereas, in plant extracts treated rats the glycogen content was decreased. Similarly, with the combination treatment (EE +PTZ) the glycogen content was increased, to lesser extent than the PTZ treatment. The results are presented in Figure (2, 3).

The glycogen levels were increased in PTZ treated animals due to mobilization of stored reserves and mobilization of glycogen from liver to the skeletal muscle in order to meet the energy demands during epileptic condition. On par with the glycogen, glucose levels were

also increased in all muscles during PTZ-induced epilepsy which might be due to increased conversion of glycogen to glucose for the onward glycolytic pathway.

On Contrary to this, glucose levels were decreased during treatment with CA extract and lesser increment in combination treatment which suggest that lesser utilization of glucose through anaerobic glycolysis. However, the elevated levels of TCA cycle activity during treatment with CA extracts and in combination treatment suggest maintenance of normal glycolytic activity that contribute to the formation of pyruvate and subsequent TCA cycle activity; in addition to the mobilization of glycogen from liver to all the metabolic tissues including muscle. Since the anticonvulsant drugs suppress the normal physical activity, it is possible that the energy yielding components such as carbohydrates, lipids etc might be utilized to a lesser extent despite the maintenance of normal metabolic activity.

CONCLUSION

The present findings demonstrate that there is significant diminution in the glycolytic and oxidative potential of all functionally different muscles of rat during PTZ-induced epilepsy. Ethanolic extracts of *Centella asiatica* considerably reduce the risk of metabolic dysfunction that occurred during epilepsy. Thus, these extract and more particularly the bioactive factors present in the antiepileptic treatment. However, further in depth studies are required to understand the physiological mechanism of different bioactive compounds present in the CA extracts and to suggest that the therapeutic approaches of these compounds with particular reference of anticonvulsant and neuroprotective activity.

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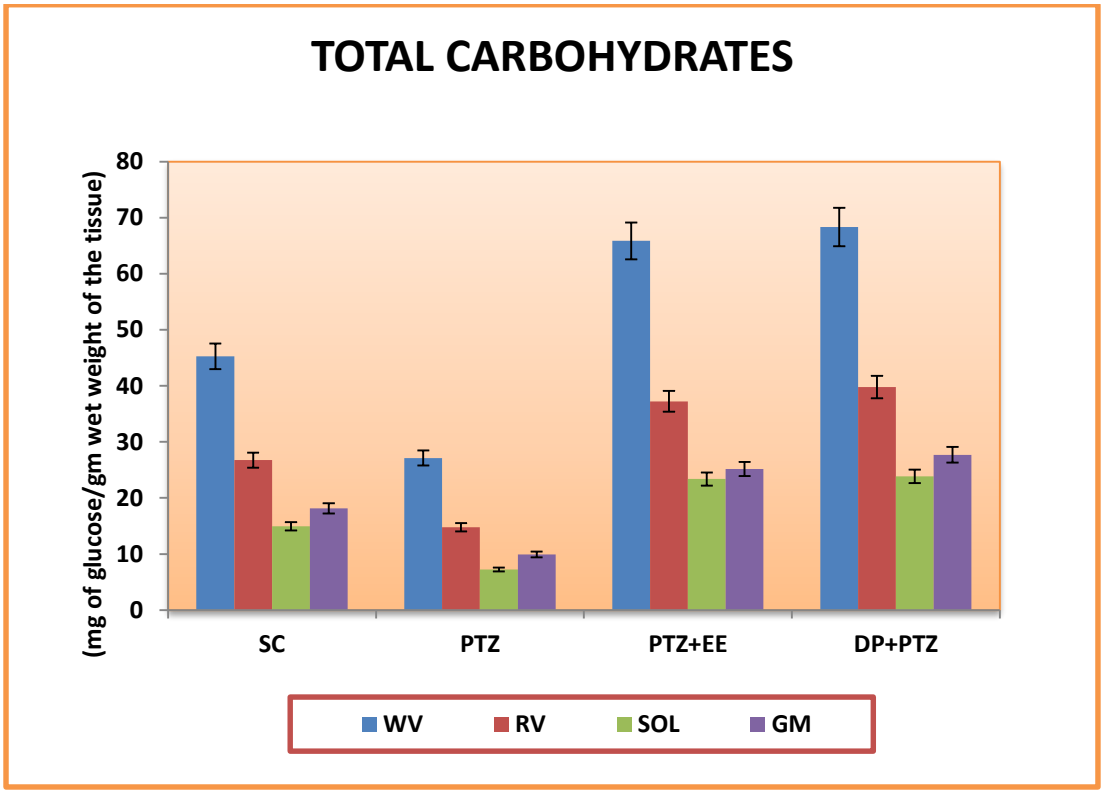
Figure 1: Changes in the Total carbohydrate content in different types of rat muscles during PTZ- induced epilepsy and pre-treatment with ethanolic extract of *Centella asiatica*.



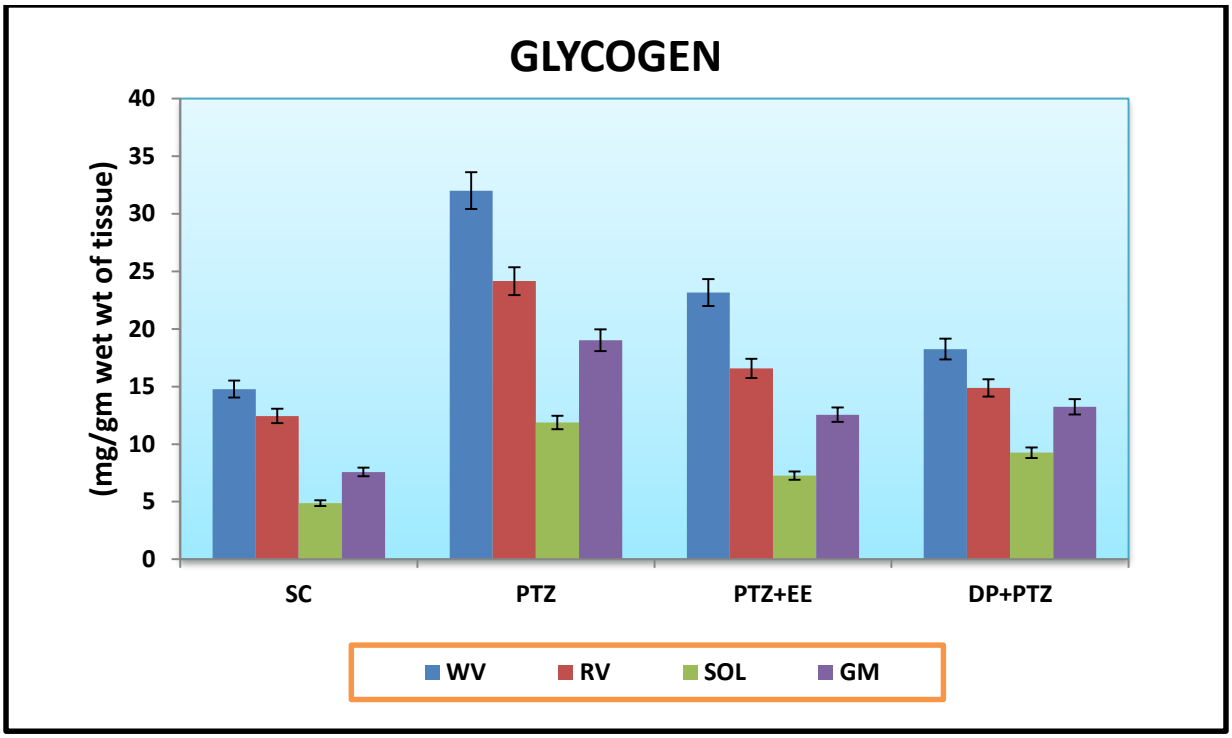
WV=White vastus; RV= Red Vastus; SOL=Soleus; GM= Gastrocnemius Muscle

SC=Saline Control; PTZ =Pentylene tetrazole; EE= Ethanol Extract

All the values are mean, ±SEM of six individual observations.

(Values are expressed in mg of glucose/gm wet weight of the tissue)

Figure 2: Changes in the Glycogen content in different types of rat muscles during PTZ-induced epilepsy and pre-treatment with ethanolic extract of *Centella asiatica*.



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All the values are mean, ±SEM of six individual observations.
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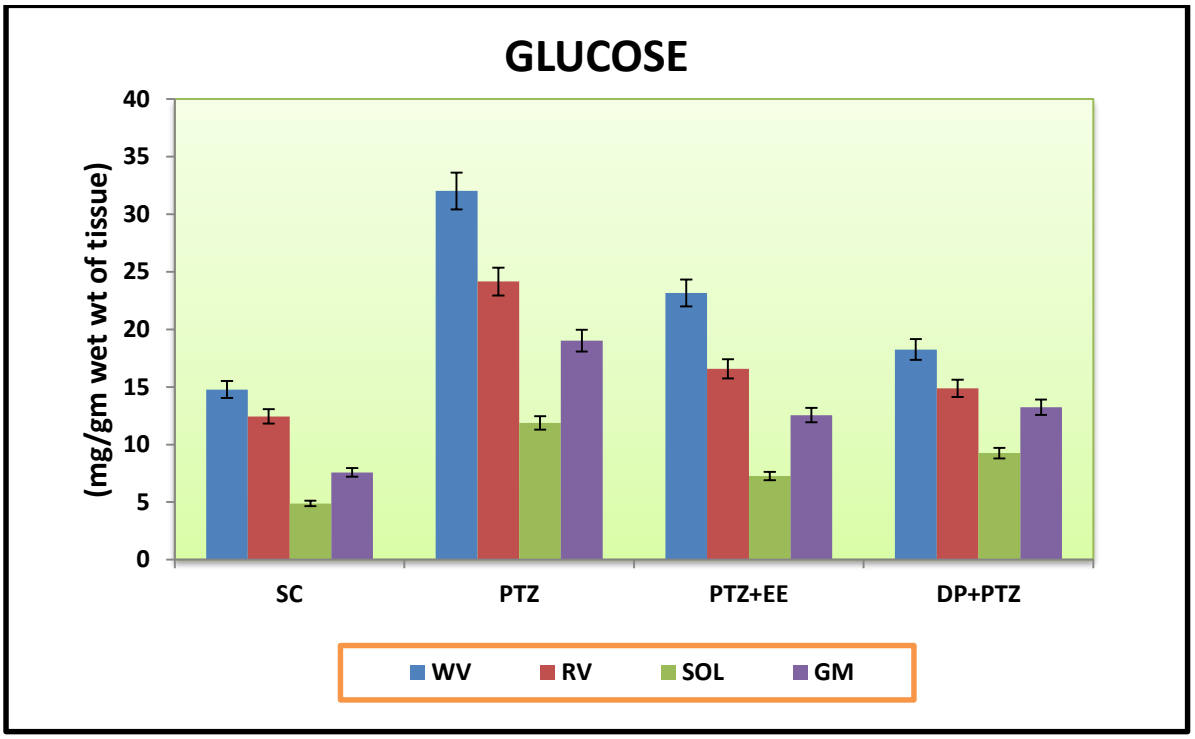
Figure 3 : Changes in the Glucose levels in different types of rat muscles during PTZ-induced epilepsy and pre-treatment with ethanolic extract of *Centella asiatica.*



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Antiepileptic Ayurvedic Medicinal Herb: *Centella Asiatica*

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ABSTRACT
Centella asiatica (gotukola) has been used as a medicine in the Ayurvedic tradition of India for thousands of years. It is listed in the historic Susruta samhita, an ancient Indian medicinal text. In China, gotukola is one of the reported "Miracle elixirs of life". In 19th century, Gotukola and its extracts were incorporated into the Indian pharmacopeia and considered as the Food for the brain. This study evaluated the anticonvulsant effect of chloroform extract of *Centella asiatica* (CA) with particular reference to carbohydrate metabolism in different types of rat muscles. The rats were randomly divided into 4 groups having 6 in each group: i.e. Control group received Saline, PTZ-induced epileptic group (60 mg/kg b.w op/ 1 day), Epileptic group pretreated with chloroform extract (CE), and Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/ip/ day). The CA extract is administered at the dose of 200 mg/kg body weight orally for one week. The experimental results were observed that the decreased content of Total carbohydrates in the muscles i.e. White Vastus (WV), Red Vastus (RV), Soleus (Sol) and Gastrocnemius (GN); increased the glycogen and glucose levels during PTZ-induced epilepsy in all the muscles. The reversal changes were observed on pre- treatment with the chloroform extract and diazepam. Hence, it is evident that the different bioactive factors of *Centella* offered protection against PTZ-induced epilepsy.

Keywords: Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, carbohydrate, rat muscle.

INTRODUCTION
Centella asiatica (CA) is a small herbaceous annual plant of the family Apiaceae, native to Asia, also known as Gotu Kola. The active constituents of *Centella* include triterpenoid glycosides i.e. asiatic acid, asiaticoside, madecassic acid, madecassoside, oxyasiaticoside, and centelloside[1,2] ; saponin glycosides (1.4-3.4%) (brahmiside, brahminoside); flavonol glycosides (quercetin-3-glycoside and Kampferol-3-glycoside); flavonoids viz., naringin, quercitin, rutin, cathecin, kampeferol and apigenin; phytosterols such as ß-sitosterol, stigmasterol and camposterol and a volatile oil consisting of vallerin, camphor, cineole and terpene acetate that comprises 35% of the total oil content (Gotu kola, centella asiatica, the Goddes of the Supreme Wisdom). Gotukola also contains naturally occurring vitamins A, B, C, G, K, tannins (24.5%); essential oils (0.8-1%); monoterpenes, sesquiterpenes, several aminoacids (lysine, alanine, phenylalanine, serine, aspartic acid, glutamic acid); fatty acids (palmitic, oleic and linoleic acids); resin (8.9%); an alkaloid named hydrocotyline and elements Calcium, Magnesium and Sodium[3].

Extracts of *Centella asiatica* have also successfully treated in surgical wounds, skin grafts, gangrene, and traumatic injuries[4]; chronic skin lesions and leprosy wounds[5]. *Centella asiatica* showed

wound healing activity[6] ; anti-anxiety activity[7] ; anti-hepatoma activity[8] ; cognition-enhancement in rats[9]. *Centella asiatica* was effective in improving microcirculation in venous hypertension and diabetic microangiopathy[10] . It was also used in the treatment of tuberculosis, syphilis, amoebic dysentery and common cold, also known as anti-aging plant[11] . It also showed protection against radiation induced damage in liver[12], lead poisoning in CNS[13] , age related oxidative damage and colon tumorigenesis. It was also used in the treatment of anaemia, blood disorders, bronchitis, urinary disorders and splenomegali. It is also an active constituent in ayurvedic formulations like Mentat, Memorin, Mentalin, Mental Alertness, Abana (Heart care), Geriforte (Gericare), Anxocare etc.

Hence, the present study selected biochemical parameters in carbohydrate metabolism in different muscles of rat during Pentylenetetrazole induced epilepsy and antiepileptic effect of chloroform extract of medicinal plant, *Centella asiatica*.

MATERIALS AND METHODS

Procurement and Maintenance of Experimental Animals

Male adult Wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28 ± 2^0C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water ad libitum.

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The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 1538/01/A/CPCSEA/ dt:14.08.2015 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2016.

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Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688).

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The active principles of the leaves of plant were extracted into chloroform, since this solvent was predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants [14,15]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with chloroform.

Induction of Epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) in saline [16,17].

Administration of Test substance

Ethnolic extract of CA (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ [18]. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA [19]. The volume of administration was kept at 1ml/kg/ animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Drugs ,Chemicals and apparatus

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma, Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India). Pentylenetetrazole and diazepam were obtained from Sigma Aldrich (St. Louis, MO, USA). In the present investigation Barnstead Thermoline water purification plant for nanopure water, Kubota KR centrifuge and Hitachi U-2000 Spectrophotometer and other standard equipments were used for biochemical analyses.

Isolation of Tissues

The animals were sacrificed after the treatment by cervical dislocation. The muscle was isolated immediately and placed on a chilled glass plate. Functionally different muscles such as white vastus (WV), red vastus (RV), soleus (SOL) and gastrocnemius (GM) muscles were separated and frozen in liquid nitrogen (-180^{0}C) and stored at -40^{0}C until further use. At the time of analyses the tissues were thawed and used. Selected parameters were estimated by employing standard methods.

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Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software (11.5 ver.) for different parameters. Difference between control and experimental assays were considered as significant at $P<0.05$.

RESULTS & DISCUSSION

Total Carbohydrates

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On treatment with chloroform extract the total carbohydrate levels were increased which might be due to the synthesis of carbohydrates through glycogenesis and gluconeogenesis. Although not to the same extent, the total carbohydrates were increased in combination treatment (PTZ+CE extract) suggesting that extract help to replenish the loss of carbohydrates that occur during epileptic seizures[24]. Table(1).

GLYCOGEN & GLUCOSE

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having glucose available for further use. Muscle glycogen is present to serve as a fuel reserve for the synthesis of ATP within that tissue for increased muscular activity.

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The glycogen levels were increased in PTZ treated animals due to mobilization of stored reserves and mobilization of glycogen from liver to the skeletal muscle in order to meet the energy demands during epileptic condition. On par with the glycogen, glucose levels were also increased in all muscles during PTZ-induced epilepsy which might be due to increased conversion of glycogen to glucose for the onward glycolytic pathway.

On Contrary to this, glucose levels were decreased during treatment with CA extract and lesser increment in combination treatment which suggest that lesser utilization of glucose through anaerobic glycolysis. However, the elevated levels of TCA cycle activity during treatment with chloroform extract and in combination treatment suggest maintenance of normal glycolytic activity that contribute to the formation of pyruvate and subsequent TCA cycle activity; in addition to the mobilization of glycogen from liver to all the metabolic tissues including muscle. Since the anticonvulsant drugs suppress the normal physical activity, it is possible that the energy yielding components such as carbohydrates, lipids etc might be utilized to a lesser extent despite the maintenance of normal metabolic activity.

CONCLUSION

The present findings demonstrate that there is significant diminution in the glycolytic and oxidative potential of all functionally different muscles of rat during PTZ-induced epilepsy. Chloroform extracts of Centella asiatica considerably reduce the risk of metabolic dysfunction that occurred during epilepsy. Thus, these extract and more particularly the bioactive factors present in the antiepileptic treatment. However, further in depth studies are required to understand the physiological mechanism of different bioactive compounds present in the CA extracts and to suggest that the therapeutic approaches of these compounds with particular reference of anticonvulsant and neuroprotective activity.

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Table1: Changes in the **Total carbohydrates** content in different muscles of rat during PTZ-induced epilepsy and treatment with chloroform extract of Centella asiatica.

Muscle	Saline control	Chloroform Extract (CE)	Pentylenetetrazole (PTZ)	PTZ+Diazepam
White Vastus	5.092	8.401*	4.440*	6.865*
	±0.010	±0.011	±0.007	±0.013
		(64.96)	(-12.81)	(34.80)
Red Vastus	8.681	11.339*	7.067*	10.132*
	±0.017	±0.023	±0.016	±0.031
		(30.60)	(-18.59)	(16.70)
Soleus	6.269	10.399*	5.205*	9.158*
	±0.020	±0.022	±0.016	±0.016
		(65.87)	(-16.96)	(46.08)
Gastrocnemius	7.033	10.007*	5.704*	8.721*
	±0.031	±0.015	±0.018	±0.014
		(42.28)	(-18.89)	(23.99)

All the values are mean, ±SE of six individual observations.

Values in '( ) 'parentheses are % change over saline control .

*Values are significant at $P < 0.05$ in Scheffe test.

(Values are expressed in mg/g wet wt of the tissue)

Table 2: Changes in the **Glucose** content in different muscles of rat during PTZ-induced epilepsy and treatment with chloroform extract of Centella asiatica.

Muscle	Saline control	Chloroform Extract (CE)	Pentylenetetrazole (PTZ)	PTZ+Diazepam
White Vastus	0.911	0.889*	1.219*	0.891
	±0.054	±0.057	±0.049	±0.048
		(-2.486)	(33.71)	(-2.17)
Red Vastus	0.766	0.742*	1.072*	0.744*
	±0.007	±0.007	±0.010	±0.008
		(-3.132)	(39.98)	(-2.827)
Soleus	0.736	0.878*	1.041*	0.743
	±0.008	±0.008	±0.003	±0.006
		(19.40)	(41.50)	(0.951)
Gastrocnemius	0.694	0.881*	1.019*	0.697
	±0.010	±0.004	±0.008	±0.008
		(26.99)	(46.85)	(0.552)

All the values are mean, ±SE of six individual observations.

Values in ’( ) ‘parentheses are % change over saline control .

*Values are significant at P < 0.05 in Scheffe test.

(Values are expressed in mg/g wet wt of the tissue)

Table3: Changes in the **Glycogen** content in different muscles of rat during PTZ-induced epilepsy and treatment with chloroform extract of Centella asiatica.

Muscle	Saline control	Chloroform Extract (CE)	Pentylenetetrazole (PTZ)	PTZ+Diazepam
White Vastus	0.945	0.887*	1.243*	0.914
	±0.024	±0.012	±0.012	±0.011
		(-6.11)	(31.56)	(-3.29)
Red Vastus	0.957	0.933*	1.263*	0.935
	±0.007	±0.007	±0.010	±0.008
		(-2.50)	(32.00)	(-2.263)
Soleus	0.927	1.069*	1.232*	0.934
	±0.008	±0.008	±0.003	±0.006
		(15.40)	(32.95)	(0.755)
Gastrocnemius	0.885	1.072*	1.210*	0.883
	±0.010	±0.004	±0.008	±0.008
		(21.16)	(36.74)	(0.433)

All the values are mean, ±SE of six individual observations.

Values in ’( ) ‘parentheses are % change over saline control .

*Values are significant at P < 0.05 in Scheffe test.

(Values are expressed in mg/g wet wt of the tissue)

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RESEARCH ARTICLE

MECHANISM AND REGULATION OF EPILEPSY

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ABSTRACT

Epilepsy is the condition in which seizures recur regularly, throughout one's lifetime. About 1% of the population will develop epilepsy and for about half of these people, a cause can be established. Many processes disrupt normal brain functions and induce either a single seizure or a few seizures over a relatively short period of time. These processes include infections, high fever, brain tumors, drugs, strokes, bleeding in the brain, trauma to the brain and low blood glucose, sodium, or calcium. Seizures caused by these processes usually cease when the underlying problem is solved and therefore are not classified as epilepsy. Only when the underlying cause cannot be found or treated, and the seizures recur indefinitely, is epilepsy diagnosed. Clinically, Epilepsy can be classified into various categories because it helps them decide how best to treat the seizures. Epilepsy is first divided into two types general and partial. General seizures affect the entire brain at once; partial seizures affect only a small part of the brain. As the partial seizure develops, it may remain localized to a small part of the brain or it may spread and affect the entire brain. In either case it is still called a partial seizure because it began in only part of the brain. This investigation reveals the neuronal and chemical mechanism of epilepsy.

Key words: Seizures, Neurotransmitter, GABA, NMDA.

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INTRODUCTION

The word "epilepsy" conveys different meanings to different people. To the physician it is a condition of the brain that is manifested by seizures that occur more or less regularly throughout a person's lifetime. Seizures are disruptions of normal brain activity. The brain is a complex organ that functions by creating and sending messages in the form of electrical signals. These electrical messages can go from the brain to the body to control, for example, the muscles. They also can go from the body to the brain, giving the brain sensory information such as sight, sound, touch, taste and temperature. Most of the brain's electrical messages, however, are sent from one part of the brain to another. These internal messages account for our ability to think, perceive sensations and to be conscious, self-motivated individuals. To function normally, the brain's messages need to be sent from place to place in an orderly manner. Seizures and epilepsy:

Seizures are a fairly common experience. About 5% to 7% of the U.S. population will suffer from a seizure during their lifetime, but simply having an isolated seizure does not represent epilepsy. Epilepsy is the condition in which seizures recur regularly, throughout one's lifetime. About 1% of the population will develop epilepsy and for about half of these people, a cause can be established. For the remainder, the cause of their epilepsy is unknown.

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Many processes disrupt normal brain functions and induce either a single seizure or a few seizures over a relatively short period of time. These processes include infections, high fever, brain tumors, drugs, strokes, bleeding in the brain, trauma to the brain and low blood glucose, sodium, or calcium. Seizures caused by these processes usually cease when the underlying problem is solved and therefore are not classified as epilepsy. Only when the underlying cause cannot be found or treated, and the seizures recur indefinitely, is epilepsy diagnosed.

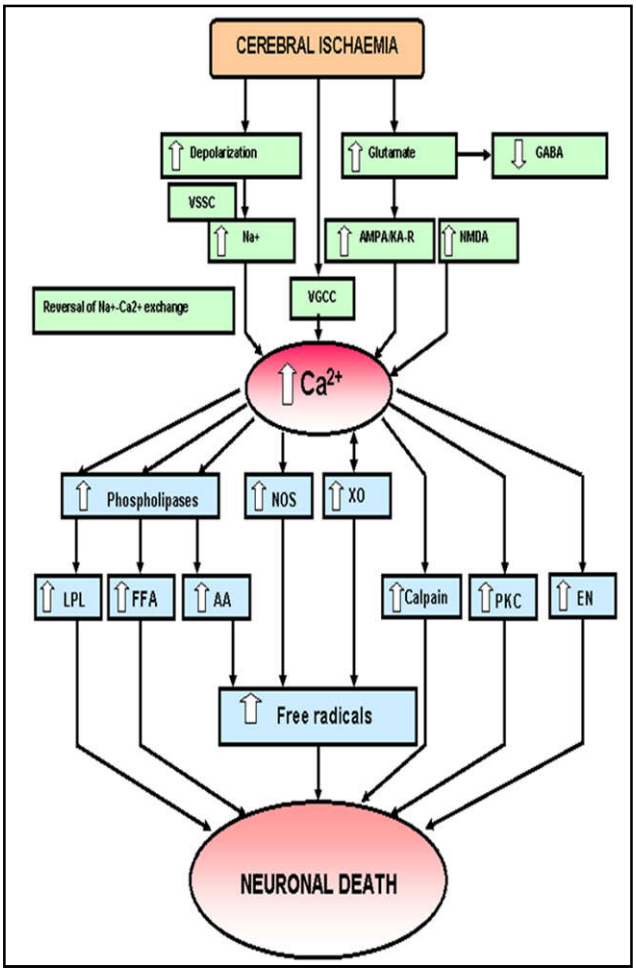
The causes of epilepsy: Physical injury to the brain can cause epilepsy. Many people, after having suffered a stroke for example, develop epilepsy which originates in the part of the brain that was injured by the stroke. Penetrating injuries to the brain also can cause epilepsy. Such injuries might occur following a skull fracture if bone fragments are thrust into the brain. Epilepsy can also be caused by events that are far more subtle than physical insult to the brain. For example alterations in the brain's chemistry can cause seizures. This can be brought about by some street drugs, or exposure to certain toxic chemicals while on the job. Unfortunately, the common thread that ties all of these different causes together - the ultimate cause of epilepsy - is not known. There is still a lot to be learned about seizures and epilepsy. Of concern to everyone with epilepsy is the question of inheritance. While there are some 200 different types of epilepsies where a genetic factor has been identified, these are all very rare forms of the condition. Unless one has been diagnosed with one of these rare forms of epilepsy, there is very little chance of passing the condition on to one's offspring. Of the common types of

epilepsy, only general absence seizures tend to run in families and in that case the risk factor is only about 5%.

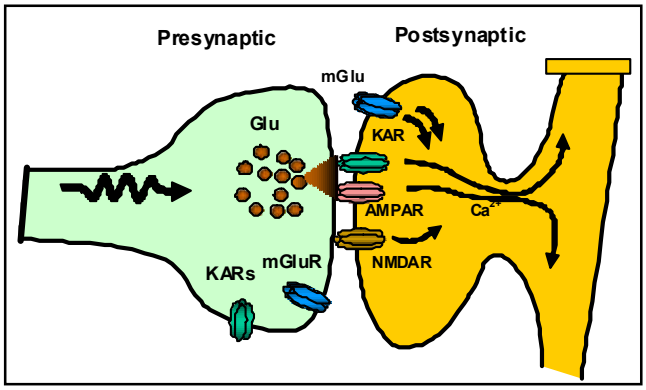
Mechanism of Epilepsy: Neuronal cell damage or death is the main symptom of neurodegenerative disorders such as epilepsy, cerebral ischemia etc. Both NMDA and non-NMDA receptors play an important role in seizure induced brain damage. Sommer (1980) discovered an area of the hippocampus that is vulnerable to injury in patients suffering from prolonged seizures (status epilepticus) which consists of the pyramidal cell fields in CA1 region. It was reported that hippocampal CA1 region contains a very high density of NMDA receptors (Geddes *et al*., 1986) in the entire brain (Monaghan and Cotman, 1985). Reduced cellular energy metabolism during ischemia and epilepsy causes increased release and decreased reuptake of glutamate, as well as increased extra cellular K+ concentrations due to inhibition of the Na+/K+ -ATPase (Feldman *et al*., 1996). Intracellular Ca2+ influx and NMDA receptor antagonists can attenuate the neuronal damage and death (Steinberg, Saleh and Kunis, 1988). Persistent glutamate activation of NMDA receptors with simultaneous membrane depolarization leads to a prolonged opening of NMDA receptor channels, permitting massive Ca2+ influx across the membrane (Glutamate-Calcium nertotoxicity hypothesis).

Depolarization is also thought to cause additional Ca2+ entry into the cell through voltage-operated Ca2+ channels (VOCC). Elevation in intracellular Ca2+ levels activate a variety of Ca2+ dependent processes, including specific proteases and endonucleases that can breakdown the cellular DNA (Lipton and Rosenberg, 1994); phospholipase A2 (PLA2) stimulates the release of arachidonic acid (AA) which in turn can lead to the formation of free radicals and subsequent oxidative cell damage; Nitric oxide synthase (NOS), which catalyzes the formation of nitric oxide can also participate in the generation of harmful free radicals (Coyle and Puttfarcken, 1993); and Ornithine decarboxylase (ODC) resulting in tissue injury. Scatton (1994) reported that excess accumulation of Ca2+ in mitochondria can also lead to severe damage to brain regions. In recent years in vitro electrophysiological models such as cultured spinal cord neurons, hippocampal and cortical slices have been developed to study the mechanism of epileptogenesis.

GABA receptor agonist, bacuculline, picrotixin or pencillin in hippocampal slice cultures or potassium channel blockers such as tetraethyl ammonium induce in vitro epileptogenesis. In vitro epileptiform events in adult slices can also induced by lowering extracellular Mg2+ (Zang *et al.,* 1995), Ca2+ (Konnerth *et al.,* 1984), and high K+ concentrations (Leschinger *et al*., 1993). Interruption of AMPA and NMDA – mediated transmissions causes repeated high-frequency excitation (Croucher and Bradford, 1990) and increasing extracellular glutamate (Glutamate injury induced epileptogenesis) may also lead to epileptogenesis in in vitro models (Sun *et al*., 2001). From the earlier reports, it has been understood that impairment of ion-channel function and GABA mediated transmission or reciprocal increase in excitation due to glutamatergic and cholinergic influences may lead to epileptic characteristics in experimental animals (Avoli *et al*., 1993).



Regulation of Epilepsy: Although glutamate is required for normal brain function, the presence of excessive amounts of glutamate can lead to excitotoxic cell death (Lipton and Rosenberg, 1994). Excitotoxicity is mediated by various types of glutamate receptors (Glu-R) particularly N-methyl-D-aspartate (NMDA), AMPA or kainic acid in different neurodegenerative disorders. Activated NMDA receptor channels allow an influx of Ca2+ which in excess can activate a variety of potentially destructive processes (Standaert and Young, 1995). Blockade of voltage dependent Ca2+ channels and release of extracellular Mg2+ channels, can regulate the activity of NMDA Channels that participate in the release of transmitters. Prevention of repetitive firing of a neuron is possible by blocking the voltage-activated Na+ channels in epileptics (McNamara, 1995). Through the inhibition of inhibitory neurotransmitter (GABA), the resting membrane potential can be regulated and thus can reduce the probability of glutamate excitation (McNamara, 1995).



Treatment of Epilepsy: Epilepsy is a disease that has been recognized since Biblical times. Until the 20th century there has been no effective medical treatment. Over approximately the past seventy years, three promising lines of therapy have emerged; diet, drugs and surgery. Diet is the oldest form of therapy but was largely replace by drugs during the 1920's because drug therapy proved to be more effective for most people. Surgery was first used as a therapy for epilepsy in the 1930's. The results were mixed. There were some notable successes. However, there were also many notable failures. Even as late as the 1950's, surgery for epilepsy was largely experimental in nature and fraught with many unexpected side effects. The goal of treatment of epilepsy is the elimination of seizures without burdening the patient with undesirable side-effects. The three principal weapons used against epilepsy are anti-convulsant drugs, neurosurgery and diet. The primary treatment for epilepsy is the use of antiseizure medicines called anticonvulsant or antiepileptic drugs (AED) to bring seizures under control. AEDs can reduce the occurrence of seizures or prevent them from occurring, but they do not cure epilepsy. The selection of an appropriate AED is based on diagnosis of the epileptic syndrome of the patient. AEDs have initial starting doses, and subsequent titration is based on response to medication and side-effect profile (Nair, 2003). Volprate and Clonazepam may be active agents which are effective against Myoclonic, Akinetic, and atonic seizures in young children (McNamara, 1995). Approximately 50% of all women with epilepsy have increased seizure frequency during pregnancy. Infant mortality is higher for epileptic mothers. Children of epileptic mothers who received antiseizure medication during the early months of pregnancy have an increased incidence of a variety of birth defects (McNamara, 1995). AEDs like carbamazepine and phenytoin induce the malformation in offspring of epileptic mothers (Lindhout *et al*., 1984; Jones *et al*., 1989).

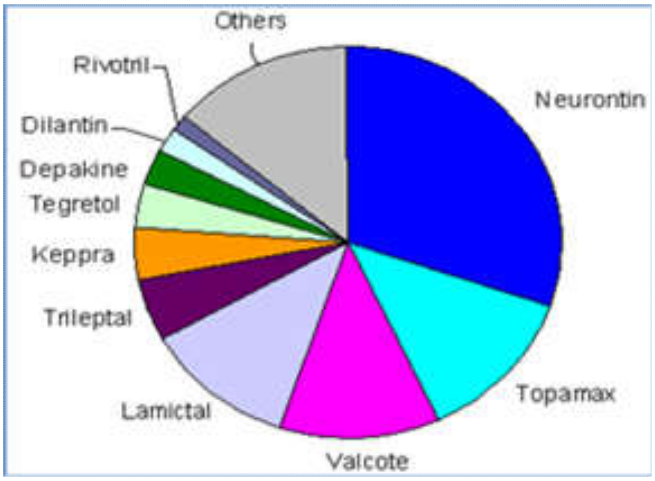
Surgery: For those people whom drug therapy is ineffective, surgery may be appropriate. Approximately half of the people who have surgery for uncontrolled epilepsy become seizure free. The goal of epileptic surgery is to remove the part of the brain that is damaged and causing the seizures. Not everyone with intractable epilepsy can be treated with surgery. Neurosurgery carries risks that ordinary surgery does not because, unlike the rest of the body, the brain is not able to repair or replace itself during the healing process. Brain tissue, once removed, cannot be replaced and the function it performed is only marginally restored. Neurosurgery is similar to an amputation; the stump of the severed limb is healed, but the limb is not replaced. Neurosurgeons have to be extremely careful about removing pieces of the brain. For example, if the damaged part of the brain that is causing the seizures is in or near a part of the brain that directs speech, removing it is likely to render the patient mute. Being unable to speak, even though one has the consciousness, intellect and desire to speak, is obviously an unacceptable side-effect. Therefore, surgery for epilepsy can only be performed if the tissue to be removed is not near any "eloquent cortex", that is to say, parts of the brain that perform essential functions like speech, memory, and major sensory and motor functions. Fortunately, most seizures are caused by damage to parts of the brain that can be safely removed, with little noticeable loss of function. Before surgery can be performed, however, an elaborate testing and evaluation process is necessary to be sure that the damaged tissue can be safely removed.

Surgical intervention for intractable epilepsy is one of the great success stories of the last decade and it is now a standard treatment option. In spite of this, surgery is the most under-utilized standard treatment for epilepsy. There are approximately 5,000 new cases of epilepsy each year that could benefit from surgery. In addition to the new cases each year, there are an estimated 20,000 people currently suffering from intractable epilepsy who could benefit from surgery. Currently, only about 500 actually get surgical treatment each year. In patients who do not respond to medication, epilepsy surgery is a potential mode of treatment that can offer up to a 70% to 90% chance of seizure freedom in some patients. Other novel modes of therapy include the vagal nerve stimulator (VNS), which is usually reserved for those patients with intractable epilepsy who are not surgical candidates (Nair, 2003). Neurocognitive side effects include dizziness, drowsiness, unsteadiness, blurred vision, ataxia, tremor, nystagmus, impaired memory, and fatigue (Nair, 2003). Though various AEds are available clinically, management of epilepsy is a very complex task because of co-existing neuropsychiatric complications (Krishnamoorthy, 2001).

Diet: Some type of epilepsy that cannot be controlled by drugs respond to a very special diet known as a ketogenic diet. This diet fundamentally alters the way the body uses energy from food. Normally the body breaks down carbohydrates we eat into glucose (sugar) and then uses the glucose as its primary source of energy. If fats are substituted for carbohydrates in the diet, the body cannot make glucose. Instead it makes another set of molecules known as ketones which are used as the primary source of energy. The brain, which is normally very dependent on glucose, must also switch its metabolism dramatically to make use of ketones instead of glucose. For reasons that are unknown, this alteration in brain metabolism makes the brain less susceptible to seizures. The ketogenic diet is extremely difficult to maintain. The patient must have a lot of fat in the diet and virtually no carbohydrates. To be effective the diet must be maintained indefinitely. Since the body does not like to generate ketones, it will rapidly convert to glucose utilization if too much carbohydrate is consumed. Because of these difficulties, most people are not successful, over the long term, with the ketogenic diet. Nevertheless, it is a treatment option that needs to be considered in difficult cases.

Antiepileptic Drugs (AEDs): Considering the multifactorial neurochemical and neurophysiological malfunctions consequent to the epileptic seizures, some antiepileptic drugs (AEDs) are designed to mitigate the debilitating aspects of epilepsy. Barbiturates and benzodiazepines control high frequency repetitive firing of action potentials interacting with voltage-gated sodium channels (McLean and Macdonald, 1986) and enhance GABAergic inhibition by binding to an allosteric regulatory site on GABA receptor (Olsen, 1987). Phenytoin and carbamazepine interact with voltage-dependent Na+ channels and reduce the frequency of sustained repetitive firing of action potentials in neuronal cell culture (Macdonald and McLean, 1986) and hippocampal neurons (Kuo and Bean, 1994). Classical AEDs like Carbamazapine, Phenobarbital, Phenytoin and Volproate as well as many newer AEDs like felbamate, gabapentin, lamotrigine, tiagabine, vigabatrine alleviate neuronal damage and delay the development of epileptogenesis (McNamara, 1995; Pitkanen, 2002). Ethosuximide, dimethidine (metabolite of trimithidine) has their action by reducing the T-type Ca2+current in thalamic relay neurons (Coulter *et al.,* 1989). Valproic acid (VPA) has

been found to block sustained high frequency repetitive firing of neurons in culture (McLean and Macdonald, 1986) interacting with T-type calcium channels (Kelly et al., 1990). Felbamate, an anticonvulsant active at NMDA receptors (Mazarti *et al*., 2002), has been shown that drugs that activate ATP-dependent K+ currents shows powerful antiepileptic effects (Macdonald and McLean, 1986). Gabapentine (GBP), a cyclic GABA analog, acts on GABAergic neurotransmitter system and increases GABA turnover in different regions of rat brain (Loscher *et al.,* 1991). Lamotrigine (LTG), a phenyltriazine, inhibits the voltage-dependent sodium channel, which indirectly prevents the presynaptic release of glutamate (Leach *et al*., 1986). Oxcarbazepine (OCBZ) has been shown to reduce sustained high-frequency repetitive firing of voltage-dependent sodium action potential in spinal cord neurons of mouse (Wamil *et al*., 1994). Vigabatrin, an effective anticonvulsant causes selective irreversible inhibition of GABA transaminase (Lippert *et al.,* 1977).However, there are also anecdotal observation that many multiple AED regimens employed in ameliorating seizures generally met with partial success and suffer from substantial problems such as pharmacoresistance (development of tolerance) (Boggs *et al*., 2000), neurotoxic effects and idiosyncratic reactions such as skin rashes etc. (Loscher and Schmidt, 2002). Although the prognosis for seizure control is good in at least 60% of patients, up to 40% of individuals suffer from intractable, pharmacoresistant epilepsy (Kwan and Brodie, 2000). Chronic toxicity was observed with some of the older AEDs such as osteoporosis, gingival hyperplasia, and alterations in reproductive endocrine function. Some specific problems have also been found with some newer AEDs. Hopefully new AEDs that act on different neurotransmitter receptors or ion-channels will result in improved control of seizures and drugs that are active on ion-channels have greater potential in restoring the function of epileptic neurons to normalcy.



Conclusion

The present preliminary findings demonstrate that there is significant recovery through AEDs and selected diet. Thus, these AEDS along with diet may be beneficial in the antiepileptic treatment. However, further in depth studies are required to understand the physiological mechanism of different types of seizures and to suggest that the therapeutic modality of the AEDs with particular reference of anticonvulsant and neuroprotective activity. Correct diagnosis and treatment is required to the epilepsy patients.

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NEUROPROTECTIVE PROPERITY OF *CENTELLA ASIATICA* AGAINST PENTYLENE-TETRAZOLE INDUCED EPILEPSY IN RAT BRAIN WITH PARTICULAR REFERENCE TO LIPID METABOLISM

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Keywords:

Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, Lipid peroxidation

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ABSTRACT: This study evaluated the anticonvulsant effect of different extracts of *Centella asiatica* (CA) with particular reference to lipid metabolism in different regions of rat brain. The rats were randomly divided into 4 groups having 6 in each group: *i.e.* Control group received saline, PTZ-induced epileptic group (60mg/kg b.w op/ 1 day), epileptic group pretreated with chloroform extract (CE), epileptic group pretreated with aqueous (AE) extract and epileptic group pretreated with diazepam (DP; Reference control) (2 mg/kg b.w/ip/ day). The CA extract is administered at the dose of 200 mg/kg body weight orally for one week. The experimental results were observed that the decreased content of phospholipids in the entire brain regions *i.e.* Cerebral cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons medulla (PM); total cholesterol, triglycerides and increased content of Lipid peroxidation in epileptic rats. The reversal changes were observed on pre-treatment with the chloroform extract of CA and diazepam. Hence, it is evident that the different bioactive factors of CA offered protection against PTZ-induced epilepsy.

INTRODUCTION: Cholesterol is an essential component for neuronal physiology not only during development stage but also in the adult life. Cholesterol metabolism in brain is independent from that in peripheral tissues due to blood-brain barrier [1]. Lipids serve several functions in the biological systems such as structural components of the membranes, storage and transport forms of metabolic fuel, protective coating on the surface concerned in cell recognition, species specificity and tissue immunity [2]. Epilepsy is a sudden surge electrical activity in the brain [3]. It is well known that the epileptic seizures result from excessive discharge in a population of hyper excitable neurons.

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Despite the multiple molecular mechanisms have been proposed in generating and spreading epileptic discharges, it has been well established that impaired GABAergic activity exaggerated glutamatergic neurotransmission primarily contribute to the various types of epilepsies [4]. Glutamate is required for normal brain function; the presence of excessive amounts of glutamate can lead to excitotoxic cell death [5].

Through the inhibition of inhibitory neurotransmitter (GABA), the resting membrane potential can be regulated and thus can reduce the probability of glutamate excitation [6]. Hopefully new antiepileptic drugs act on different neurotransmitter receptors or ion-channels will result in improved control of seizures and drugs that are active on ion-channels have greater potential in restoring the function of epileptic neurons to normalcy [7]. CA showed decrement in seizure score, improvement in learning deficits induced by PTZ and increased latencies in passive avoidance behavior [8].

It showed protection against electroshock induced convulsions, pentylenetetrazole and strychnine induced chemo convulsions [9]. It also showed a reduction in lipid peroxidation, spontaneous motor activity, potentiation in diazepam withdrawal-induced hyperactivity, hypothermia and potentiation of pentobarbitone sleeping time [10]. However, there are also anecdotal observations that multiple antiepileptic drugs regimens employed in ameliorating seizers generally met with partial success and suffer from substantial problems such as neurological disorders.

During the past few years, considerable progress has been made towards identifying active factors from indigenous medicinal plants for different human ailments including neurodegenerative disorders such as Alzheimer's disease, parkinsonism, epilepsy etc. *Centella asiatica* considerably increased the seizure threshold and reversed the neuro chemical abnormalities occurred in cholinergic system, monoamine neurotransmitter system and metabolism of glutamate and energy during PTZ- induced epilepsy. Keeping in view of this the present investigation is aimed at studying the modulations of lipid metabolism during PTZ-induced epilepsy and antiepileptic treatment with *Centella asiatica*.

MATERIALS AND METHODS:

Procurement and Maintenance of Experimental Animals: Male adult wistar rats weighing 150 ± 25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28 ± 2 ^{0}C temperatures with photoperiod of 12 hours light and 12 hours dark and 75 % relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*.

Ethical Guidelines: The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/A/CPCSEA/dt:17.07.2006 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2006.

Selection of Drug: Pentylenetetrazole (PTZ), an anticonvulsant drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Collection of the Plant Material: *Centella asiatica* (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V. University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S. V. University, Tirupati (Voucher no. 1688).

Preparation of Plant Extracts: The active principles of the leaves of plant were extracted into Chloroform, since this solvent was predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants [11, 12]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with chloroform.

Induction of Epilepsy: Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60 mg/Kg body weight) in saline [13, 14].

Administration of Test Substance: Each fraction of CA extract (200 mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ [15]. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA [14]. The volume of administration was kept at 1 ml/kg/ animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2 mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Drugs, Chemicals and Apparatus: All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma, Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India). Pentylenetetrazole and diazepam were obtained from Sigma Aldrich (St. Louis, MO, USA). In the present investigation Barnstead Thermoline water purification plant for nanopure water, Kubota KR

centrifuge and Hitachi U-2000 Spectrophotometer and other standard equipments were used for biochemical analyses.

Isolation of Tissues: The animals were sacrificed by cervical dislocation and different brain regions such as Cerebral Cortex (CC), Cerebellum (CB), Pons Medulla (PM) and Hippocampus (HC) were isolated, frozen in liquid nitrogen and were stored at -80 ^{0}C.

Experimental Design for Screening of Plant Extracts for Anticonvulsant Activity: The rats were randomly divided into 4 groups having 6 in each group: *i.e*. Control group received Saline, PTZ-induced epileptic group (60 mg/kg b.w./ i.p/ 1 day), Epileptic group pretreated with chloroform extract (CE) and epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/i.p). The chloroform extract was administered at the dose of 200 mg/kg body weight orally for one week.

Biochemical Analysis: Phospholipids were estimated by the method of Zilversmidth and Davis [16]. The total cholesterol and triglycerides contents were estimated by the method of Natelson [17]. MDA levels were estimated by Ohkawa *et al*., [18].

Statistical Treatment of Data: All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of variance (ANOVA) were done using SPSS statistical software (11.5 ver.) for different parameters. Difference between control and experimental assays were considered as significant at $P<0.05$.

RESULTS:

Phospholipids: The phospholipids content was decreased significantly in all the brain regions in induced epileptic rats (PTZ), with highest decrease noted in the hippocampus (HC). Pretreatment with CA extract *i.e*. CE and diazepam (Reference control) were resulted in significantly increased phospholipids content in all the brain regions and highest increment is noted in HC (**Table 1**).

Total Cholesterol: When compared with saline control, PTZ-induced animals had significantly decreased the total cholesterol in all the brain regions, with highest decrease noted in the hippocampus (HC). Pre-treatment with CA extract *i.e*. CE and diazepam (Reference control) were resulted in significantly increased total cholesterol content in all the brain regions. (**Table 2**)

Triglycerides: The Triglycerides content was decreased significantly in all the brain regions in induced epileptic rats (PTZ), with highest decrease noted in the hippocampus (HC). Pre-treatment with CA extract *i.e*. CE, and were resulted in significantly increased Triglycerides content in all the brain regions and highest increment was noted in HC (**Table 3**).

Lipid Peroxidation: The malondialdehyde content was significantly increased in all the areas of brain during PTZ- induced epilepsy, the highest elevation was noted in hippocampus (HC). Meanwhile pre-treatment with the extract of CA, showed significant decrease malondialdehyde content in all the brain regions, the highest decrement was noted in HC (**Table 4**).

TABLE 1: CHANGES IN THE PHOSPHOLIPIDS CONTENT IN DIFFERENT REGIONS OF RAT BRAIN DURING PTZ- INDUCED EPILEPSY AND PRE-TREATMENT WITH DIFFERENT EXTRACTS OF *CENTELLA ASIATICA*

Brain Region	SC	PTZ	PTZ+CE	DP+PTZ
CC	48.398	30.256*	68.983*	71.471*
	±0.198	±1.016	±0.264	±0.657
		(-37.48)	(42.53)	(47.67)
CB	29.87	17.921*	40.348*	42.928*
	±0.358	±0.845	±0.385	±0.833
		(-40.00)	(35.07)	(43.71)
HC	18.057	10.360*	26.519*	26.975*
	±0.123	±0.818	±0.821	±1.187
	(-42.62)	(46.86)	(49.38)	(-42.62)
PM	21.281	13.056*	28.283*	30.820*
	±0.227	±0.720	±0.808	±0.511
		(-38.65)	(32.90)	(44.82)

All the values are mean, ± SEM of six individual observations. Values in '( )'parentheses are % change over saline control. *Values are significant at $P < 0.05$ in Scheffe test. (Values are expressed in mg of phospholipids/g wet wt of the tissue)

TABLE 2: CHANGES IN THE TOTAL CHOLESTEROL CONTENT IN DIFFERENT REGIONS OF RAT BRAIN DURING PTZ-INDUCED EPILEPSY AND PRE-TREATMENT WITH DIFFERENT EXTRACTS OF *CENTELLA ASIATICA*

Brain Region	SC	PTZ	PTZ+CE	DP+PTZ
CC	42.775	31.383*	60.994*	63.348*
	±0.456	±0.849	±0.507	±0.668
		(-26.63)	(42.59)	(48.09)
CB	80.271	42.425*	106.655*	116.196*
	±0.292	±0.942	±0.475	±0.551
		(-47.14)	(32.86)	(44.75)
HC	60.039	30.018*	93.453*	95.344*
	±0.719	±0.647	±0.617	±0.596
		(-50.00)	(55.65)	(58.80)
PM	97.279	60.815*	148.383*	143.658*
	±0.397	±0.616	±0.832	±0.933
		(-37.48)	(52.53)	(47.67)

All the values are mean, ± SEM of six individual observations. Values in '( )'parentheses are % change over saline control. *Values are significant at P < 0.05 in Scheffe test. (Values are expressed in mg of phospholipids/g wet wt of the tissue)

TABLE 3: CHANGES IN THE TRIGLYCERIDES CONTENT IN DIFFERENT REGIONS OF RAT BRAIN DURING PTZ- INDUCED EPILEPSY AND PRE-TREATMENT WITH DIFFERENT EXTRACTS OF *CENTELLA ASIATICA*

Brain Region	SC	PTZ	PTZ+CE	DP+PTZ
CC	0.396	0.292*	0.467	0.523*
	±0.021	±0.021	±0.022	±0.031
		(-26.26)	(17.99)	(31.95)
CB	0.555	0.389*	0.745*	0.744*
	±0.015	±0.011	±0.029	±0.017
		(-29.94)	(34.25)	(34.04)
HC	0.901	0.563*	1.257*	1.330*
	±0.023	±0.013	±0.012	±0.032
		(-37.51)	(39.56)	(47.64)
PM	0.458	0.316*	0.569	0.622*
	±0.014	±0.033	±0.008	±0.025
		(-30.90)	(24.23)	(35.85)

All the values are mean, ± SEM of six individual observations. Values in '( )'parentheses are % change over saline control. *Values are significant at P < 0.05 in Scheffe test. (Values are expressed in mg of triglycerides / g wet wt of the tissue)

TABLE 4: CHANGES IN THE LIPID PEROXIDATION CONTENT IN DIFFERENT REGIONS OF RAT BRAIN DURING PTZ-INDUCED EPILEPSY AND PRE-TREATMENT WITH DIFFERENT EXTRACTS OF *CENTELLA ASIATICA*

Brain Region	SC	PTZ	PTZ+CE	DP+PTZ
CC	29.866	40.566*	19.756**	17.356*
	±1.877	±1.252	±0.935	±1.077
		(35.82)	(-33.85)	(-41.88)
CB	62.794	84.221*	37.535*	33.320*
	±2.021	±1.187	±0.881	±1.477
		(34.12)	(-40.22)	(-46.93)
HC	122.01	180.124*	66.047*	56.399*
	±2.123	±1.474	±1.037	±1.649
		(47.63)	(-45.86)	(-53.77)
PM	96.751	131.165*	67.757*	56.605*
	±6.993	±1.101	±1.456	±1.150
		(35.57)	(-29.96)	(-41.49)

All the values are mean, ± SEM of six individual observations. Values in '( )' parentheses are % change over saline control. *Values are significant at P < 0.05 in Scheffe test. (Values are expressed in µ moles of malondialdehyde formed / gram wet wt of the tissue).

DISCUSSION: The treatment with extracts of *Centella asiatica* and diazepam restored the levels of cholesterol in different regions of brain of epileptic rats. The membrane micro domains are rich in cholesterol; the alterations in cerebral cholesterol in induced epilepsy could alter the cellular signaling pathways which possibly play a pivotal role in the neuro degeneration process [19]. Increase in cholesterol levels were reported in rats fed both CA extract and powder during H_2O_2 induced oxidative stress [20]. The decreased levels of triglycerides in different regions of brain during PTZ-induced epilepsy might be due to enhanced lipolysis through lipase activity. It is well established that glutamate excitotoxicity and oxidative stress contribute to neuronal degeneration in acute conditions such as stroke, epilepsy, trauma, hypoxia and hypoglycemia and chronic neuro-degenerative diseases such as Parkinson's disease, Alzheimer's and Huntington's disease [21].

Since the bioactive factors of CA significantly attenuate the glutamate induced excitation and oxidative stress, it is possible that the CA extract possibly ameliorate the deregulated lipid metabolism in general and cholesterol metabolism in particular, thus protecting the progressive cell damage that occurs in induced epilepsy [22]. Lipid peroxidation is a complex process generating reactive radicals, which is regarded as an etiologic or pathogenic factor in several diseases of central nervous system including epilepsy.

The cell membranes, enriched with polyunsaturated fatty acids (PUFAs), are more prone to free radical mediated lipid peroxidation. Lipid peroxidation of cell membranes causes a loss of the fluid properties of the membrane as well as increase in membrane permeability [23]. Lipid peroxidation products are constantly involved in some of the pathophysiological effects associated with oxidative stress in cell and tissues. Unlike reactive free radicals, aldehydes can produce lipid peroxidation products, which are rather long lived and can therefore diffuse from the site of their origin, reaching and attacking intracellular and extra cellular targets [24]. They disrupt various important structural and protective functions associated with bio-membranes in various *in-vivo* pathologic events and are implicated as a result of this oxidation [25].

The key functions of nerve cells, such as creation and maintenance of transmembrane potential, reception and subsequent transmission of signal, synthesis and regulation of signal transducers, and uptake and secretion of neurotransmitters are highly susceptible to excessive accumulation of endogenous products of lipid peroxidation in neuronal membrane structures.

Hence, lipid peroxidation is regarded as an etiologic or pathological factor in myriad number of neurological disorders such as Parkinson's disease, Downs's syndrome, schizophrenia, epilepsy *etc*. oxidative damage induced by lipid peroxidation has been recognized as key factor for the occurrence of many human diseases [26]. It has been hypothesized that COx enzyme induction leads to an increase in various prostaglandins, particularly PGE2 which facilitates the massive release of glutamate from nerve terminals and astrocytes and subsequently increase the free radical production leading to oxidative stress followed by apoptosis of GABAergic neurons ending in epileptic discharges [27].

Similar increases in MDA, XO and NO levels have also been recorded in the brains of mice treated with PTZ [28]. Liu *et al*., [29] have indicated that the made cassoside, the active constituent of CA, decreased nitric oxide (NO) levels and malanoldehyde (MDA) content in the burn skin tissue. Decreased MDA content and an increase in glutathione and catalase activities have been reported in rats treated with aqueous extract of CA in intra cerebroventricular streptozotocan model of Alzheimer 's disease in rats. Decreased lipid peroxidation and increased enzymatic and non-enzymatic antioxidants have been elucidated by the asiaticoside derived from CA [30].

CONCLUSION: The present findings in conjugation with the earlier reports it is speculated that the bioactive factors of CA has the propensity to a modulate excitotoxic glutamate induced oxidative impairments in the brain and may be efficiently employed as a neuroprotective adjuvant to abrogate the oxidative stress that occur during induced epilepsy. However, further in depth studies are required to understand the physiological mechanism of different bioactive compounds present in the CA extracts and to suggest that the

therapeutic modality of these compounds with particular reference of anticonvulsant and neuroprotective activity.

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NEUROPROTECTIVE UPSHOT OF CENTELLA ASIATICA AGAINST PENTYLENETETRAZOLE INDUCED EPILEPSY IN RATS WITH REFERENCE TO PROTEIN METABOLISM

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Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, protein.

ABSTRACT

Centella asiatica (CA) is being used in traditional medicine in the treatment of several neurological disorders including epilepsy. The present study is carried out to investigate the anticonvulsant effect of different extracts of CA with particular reference to protein metabolism in different regions of rat brain (Cerebellum, Cerebral Cortex, Hippocampus and Pons-medulla) during Pentylenetetrazole (PTZ)- induced epilepsy. The rats were randomly divided into 8 groups having 6 in each group: 1. Control group received Saline, 2. PTZ-induced epileptic group (60 mg/kg b.w./ i.p/ 1 day) 3.Epileptic group pretreated with n-Hexane extract (n-HE), 4. Epileptic group pretreated with Chloroform extract (CE), 5. Epileptic group pretreated with n-butanol extract (n-BE), 6. Epileptic group pretreated with Ethyl acetate (EAE) extract, 7. Epileptic group pretreated with Aqueous(AE) extract and 8. Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/i.p). The CA extracts were administered at the dose of 200 mg/kg body weight orally for one week. Selected parameters representing protein (total, soluble and structural proteins) metabolism was studied in different regions of brain during induced epilepsy and on pre-treatment with different extracts of CA. PTZ treatment in a convulsive dose of 60 mg/kg significantly reduced total and soluble proteins content in all the brain regions compared to controls. Whereas significantly higher levels found in the PTZ treated epileptic group. Treatment with different extracts of CA reversed the alterations that have occurred during PTZ-induced epilepsy. Hence, it is evident that the different bioactive factors of CA offered protection against PTZ-induced epilepsy.

INTRODUCTION

Proteins are the most abundant bio-chemical compounds of the living organisms, which have a pivotal role to play in cellular metabolism. They constitute about one fifth of an animal body on wet weight basis (Swaminathan, M 1983). Proteins have important activities including catalysis of metabolic reactions and transport of vitamins, minerals, oxygen and fuels. Functionally proteins exhibit a great diversity and constitute heterogeneous group having diverse physiological functions as structural elements, in contractile systems, for nutrient storage, as vehicles of transport, as hormones, as catalysts, as toxins and as protective agents (Nelson and Cox, 2005).

Hence, an important goal of molecular medicine is the identification of proteins whose presence, absence, or deficiency is associated with specific physiologic states or diseases (Murray *et al*., 2007). Proteins play a dual role as a building material and as a source of energy for the organism. It provides the organism with energy liberated through its breakdown and utilized in life processes (Babsky *et al*., 1985). Free amino acids are considered to act as a connecting link between protein and carbohydrate metabolism (Murray *et al*., 2007). Transaminases are important enzymes in animal metabolism which are intimately associated with amino acid synthesis and lysis. Among these, aspartate and alanine transaminases (AAT and ALAT) are widely distributed in the cells of all animals. The AAT catalyses the interconversion of aspartic acid and a- ketoglutaric acid to oxaloacetic acid and glutamic acid. While ALAT catalyses the interconversion of alanine and α -ketoglutaric acid to pyruvic acid and glutamic acid. Branched chain amino acids (BCAAs) leucine, isoleucine and valine are essential amino acids and the precise regulation of these amino acids depends on endogenous proteolysis (Haymond *et al*., 1983). The metabolism of BCAAs is intiated by respective branched chain aminotransferases (BCAT) resulting in glutamate and corresponding branched chain keto acids (Odessey, R and Gold berg, 1979). These transaminases also function as a link

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between protein and carbohydrate metabolisms and the net outcome is incorporation of keto acids into the TCA cycle; besides these enzymes are the first in their catabolic pathways and thus limit the overall reaction rates.

In view of the importance of protein metabolisms, the present study is taken up to study the alterations in protein profiles and their turnover in different regions of brain during PTZ-induced epilepsy and on antiepileptic treatment using different extracts of *Centella asiatica.*

MATERIAL AND METHODS

Procurement and Maintenance of Experimental Animals

Male adult Wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±2^{0}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA

438/01/a/cpcsea/dt:17.07.2006 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2006.

Drugs and Chemicals

Pentylenetetrazole and diazepam were obtained from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were analytical grade.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of Plant Extracts

The active principles of the leaves of plant were extracted into different solvents, Methanol, Water, n-Hexane, Chloroform, Ethyl acetate and n-Butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants (Sowmyalakshmi *et al*., 2005; Vattanajun *et al*., 2005). Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for these studies.

Induction of Epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole

(60mg/Kg body weight) in saline (Santos *et al*., 2002; Rizwan *et al*., 2003).

Administration of Test substance

Each fraction of CA extract (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ (Saxena and Flora, 2006). A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA (Vattanajun *et al*., 2005). The volume of administration was kept at 1ml to the animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Experimental design for screening of plant extracts for anticonvulsant activity

The rats were divided into 8 groups i.e., Group1-Normal saline treated control rats (SC), Group 2-Rats treated with PTZ (Epileptic group), Group 3-Epileptic rats pretreated with n-Hexane Extract (nHE+PTZ), Group 4-Epileptic rats pretreated with Chloroform Extract (CE+PTZ), Group 5-Epileptic rats pretreated with Ethyl acetate Extract (EAE+PTZ), Group 6-Epileptic rats pretreated with n-Butanol Extract (nBE+PTZ), Group 7-Epileptic rats pretreated with Aqueous Extract (AE+PTZ) and Group 8-Epileptic rats pretreated with Diazepam (DP+PTZ). Each group consisted of 6 rats and used for studying the effects of different fractions/extracts of plant, *Centella asiatica*.

Isolation of Tissues

After stipulated duration, the animals were sacrificed by cervical dislocation and different brain regions such as Cerebral Cortex (CC), Cerebellum (CB), Pons Medulla (PM) and Hippocampus (HC) were immediately isolated, frozen in liquid nitrogen and were stored at -80^0 C until analysis.

Biochemical Analysis

The total, soluble and structural protein content was estimated by the method of Lowry *et al.,* 1951.

Statistical Analysis

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

RESULTS

Proteins

Total and Soluble proteins were decreased with non-significant changes in structural proteins in different regions of rat brain during PTZ-induced epilepsy. An increase in the levels of

different protein fractions were recorded during treatment with different CA extracts.

During PTZ-induced epilepsy Cerebral cortex (CC) recorded highest depletion in total protein content (-40.34) followed by Cerebellum (CB) (-24.33), Pons medulla (PM) (-20.35) and Hippocampus (HC) (-11.65). Whereas the total protein content was increased in all the brain regions of epileptic rats pre-treated with different extracts of CA and diazepam (Table 1).

and Cerebellum (CB) (-16.64). Whereas, the soluble protein content was increased in all the brain regions in the epileptic animals pre-treated with different extracts of CA and diazepam (Table 2).

During the PTZ-induced epilepsy Pons medulla (PM) recorded highest depletion in structural protein content (-19.87) followed by Cerebral cortex (CC) (-18.13), Hippo campus (HC) (-17.74) and Cerebellum (CB) (-13.85).

Table 1 Changes in Total protein content in different regions of rat brain during PTZ- induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*.

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+DP
CC	79.205 ±1.775	47.235* ±2.935 (-40.36)	99.222 ±11.705 (25.27)	111.099* ±21.250 (40.26)	96.434 ±3.628 (21.75)	109.751* ±10.016 (38.56)	107.675* ±5.968 (35.94)	117.083* ±2.137 (47.82)
CB	84.850 ±1.775	64.206* ±4.224 (-24.33)	87.428 ±4.899 (3.03)	107.675* ±2.252 (26.90)	116.712* ±2.249 (37.55)	93.850* ±4.352 (10.60)	115.623* ±2.874 (36.26)	113.364* ±2.001 (33.60)
HC	91.874 ±1.775	81.162* ±2.754 (-11.65)	97.668* ±2.250 (6.30)	115.66* ±1.038 (25.89)	106.685* ±1.457 (16.12)	101.874* ±2.354 (10.88)	119.448* ±3.233 (30.01)	122.981* ±2.752 (33.85)
PM	67.840 ±1.775	54.034* ±1.405 (-20.35)	76.947* ±2.048 (13.42)	81.328* ±1.972 (19.88)	89.516* ±4.736 (31.95)	81.781* ±0.773 (20.55)	91.478* ±3.885 (34.84)	91.985* ±2.849 (35.59)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over saline control
*Values are significant at P < 0.05 in Scheffe test. (Values are expressed in mg/g wet wt of the tissue)

Table 2 Changes in Soluble protein content in different regions of rat brain during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+DP
CC	46.705 ±1.383	29.424* ±0.965 (-37.00)	52.118* ±1.296 (11.59)	54.506* ±1.567 (16.70)	57.326* ±1.104 (22.74)	49.873 ±1.455 (6.78)	60.352* ±3.275 (29.22)	60.001* ±1.478 (28.46)
CB	51.935 ±1.175	43.292* ±1.890 (-16.64)	54.507 ±3.244 (4.95)	57.903* ±0.808 (11.49)	63.656* ±0.834 (22.56)	68.466* ±1.178 (31.83)	69.112* ±1.283 (33.07)	73.987* ±1.502 (42.46)
HC	52.406 ±1.821	43.207* ±1.648 (-17.55)	54.901 ±1.754 (4.76)	57.552* ±0.152 (9.82)	62.863* ±1.244 (19.95)	67.827* ±2.092 (29.42)	69.781* ±1.313 (33.15)	71.160* ±1.610 (35.78)
PM	35.351 ±1.383	18.070* ±0.965 (-48.88)	40.764* ±1.296 (15.31)	43.152* ±1.567 (22.06)	45.972* ±1.104 (30.04)	38.519 ±1.455 (8.96)	48.998* ±3.275 (38.60)	48.647* ±1.478 (37.61)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over saline control
*Values are significant at P < 0.05 in Scheffe test.
(Values are expressed in mg/g wet wt of the tissue)

Table 3 Changes in Structural protein content in different regions of rat brain during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*.

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+DP
CC	48.835 ±1.381	39.978* ±0.204 (-18.13)	53.213 ±0.248 (8.96)	58.069* ±1.746 (18.90)	58.288* ±2.297 (19.35)	54.892 ±1.297 (12.40)	56.608 ±2.456 (15.91)	55.506 ±0.765 (13.66)
CB	56.447 ±0.527	48.627 ±0.555 (-13.85)	59.883 ±0.389 (6.08)	63.323 ±1.285 (12.18)	62.762 ±1.208 (11.18)	65.637 ±1.228 (16.28)	61.014 ±1.584 (8.09)	63.851 ±1.406 (13.11)
HC	65.684 ±1.715	54.030 ±1.121 (-17.74)	67.806 ±1.787 (3.23)	70.678 ±1.812 (7.60)	72.230 ±1.433 (9.96)	75.123 ±0.738 (14.37)	70.533 ±0.942 (7.38)	77.951* ±1.312 (18.67)
PM	49.399 ±2.683	39.579* ±2.134 (-19.87)	51.920 ±0.523 (5.10)	56.087 ±0.512 (13.53)	57.447 ±1.032 (16.29)	55.764 ±1.578 (12.88)	57.139 ±1.103 (15.66)	55.199 ±0.670 (11.74)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over saline control
*Values are significant at P < 0.05 in Scheffe test. (Values are expressed in mg/g wet wt of the tissue)

During PTZ-induced epilepsy, Pons medulla (PM) recorded highest depletion in soluble protein content (-48.88) followed by Cerebral cortex (CC) (-37), Hippo campus (HC) (-20.35)

Whereas, pre-treatment with CA extracts and diazepam caused an increase in the structural protein content in all the brain regions (Table 3).

DISCUSSION

In the present investigation the total and soluble proteins were decreased significantly in all the brain regions during PTZ-induced epilepsy. Among all the protein fractions, the soluble protein content was found to be decreased more than that of insoluble protein.

Pretreatment with different extracts of CA caused a marked elevation of total and soluble proteins with moderate elevation in insoluble or structural protein content. The present findings are in coherence with the observation of Oliveira *et al.,* 2004 and Fighera *et al.,* 2006 who reported the reversal of PTZ-induced alterations by *Centella asiatica*. Keeping in view of neuroprotective role of CA, it is expected that different extracts of CA might have prevented the cellular damage by inhibiting the proteolytic activity as evidenced by elevation in protein content. It is well established that glutamate is a major excitatory neurotransmitter which plays a central role in epileptogenesis (Da Silva *et al*., 2007) and has been implicated in spreading seizure activity (Feng *et al*., 2005).

Earlier studies have shown that seizure associated glutamate release is doubled in the epileptogenic human hippocampus (During and Spencer, 2000). Chapman, 2000 has reported the involvement of excitatory glutamatergic mechanism during acute and transient seizures in chronic epilepsy models such as amygdale-kindled rats or induced status epilepticus. Similar increase in glutamate release was also observed in different types of epilepsy (Bikjdaouene *et al*., 2004; Petroff *et al*., 1999).

From the observed decrease in protein metabolism during PTZ induced epilepsy, it is presumed that the bioactive factors present in different extracts of CA possibly modulate the different pathways related to glutamate metabolism thus reducing the endogenous production and accumulation of glutamate as one of the aspect of antiepileptic treatment.

CONCLUSION

The observed changes in Proteins, it is presumed that the bioactive factors present in different extracts of *Centella asiatica* possibly modulate the different pathways related to glutamate metabolism thus reducing the endogenous production and accumulation of glutamate as one of the facets of antiepileptic treatment.

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National Seminar on Molecular and Genetics Basis of Neurological Disorders

(MGBND 2014)

December 19-20, 2014
Sponsored by UGC-SERO

PROCEEDINGS

Editors

Dr. Kanchi. Siva Prasad
Prof. S. Krupanidhi
Dr. M. Ananda Rao
Dr. K. S. Sairam

Organized by

Department of Zoology
SRI V.S.S.C. GOVERNMENT DEGREE COLLEGE
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SPSR NELLORE, ANDHRA PRADESH, INDIA

Proceedings of National Seminar on Molecular and Genetics Basis of Neurological Disorders, December 19-20, 2014, Department of Zoology, Sri VSSC Govt. Degree College, Sullurpeta, SPSR Nellore Dt.

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Message from Regional Joint Director

India faces a lot of challenges regarding health sciences particularly neurology. It is indeed very relevant that a National Seminar on “Molecular and Genetics Basis of Neurological Disorders” is being held at Sri VSSC Government Degree College Sullurpeta on December 19-20, 2014. The seminar has received a huge response and received many research papers, and organizers have selected all the papers for inclusion in the proceedings of the seminar. It is inspiring to note that a number of papers have been received from Degree Colleges and Universities. Professors and many other senior Lecturers / researchers will deliver invited talks in the seminar. I hope that the deliberations in the seminar will help students / researchers from and the seminar will provide a platform for inculcating the scientific attitude from all of the students. I wish the seminar all success.

(Dr. M. Prasad Rao)

Message from Guest of honor

Prof. S. Krupanidhi
Head, Dept. of Biotechnology
Vignan's University, VFTRU
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MESSAGE

It gives me an immense joy that an young faculty took the lead in initiating and organizing a UGC sponsored national seminar on the interesting and upcoming topic "*Molecular and Genetics Basis of Neurological Disorders*" On 19th and 20th December 2014. The intricacies of Neuroscience are so complex. Several disciplines including medical science are attempting to unravel the hidden principles in neuroscience. It is basically skill oriented. Students need to be trained in this line. I congratulate the Principal, Head of the department of zoology and the organizing committee and students on this memorable occasion and I wish that this seminar is successful.

Prof S. Krupanidhi

SRI V.S.S.C. GOVERNMENT DEGREE COLLEGE SHAR ROAD, SULLURPET – 524 121

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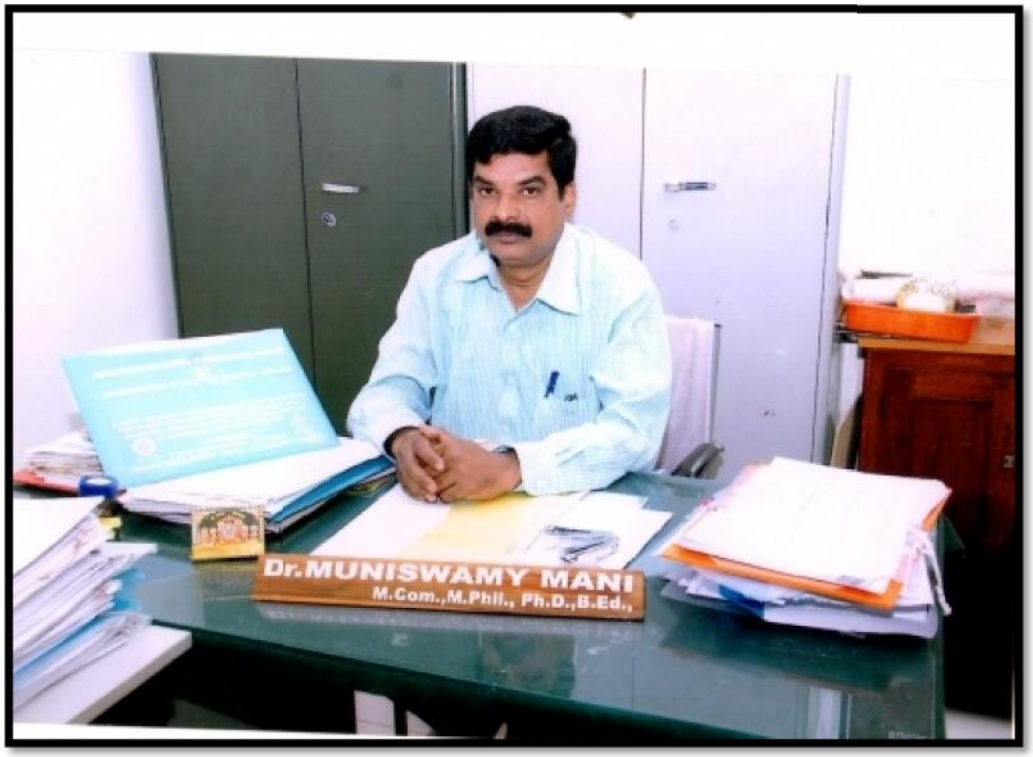
December 12, 2014

Message from Principal



The National Seminar on *"Molecular and Genetics Basis of Neurological Disorders"* organized by the department of Zoology of our college makes me glad to be a part with you in this grand occasion. I hope it is one of the rare opportunities for academicians/ Lecturers/ young scientists / students and those who are interested in this field, provides opportunities to participate and to share their ideas thereby to take constructive suggestions on the emerging trends in the relevant field. On behalf of our institution and my team I extend my cordial welcome to all the participants.

Dr. K. S. Sairam BHMS
Homoeopathic Physician
Sullrupeta.

We can get desired results in various aspects by encouraging and involving Science Graduates/P.Gs/Research Scholars along with the specialist in the Medical field in addressing common Neurological Disorders for their Preventive and Promotional Health care management with multi dimensional approach.

GOVERNMENT DEGREE AND P.G. COLLEGE

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MESSAGE

I am happy to note that the department of zoology, headed by Dr. K. Sivaprasad, Sri V.S.S.C. Government Degree College, **SULLURUPETA** is organizing **National Seminar on "Molecular and Genetics Basis of Neurological Disorders" on 19-12-2014 & 20-12-2014.** In the present scientific scenario Molecular genetics and Neurological studies are occupying prime importance. The neurological disorder rate is at higher pace in the present population. I strongly believe that the deliberations of this seminar will definitely come out with some ideas in the direction of filling the lacunae in the treatment of neurological disorders. I wish the Principal and the organizers a grand success.

(Dr. M. Ananda Rao)

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Dr. K. Siva Prasad, Lecturer in Zoology

Message from Organizing Secretary

The National Seminar "Molecular and Genetics basis of Neurological Disorders" aims to provide a common platform for all scientific community and academia. A number of persons from colleges and universities are participating in this seminar. It is also expected to be a good get-together of senior and young scientists. Department of Zoology, SVSSC Government Degree College, Sullurpeta has good infrastructure to support research activities. Although the present seminar is a National Seminar, its scope is wider. We got huge response and received about 58 papers. All papers were subjected to peer review process and finally 20 papers are finding place in the proceedings.

The financial support for the seminar has been provided by University Grants Commission, South East Regional organization, Hyderabad. We acknowledge them.

We wish to thank all the authors, reviewers and invited speakers, members of advisory board and organizing team, student-volunteers and all other who have contributed in the successful organization of the seminar.

We are very grateful to Sri. M. Prasad Rao RJD Guntur.

My deep and profound gratitude's are due to Prof. S. Krupanidhi, Vignan University, Dr. YV Rami Reddy, Dr. S. Kishore, SV University.

I wish to express my deepest sense of gratitude to my judicious and sagacious Principal Dr. Muniswamy Mani, whose keen, able, friendly advice and encouragement culminated this tedious seminar in success. His transfixing instructions and continuous support provided great impetus throughout the way of this seminar.

I express my sincere gratitude to Vice-principal Dr. N.Thyagaraju.

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(Kanchi. Siva Prasad)

Organizing Secretary.

INDEX

Public health and Neurological disorders

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WHO defined health as 'a state of complete physical, mental and social-well being and not merely the absence of infection or disease'.

Public health is an offshoot of the government and deals with science and practice of protecting and improving the health of the population/community through promotion, health education and management of communicable and non-communicable diseases including neurological disorders.

"Healthy people in healthy communities" is the ultimate goal of all the public health policies-which are aimed at promoting physical and mental health and preventing diseases, injury and disability.

Neurological research provides scope to several different disciplines namely Morphology, Physiology, Pharmacology, Psychology: behavioural and cognitive science. Psychology looks the brain as a "Black Box" where only the determined inputs are given and varied outputs are analyzed and not bothered about neural networks. However, the other listed disciplines deal with the symptoms and behavior.

The Global Burden of Disease study, is an ongoing international collaborative project between WHO, the World Bank and Harvard school of public health. This program produced evidences that these neurological disorders are the greatest threats to public

health. Further, it is anticipated that the neurological burden is expected to become even more serious and unmanageable problem in all countries in this informatics era.

What are neurological disorders?

Neurological disorders are diseases of the central and peripheral nervous system i.e., the brain, spinal cord, cranial nerves, peripheral nerves, nerve roots, autonomic nervous system, neuromuscular junction and muscles. These disorders include epilepsy, Alzheimer disease and other dementias, cerebrovascular diseases including stroke, migraine and other headache disorders, multiple sclerosis, Parkinson's disease, neuroinfections, brain tumors, traumatic disorders of the nervous system such as brain trauma, neurological disorders as a result of malnutrition and muscular dystrophy.

Mental disorders, on the other hand, are "psychiatric illnesses" or diseases which appear primarily as abnormalities of thought, feeling or behaviour, producing either distress or impairment of function.

Hundreds of millions of people worldwide are affected by neurological disorders. Approximately 6.2 million people die because of stroke each year; over 80% of deaths take place in low- and middle-income countries. More than 50 million people have epilepsy worldwide. It is estimated that there are globally 35.6 million people with dementia with 7.7 million new cases every year - Alzheimer's disease is the most common cause of dementia and may contribute to 60–70% of cases. The prevalence of migraine is more than 10% worldwide.

These neurological disorders are either genetic disorders or metabolic diseases of the nervous, sensory, hormonal and muscular systems. There are more than 600 neurological disorders reported so far.

The role of public health in neurological disorders fulfils two roles:

1. Provides comprehensive information to the policy makers
2. Confers public health program as awareness raising tool.

Several nongovernmental organizations are working in the areas of various neurological disorders, both in professional capacity and in caring for people affected

by these malfunctions. The key constitutional responsibilities of the WHO are to foster partnership and collaborations among scientific and professional groups in order to contribute to the advancement of global health.

List of a few non-governmental organizations is:

1. Alzheimer's disease international
2. European Parkinson's disease association
3. International association of the study of pain
4. International bureau for epilepsy
5. International headache society
6. International league against epilepsy
7. Multiple sclerosis international federation
8. World federation of neurology
9. World federation of neurological society
10. World headache alliance.

Public education, popularization of science, school and collegiate education and global campaigns through the committed organizations should work to eliminate the stigma and discrimination against people with neurological disorders or otherwise it is unfortunately rampant and ubiquitous. Dignity of people with neurological disorders needs to be sustained and their quality of life is to be improved. As students of biology, endowed with the knowledge on the nervous system, it is our responsibility to carry the message to upkeep the social balance.

(**Source**: Neurological disorders: Public health challenges, World Health Organization)

STATUS OF CALPAINS IN KAINATE INDUCED TEMPORAL LOBE EPILEPSY

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ABSTRACT

Temporal lobe epilepsy is the most common type of epilepsy, seen in humans characterized by spontaneous recurrent seizures of temporal lobe origin. The spontaneous recurrent seizures leads to calcium influx into the neuronal cells, activate cell death mediators leading to death of neurons. The hippocampal neuronal loss plays critical role in genesis and progression of temporal lobe epilepsy.

In the study we sought to test the status of calpains which are one of the cell death mediators by administrating kainic acid to induce to temporal lobe epilepsy.

Kainate was administered intraperitonially to male Wistar rats of three months age at different time intervals, the brain samples were collected and the morphology of cells was studied by haematoxylin and eosin staining and found that the neuronal cells showed both apoptotic and necrotic type of cell death. Then sub cellular fractions are isolated for protein analysis and to perform western blot, so as to study the status of calpains. The result showed the up regulation of calpains. At last the activity of the calpains was also measured using azocaesin as substrate which was found to be high after 12hrs.

From the above experiments and results it is concluded that in kainate induced temporal lobe epilepsy calpains are active , up regulated and both apoptotic and necrotic type of cell death mechanisms might be operated and there is still more research required to unravel the death mechanisms whish can pave the way for better treatment by discovering new drugs.

KEY WORDS :

Epilepsy, Temporal lobe, neuronal cells, apoptosis, cell death mediators.

Introduction

"Epilepsy is a neurological disorder in which the normal pattern of neuronal activity is disturbed. (ref1, 2). Studies in developed countries suggest an annual incidence of epilepsy of approximately 50 per 100,000 of the general population. However studies in developing countries suggest that this figure is nearly double that of 100 per 100,000. One of the main reasons for the higher incidence of epilepsy in developing countries is the higher risk of experiencing a condition which can lead to permanent brain damage. These conditions include neurocysticercorsis and malnutrition. Temporal lobe epilepsy is the most common type of epilepsy in adult humans characterized clinically by the progressive development of spontaneous recurrent seizures from temporal lobe foci.Hippocampal neuronal loss in the Pyramidal cell layers of CA1 and CA3 regions and mossy fiber sprouting plays a critical role in the genesis and progression of temporal lobe epilepsy. (ref-1 2).

Glutamate is the main excitatory neurotransmitter in the CNS mediates a variety of neurological effects as a result of ionotropic glutamate receptor stimulation.

Kainate is, the structural analogue of glutamate binds irreversibly to glutamate receptors and cause excitotoxic cell death due to influx of excessive $Ca2^{+}$ into the cell and inturn activation of various cell death mediators.[ref 13] [ref 7,9] .When excitatory neurotransmitters such as glutamate are released from synapses on depolarization of the arrival of action-potential, they get cleared off by the various transporters at the synaptic cleft normally. (ref-1) But catastrophic depolarization which occurs during seizure, (ref- 4), hypoxia or hypoglycemia, compromise energy production and therefore the ability of the cell to maintain a membrane potential.

Glutamate binds and opens specific ionotropic receptor channels on post synaptic neurons. Gating of these channels provokes an influx of Ca^{2+} ions inside the cell directly (through glutamate receptors that conduct both calcium and sodium) or indirectly (through the activation of voltage-gated calcium channels). The sharp increase of intracellular calcium concentration is a principal death-signaling event that is involved in both apoptosis and necrosis [ref 3,6].

The contribution of each type of death to excitotoxicity correlates with the severity and abruptness of the increase in intracellular calcium concentration. [ref 11]. More profound change initiates necrosis, where as relatively mild increase preferentially induce apoptosis.

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While necrosis and apoptosis can be distinguished in same situations in certain dying cells show distinctive features of both apoptosis (ref- 10) and necrosis. (ref- 08). The idea of a continuum of responses ranging from apoptosis to necrosis is emerging where the relative contribution of each depends on several factors, including the energy content of the cell and the severity of the insult. (ref- 14).

Proteases in neurodegeneration and necrosis.

A wide variety of proteases are engaged in cell death processes through non-specific and limited proteolysis.These include cysteine and aspartyl proteases, lysosomal proteases, microglia proteases and the ubiquitin proteasome system. Cytosolic cysteine-proteases include caspases and calpains [ref 3,5]

Objectives

To induce temporal lobe epilepsy by administrating kainic acid.To know the morphology of cells whether they are normal or undergoing cell death after kainate administration using H and E staining.To study DNA fragmentation during cell death using agarose gel electrophoresis.To isolate subcellular fractions for protein analysis and perform western blot so as to study the status of Calpains and measure the activity of Calpains using azocaesin

.**Materials and Methods**

Sample generation :

Animal model: Taken from Andrew J.Cole,Sookyong Koh and Yi Zheng Are seizures harmful: What can we learn from animal models. Progress in Brain Research,vol.135[13-23]

a) **Experimental:** Male wistar rats of 3 months old and weight 200 grams are taken. Kainate [0.01 g/Kg wt] was administered systemically and they were divided into two groups. One group is decapitated and the brain samples are used for protein analysis, other group is perfused and their brain samples are used for histological studies. Rats were sacrificed at 3, 6, 12, 24, 48hrs, after kainite administration.
b) **Control: C**ontrol are generated by giving saline instead of kainite systemically.

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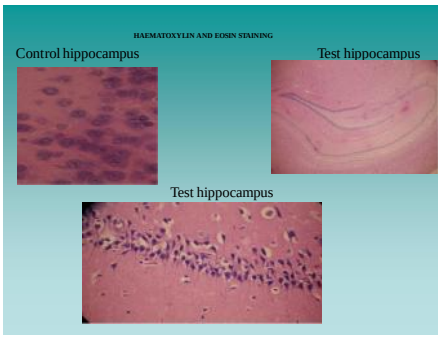
Histopathology

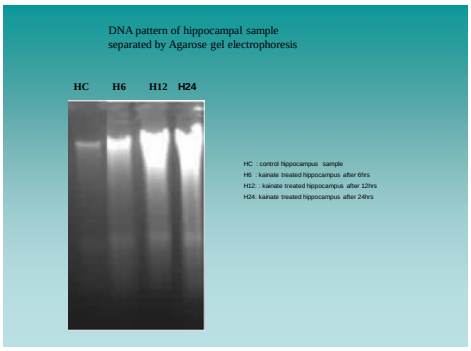
To check whether cell death is involved in Kainate induced temporal lobe epilepsy, the brain was collected after kainate administration and was subjected to H and E staining.

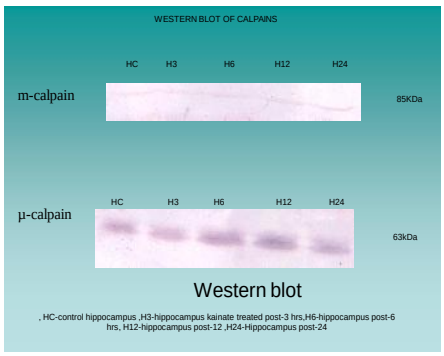


The cellular morphology of neurons was observed in the hippocampal region and was found that a large number of degenerative cells appeared in Kainate treated rats than the control rats. The control sections showed intact neuropile, cells with normally stained cytoplasm and intact nucleus. Whereas some shrunken cells with darkly stained nucleus and some lightly stained swollen cells are clearly visible in the sections treated animals. This shows clear signs of cell death and further analysis on the mode of cell death can be made based on further biochemical and microscopic observation.

DNA Gel electrophoresis

After confirming that there is cell death in kainite treated samples, agarose gel electrophoresis is done using the DNA extracted from hippocampal region to know the type of cell death involved. The DNA Gel electrophoresis showed smeared pattern of DNA in gel. This is a characteristic of necrotic cell death. The extent of smearing is shown to be greater from 12 hrs.





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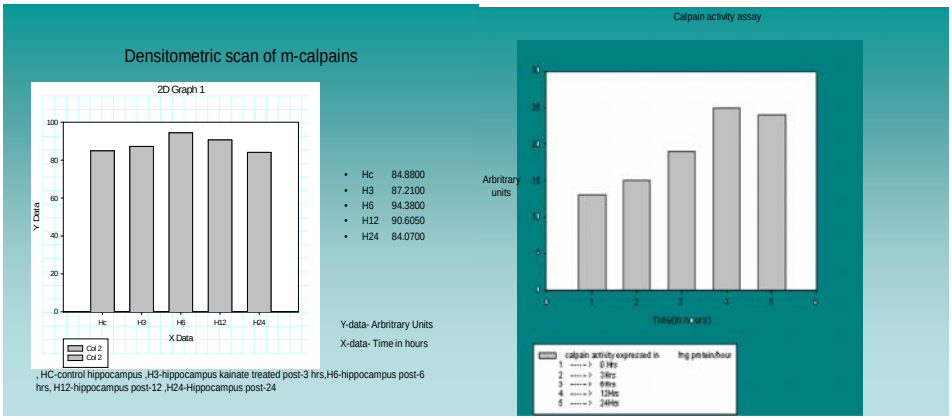
Western blot

After confirming the type of cell death subcellular fractionation is done from hippocampal region and cytosolic fractions are collected. The protein content is estimated by Lowrys method and SDS-PAGE is done to resolve various proteins in the sample. Then this gel with resolved proteins is probed with polyclonal Ab against calpains for immunoblot analysis to know whether calpains are up regulated or not. The 12.5% SDS-PAGE showed equal loading of sample. The immunoblot showed significant up regulation of µ-calpain from 12 hours on wards but m-calpain was not significantly up regulated even after 24 hours of the set of seizures.

Calpain activity assay.

This assay is done to know whether the up regulated calpains are active or not azocaesin as substrate. This assay showed that there is no significant increase in calpain activity up to 6 hrs but after 6 hrs there is a significant increase in calpain activity and reaches peak at 12 hrs.



Results and Discussion

The H and E staining showed both apoptotic and necrotic type of cell death, but DNA agarose gel electrophoresis showed smeared pattern of DNA which is a characteristic feature of necrotic cell death.The calpains which are the mediators of both apoptotic and necrotic cell death are found to be upregulated and active.From all the above results it is concluded that in kainate induced temporal lobe epilepsy both apoptotic and necrotic type of cell death mechanisms might be operated and there is still more research required to unravel the death mechanisms involved in kainate induced temporal lobe epilepsy.

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NEUROTOXINS EFFECT ON HEALTH

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ABSTRACT

Neurotoxins are substances that are poisonous or destructive to nerve tissue. Common examples of neurotoxins include lead,[ethanol (drinking alcohol), glutamate, nitric oxide(NO), botulinum toxin (e.g. Botox), tetanus toxin, and tetrodotoxin. Some substances such as nitric oxide and glutamate are in fact essential for proper function of the body and only exert neurotoxin effects at excessive concentrations. Local pathology of neurotoxin exposure often includes neuron excitotoxicity or apoptosis but can also include glialcell damage. Macroscopic manifestations of neurotoxin exposure can include widespread central nervous system damage such as intellectual disability, persistent memory impairments, epilepsy, and dementia.

KEY WORDS :

Neurotoxins, excitotoxicity, pathology, neuron .

Introduction

Exposure to neurotoxins in society is not new, as civilizations have been exposed to neurologically destructive compounds for thousands of years. One notable example is the possible significant lead exposure during the Roman Empire resulting from the development of extensive plumbing networks and the habit of boiling vinegared wine in lead pans to sweeten it, the process generating lead acetate, known as "sugar of lead".[1] In part, neurotoxins have been part of human history because of the fragile and susceptible nature of the nervous system, making it highly prone to disruption.

The nervous tissue found in the brain, spinal cord, and periphery comprises an extraordinarily complex biological system that largely defines many of the unique traits of individuals. As with any highly complex system, however, even small perturbations to its environment can lead to significant functional disruptions. Properties leading to the susceptibility of nervous tissue include a high surface area of neurons, a high lipid content which retains lipophilic toxins, high blood flow to the brain inducing increased effective toxin exposure, and the persistence of neurons through an individual's lifetime, leading to compounding of damages.[20] As a result, the nervous system has a number of mechanisms designed to protect it from internal, and external assaults, including the blood brain barrier.
The blood-brain barrier (BBB) is one critical example of protection which prevents toxins and other adverse compounds from reaching the brain.[2] As the brain requires nutrient entry and waste removal, it is perfused by blood flow. Blood can carry a number of ingested toxins, however, which would induce significant neuron death if they reach nervous tissue. Thus, protective cells termed astrocytes surround the capillaries in the brain and absorb nutrients from the blood and subsequently transport them to the neurons, effectively isolating the brain from a number of potential chemical insults.

As neurotoxins are compounds which adversely affect the nervous system, a number of mechanisms through which they function are through the inhibition of neuron cellular processes. These inhibited processes can range from membrane depolarization mechanisms to inter-neuron communication. By inhibiting the ability for neurons to perform their expected intracellular functions, or pass a signal to a neighboring cell, neurotoxins can induce systemic nervous system arrest as in the case of botulinum toxin,[3] or even nervous tissue death.[4] The time required for the onset of symptoms upon neurotoxin exposure can vary between different toxins, being on the order of hours for botulinum toxin[5] and years for lead.

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Neurotoxin classification	Neurotoxins
Na channel inhibitors	Tetrodotoxin
K channel inhibitors	Tetraethylammonium
Cl channel inhibitors	Chlorotoxin,
Ca channel inhibitors	Conotoxin
Inhibitors of synaptic vesicle release	Botulinum toxin, tetanus toxin[1]
Receptor inhibitors	Bungarotoxin Curare
Receptor agonists	25I-NBOMe JWH-018
Blood brain barrier inhibitors	Aluminium, mercury
Cytoskeleton interference	Arsenic, ammonia
Ca-mediated cytotoxicity	Lead
Multiple effects	Ethanol
Endogenous neurotoxin sources	Nitric oxide,[1]glutamate

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THERAPEUTIC POTENTIAL OF PHYTOPHENOLS IN THE TREATMENT OF NEURODEGENERATIVE DISORDERS

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ABSTRACT

Incidence of neurodegenerative disorders is on raise in India during the recent times. The changes in lifestyle due to the influence of westernization in the liberalized economy have been a major contributor and this has a devastating effect on the national economy. Increase in the incidence of Alzheimer's Disease, Parkinson's Disease, Stroke and other neurodegenerative disorders is a worrying factor in terms of costs involved in therapeutics. The synthetic drugs now used in the treatment regimen are beyond reach for vast majority and this gives scope for development of new drugs from naturally available compounds, which are widely available and cost effective, and have therapeutic potential. The possible use of botanical phenolics in the treatment of neurodegenerative disorders is reviewed in this paper.

Key Words: Neurodegeneration, Parkinson's Disease, Alzheimer's Disease, Stroke, Phenolics

INTRODUCTION

Different reactive oxygen species (ROS) like superoxide, hydroxyl and peroxyl radicals are produced in living cells under normal as well as pathological conditions[1]. Oxidative damage to DNA, proteins and lipids occurs when the rate of ROS generation go beyond the ability of antioxidant defense. Oxidative stress has been implicated in neuronal cell injury in various pathological states in central nervous system (CNS). Reactive nitrogen species (RNS) which include nitric oxide, nitroxyl anion and proxy nitrate are implicated in the pathology of neurodegenerative disorders. The effect of these RNS has been termed as "Nitrosative stress" during recent times. Both Oxidative and Nitrosative stress are implicated in the pathology of

Many neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's Disease (HD), Amyotrophic Lateral Sclerosis (ALS) and Stroke. Brain tissue is vulnerable to oxidative damage because of the fact that it utilizes high amount of oxygen for

energy generation and has low antioxidant defense enzymes. This is particularly true during aging. As a matter of fact, membranes of brain cells have abnormally high proportions of poly unsaturated fatty acids (PUFAs). Among the brain cells, neurons are highly vulnerable to toxic compounds. They are very sensitive to ischemic damage, seizure and other excitotic injuries. Oxidative damage to lipids is associated with progressive loss of membrane integrity, reduction of mitochondrial membrane potentials, and increase in plasma membrane permeability to Ca^{2+} ions [2]. Oxidative damage to proteins leads to the generation of carbonyl and nitrosylated derivatives. In addition, ROS damage to DNA results in nuclear condensation and altered gene expression, which implies that oxidative stress, is a major risk factor in the pathology of neurodegeneration. Extensive efforts have been devoted to develop novel strategies to overcome different types of damage to nerve cells [3].

Polyphenolic compounds offer beneficial effects in protecting against diseases involving oxidative stress. The mechanisms through which these compounds exert their beneficial effects are not well understood. But, there is a general consensus that these compounds have antioxidant and anti-inflammatory properties and are capable of chelating metal ions [4,5,6]. Some of these compounds may contribute to special biochemical effects which are beyond their antioxidant and radical scavenging properties. These effects may have an impact on the onset and progression of aging and neurodegenerative diseases. Proper understanding of these metabolic and signaling effects of polyphenols can pave way for new nutritional interventions in the treatment of neurodegenerative disorders [7]. In this paper, we review two botanical phenolics:

1. Resveratrol from Grapes
2. Curcumin from Turmeric

Their potential beneficial effects in the prevention and treatment of neurodegenerative disorders are discussed, with emphasis on Alzheimer's disease and notes on Parkinson's Disease and Stroke.

ALZHEIMER'S DISEASE:

Alzheimer's disease is the most common form of dementia. It is age-dependent and affects specific regions of brain that control memory and cognitive functions. Accumulation of amyloid plaques and presence of neurofibrillary tangles in neurons characterize the pathogenesis of AD [8,9]. Number of studies have revealed that oxidative stress is and early event in the development of AD [10,11,12,13]. There is evidence that soluble oligomeric form of amyloid-ß (A ß) peptides may be a key cytotoxic compound which impairs synaptic

plasticity, long before amyloid plaques are formed [14,15,16]. Cytotoxic A ß may cause neuronal cell death through ROS generation [17,18]. Evidence is available for the fat that A ß cause causes neuronal damage by targeting excitatory ionotropic glutamate receptors, in particular the N-methyl-D-aspartate (NMDA) subtype. The ability of oligomeric amyloid ß peptides and NMDA to stimulate cortical neurons and triggering signaling pathways leading to the activation of mitogen-activated protein kinases (MAPKs) and phospholipase A2 is well acknowledged [19]. Excitatory pathways involve ROS production by NADPH oxidase [20,21].

Loss of cholinergic neurons is also an important abnormality in AD pathology, in addition to changes in glutamatergic transmission. Aberrant cholinergic transmission in the basal forebrain, some cortical regions, and the hippocampus is associated with memory loss in advanced stages of AD [22]. Acetylcholine esterage inhibitors are considerd as symptomatic drugs for the treatment of AD [23]. Donepezil, rivastigmaine and galantamine are currently prescribed to treat AD patients. In general, these drugs help maintain the abilities of individuals to carry out their day to day life and are in maintain thinking, speaking, memory and other skills. But, these drugs cannot reverse the progression of the disease and are mere palliatives.

Inflammatory process which are triggered by oxidative changes may also involve in the A ß induced neurodegeneration [24]. At present, there are several AD-prevention trials are going on, aiming to use antioxidants in such a way to decrease oxidative and inflammatory damage.

There has been wide spread interest in considering natural botanical compounds as possible therapeutics for treating AD patients [25,26,27]. Plant-derived compounds are generally safer to use as compared with the synthetic drugs [28]. A number of studies used transgenic rodent models to test the neuroprotective effects of botanical compounds [29,30,31,32].

RESVERATROL:

Resveratrol is chemically 3,4',5- trihydroxystilbene. It is a polyphenolic compound found in purple grapes, pea nuts and several other plants [33]. A dietary supplement of grape skin polyphenols can offer protection against oxidative damage to the brain synaptic membranes [34,35]. This compound is more effective in protecting oxidative damage than vitamins E and C, as evidenced ny studies with rat pheochromocytoma (PC-12) cells [36]. It was shown to have protective effect against neuronal damage and excitotoxicity induced by

kainic acid in rats [37]. This compound is readily transported to the liver, brain and blood shortly after intraperitoneal injection. The most resveratrol was converted to a glycoconjugate form [38]. Many other studies in vitro and in vivo have attempted to reveal the underlying mechanisms of the neuroprotective effects of resveratrol [39,40].

Resveratrol can offer protective effects for other neurodegenerative disorders as well, besides alleviating stroke damage. This compound appears to mimic the effects of dietary caloric restriction, which has been shown to stimulate the activation of sirtuin proteins [41,42,43]. It is known to increase the life span of a number of lower organisms like yeasts, nematodes and fruit flies [44] and this effect is attributed to the activation of sirtuins which are evolutionarily conserved NAD –dependent histone deacetylases known to participate in the pathology of numerous age related disorders [45,46]. The precise mechanism of its action in activation of sirtuin I (SIRT I) and prolonging lifespan of organisms is still unclear, but there is evidence that activation of SIRT I is associated with triggering of downstream proteins like peroxisome proliferator-activated receptor coactivator-1£ (PGC-1£), the FOXO family and nuclear factor κB [45]. Several studies suggest resveratrol exerts its neuroprotective effects due its ability to activate SIRT I and other vitogenes [46] and activation of SIRT I/PGC-1 pathway was shown to protect against axonal degeneration of neurons and to decrease accumulation of amyloid peptides in nerve tissue.

Studies demonstrating the ability of resveratrol to activate SIRT1 and other vitagenes make resveratrol a promising candidate as a therapeutic agent for treating AD [47,48]. Two recently conducted studies show that the deleterious effects of high-fat and high calorie diets in mice can be mitigated by dietary supplementation with resveratrol. In one study, resveratrol reversed the shortened life span resulted the high-fat diet, and in the second study, resveratrol increased SIRT1 activation, PGC-1α deacetylation, and mitochondrial biogenesis in muscle [49]. Interestingly, a synergistic protection can be achieved when resveratrol is administered in combination with catechin (C), another plant phenolic compound [50]. In a rat model of sporadic AD, administration of resveratrol prevented cognitive impairment and oxidative stress induced by intracerebroventricular streptozotocin [51]. In another animal model of AD and tauopathy, resveratrol decreased neurodegeneration in the hippocampus and prevented learning impairment [52]. A study conducted by Wang et al. (2006) showed that consumption of red wine by Tg2576 mice can attenuate deterioration of spatial memory function and Aβ neuropathology.

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It is worth noting that besides causing in vivo effects, resveratrol can also inhibit the formation of Aβ fibrils and destabilize fibrilized Aβ [53]. Resveratrol was reported to decrease amyloid-β secretion from different cell lines [54]. In other studies, resveratrol suppressed neuroinflammation by inhibiting NADPH oxidase activity and attenuating NF-κB-induced expression of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) [55]. Taken together, these studies indicate that besides its antioxidant property, resveratrol may also exert neuroprotective effects through activation of sirtuin and vitagenes.

CURCUMIN (DIFERULOYLMETHANE):

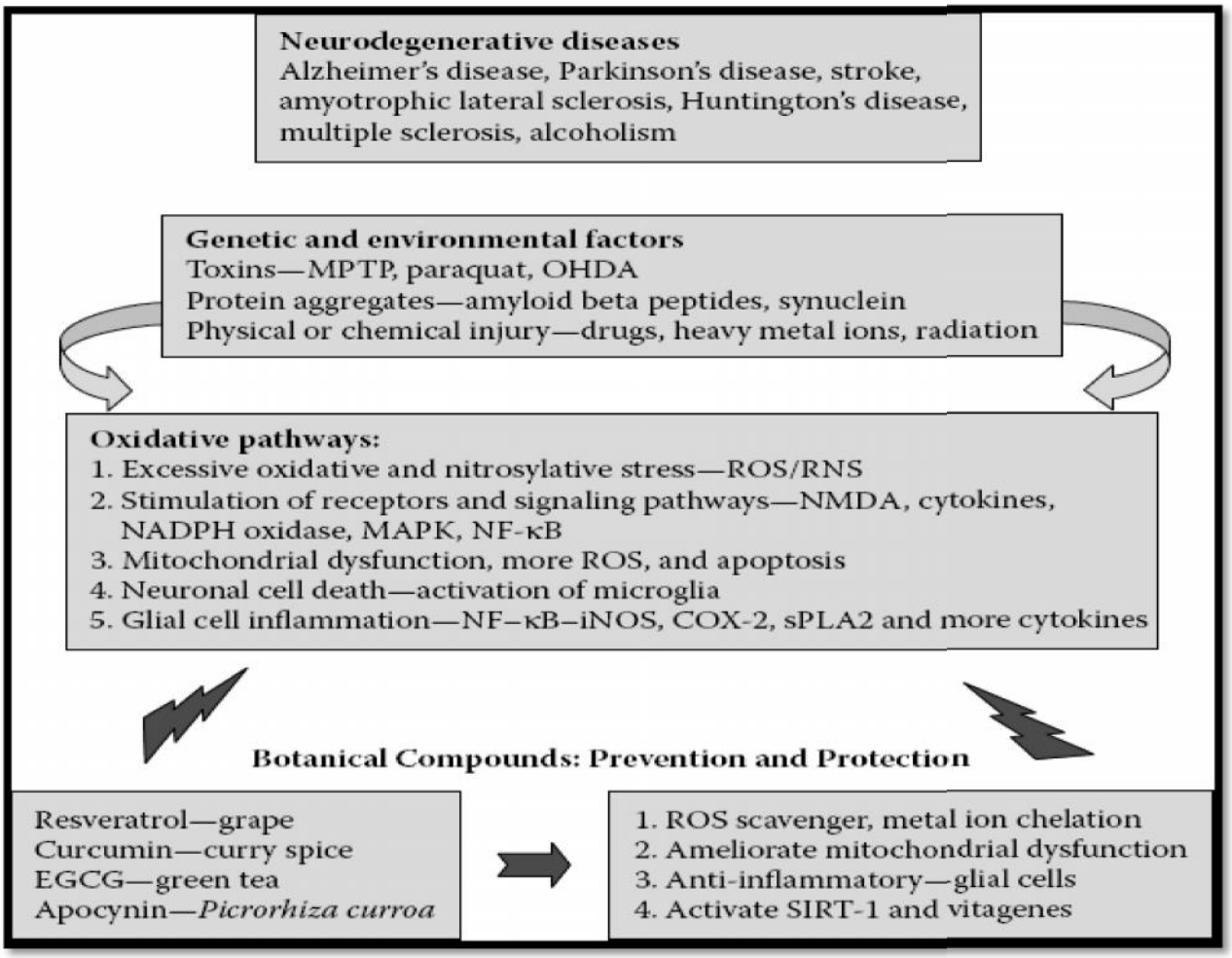
Curcumin is derived from turmeric, the powdered rhizome of the medicinal plant *Curcuma longa* Linn. It is widely used as a spice in Southeast Asian and Middle Eastern culinary. Turmeric is thought to have many medicinal properties; it is used as an antiseptic for cuts, burns, and bruises and used as an antibacterial agent. In Asian countries, curcumin can also help with stomach problems and other ailments. Besides being a strong antioxidant and anti-inflammatory agent [56], curcumin was also found to bind amyloid directly and inhibit Aβ aggregation as well as fibril and oligomer formation in vivo. Curcumin was found to inhibit the formation and extension of Aβ fibrils and to destabilize fibrilized Aβ [57].

There is a great need for well-designed studies to assess whether dietary curcumin is efficient in treating AD. In a study conducted on Tg mice, conventional non-steroidal anti-inflammatory drug (NSAID), ibuprofen, and curcumin were compared for their ability to protect against Aβ-induced damage in mice [58]. Dietary curcumin (2000 ppm), but not ibuprofen, suppressed oxidative damage and synaptophysin loss. Dietary curcumin also decreased Aβ deposits, prevented Aβ-induced spatial memory deficits in the Morris water maze test, and prevented postsynaptic density loss in Tg mice [59]. Both low and high doses of curcumin significantly lowered oxidized proteins and interleukin 1β, a pro-inflammatory cytokine that was elevated in the brains of the AD mice [60]. Besides its antiamyloidogenic, antioxidant, and anti-inflammatory abilities [61], curcumin can also alter signalling molecules and pathways in cells[62]. Clearly, more clinical trials are needed to assess the therapeutic use of curcumin for the treatment of AD [63].

THERAPEUTIC MECHANISMS OF PHYTOPHENOLS IN NEURODEGENERATIVE DISORDERS:

Many plant extracts and identified plant-derived compounds have been found useful for treatment and prevention of neurodegenerative disorders. However, their underlying molecular mechanisms and therapeutic value are still largely unknown [64]. Investigation of the health benefits of these natural compounds poses substantial challenges to modern medicine. Polyphenols are divided into different groups, depending on the number of hydroxyl groups and derivatives to the benzene rings. Flavonoids make up the largest and the most important group of polyphenols, which can be divided into subgroups such as flavanols (C, EC), flavonols (quercetin, myricetin, kaempferol), flavanons (hesperetin, naringenin), flavons (apigenin, luteolin), isoflavonoids (genistein, daidzein), and anthocyanins (cyanidin, malvidin). Depending on their molecular structure, the positions of their hydroxyl groups, and the presence of conjugated dienes, these flavonoids may have different antioxidant properties and ROS-scavenging activities.

FIGURE 1:



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The pathophysiological mechanisms underlying neurodegenerative disorders are complex and diverse, and range from oxidative stress to inflammatory responses and apoptosis (Figure 1). The complexity of cell-signalling pathways may explain the difficulties encountered in finding effective treatments. Although much progress has been made in understanding the pathogenesis of AD, current therapeutic approaches merely address symptoms. Novel therapeutic approaches using natural botanical antioxidants may be suggested to ameliorate neurotoxicity and chelate transition metals (e.g., iron and copper). Both experimental and epidemiological evidence demonstrate that flavonoid polyphenols improve age-related cognitive decline and are neuroprotective in models of PD, AD, and cerebral I/R injuries [65,66].

Many plant polyphenols have been suggested as excellent candidates for development as therapeutic agents for treatment of neurodegenerative diseases. In particular, there is increasing interest in using resveratrol for treatment of progressive neurodegenerative maladies such as AD and PD. It is recognized that some botanical compounds may selectively target a single pathway, whereas others may act globally on multiple pathways. For example, apocynin, a known inhibitor of NADPH oxidase, has been used mainly to block ROS production by NADPH oxidase, whereas resveratrol, curcumin, and EGCG can target multiple pathways. Besides its ROS-scavenging activity, resveratrol can activate SIRT1, which is related to histone acetylation and deacetylation and alteration of proteins involved in the suppression of apoptotic pathways [67]. Resveratrol can also affect Nrf2/Keap 1 pathway, which is linked with the activation of antioxidant proteins [68]. Inhibition of the NF-κB pathway by resveratrol and EGCG can decrease the production of inflammatory factors, for example, iNOS, COX-2, and secretory phospholipase A2 (sPLA2), which, in turn, can suppress the vicious cycle of cell death caused by oxidative stress. The multiple effects associated with resveratrol may offer an effective therapeutic remedy to restore neuronal homeostasis [69]. Future studies should be directed to investigate whether other phytochemicals also exhibit multiple functions [70]. Questions about bioavailability, biotransformation, synergism with other dietary factors, and their ability to cross the blood–brain barrier also need to be addressed prior to application of botanical compounds on humans. Table 1 summarizes recent studies in which different botanical compounds in different paradigms were used to test neuroprotective effects on AD, PD, and stroke models.

TABLE 1:

Studies on Botanical Compounds Used in AD, PD, and Stroke Models

Compounds	Model	Effects
		AD models
Resveratrol	i.c.v. streptozotocin	+
Curcumin	Aβ infusion	+
	Tg2576	+
EGCG	Aβ infusion, performance status	+
	Aβ infusion	+
	Tg 2576	+
		PD models
Resveratrol	6-OHDA	+
	MPTP	+
Curcumin	6-OHDA	+
	MPTP	+
EGCG	6-OHDA	−
	MPTP	+
Apocynin	Paraquat	+
		Stroke models
Resveratrol	MCAO	+
	MCAO and CCAO	+
	CCAO	+
Curcumin	MCAO	+
	CCAO	+
EGCG	MCAO	+
		±
	CCAO	+
Apocynin	CCAO and hypoxia	+
	CCAO (2X)	−
		+
Apocynin	CCAO	+
	Neonatal H/I	−

Notes: 1.CCAO: common carotid artery occlusion;
2. MCAO: Middle Cerebral Artery Oclusion
3. H/I: hypoxia/ischemia;
4. i.c.v.: intracerebral ventricular;
5. 6-hydroxydopamine (6-OHDA)-induced PD
6. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),
7. "+" indicates improvement, and
8. "−" indicates no effect or worse outcome.

(Adopted from Taylor and Francis Group, LLC)

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Elucidation of Intra-species relations of *Achatina fulica* using 16S rDNA Sequence Information

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Abstract:

In the present study, phylogenetic reconstruction of families Achatinidae and Orculidae belonging to the clade Stylommatophora, and family Onchidiidae belonging to the clade Systellommatophora was performed using 16S rDNA sequence information. It was observed that *Achatina fulica* sequenced in this study and remaining representatives from this family Achatinidae formed a single cluster. However, representative species of Orculidae instead of forming cluster with Achatinidae, clustered with the species represented from the family Onchidiidae, clade Systellommatophora. This cluster is meaningful as both the clades belong to Pulmonata.

Introduction:

Molecular phylogenetic analysis has become a valuable tool in species identification; as well in unravelling the subtle differences for which conventional systematics at times is a poor guide as it depends mainly on morphological and anatomical characters. Molecular phylogeny derives information from several molecular markers viz., 16S, 18S, ITS, 5.8S, Mt COI – I & II etc. which are useful in ascertaining suitable position for the taxa at a deeper level, where conventional taxonomy fails (Adamkewicz et.al., 1997), (Wade & Mordan, 2000).

However, one has to be careful while performing molecular phylogenetic analysis with regard to the completeness of the molecule, number of molecules that are chosen and the number of species included in the study in order to get a clear picture (Adamkewicz et.al., 1997). Classification of gastropods proposed by (Bouchet & Rocroi, 2005) was taken as a reference system in this study.

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In the present study, 16S rDNA sequences of different gastropods were retrieved from the NCBI GenBank to ascertain their phylogenetic positions using 16S rDNA sequence.

Materials and Methods:

First and foremost step in performing Molecular phylogenetic analysis is the isolation of genomic DNA of good quality. However, this has always been a difficult step with respect of gastropods due to their high protein content and co precipitation of mucopolysaccharides during isolation which contaminate the genomic DNA, thus impairing the PCR amplification (Personal observation), (Wade & Mordan, 2000) & (Sokolov, 2000). Different methods were employed by several workers to obtain good quality genomic DNA. In the present study, we have collected *Achatina fulica* from the state of Odisha (see figure 1). We have isolated the genomic DNA from the foot muscle tissue of *Achatina fulica* by slightly modifying the procedure of (Sokolov, 2000). Genomic DNA was PCR amplified for 16S rDNA and sequenced in both the directions. The sequence information thus obtained from both the strands was manually checked for complimentary matching and verified using BioEdit ver 7.2.5 (T.A. Hall 1999). A consensus sequence was generated and was submitted to NCBI for obtaining accession number. Apart from the 16S rDNA sequence of *Achatina fulica* obtained in this study, 16S rDNA gene sequences of several gastropods belonging to different taxonomic positions were retrieved from NCBI for analysis. Details regarding the sequences of species and their taxonomic positions were given in table 1 & 2.

Phylogenetic analysis was carried out using MEGA 6 (Tamura et.al., 2013). Sequences were aligned using the inbuilt *Muscle* programme with default parameters (gap opening penalty:-400, gap extension penalty 0). The resultant data obtained after multiple sequence alignment was analysed using Maximum likelihood (ML) and Neighbour joining (NJ) methods with 1000 bootstrap replications. Gap or missing data was treated as deletion during tree construction

Table 1 Organism, its location and its accession number obtained from this study

Organism	Accession Number	Taxonomic position	Country
Achatina fulica	KP317640	Clade: Stylommatophora; Inf. Group: Sigmurethra; Super family: Achatinoidea Family: Achatinidae	India: Odisha

Table 2 Organisms, their locations and their accession number retrieved from NCBI

Organism	Accession Number	Taxonomic position	Country
Achatina fulica	KM232636	Clade: Stylommatophora; Inf. Group: Sigmurethra; Super family: Achatinoidea Family: Achatinidae	India: Kerala
Achatina fulica	KC682495	Clade: Stylommatophora; Inf. Group: Sigmurethra; Super family: Achatinoidea Family: Achatinidae	USA: Florida
Achatina fulica	JQ436767	Clade: Stylommatophora; Inf. Group: Sigmurethra; Super family: Achatinoidea Family: Achatinidae	Uganda: Kampala
Achatina fulica	JQ436751	Clade: Stylommatophora; Inf. Group: Sigmurethra; Super family: Achatinoidea Family: Achatinidae	India: Nagpur
Orcula zilchi	KM188646	Clade: Stylommatophora; Sub Clade: Orthurethra Super family: Pupilloidea Family: Orculidae	Bulgaria: Burgas
Platevindex cf. mortoni	GQ985309	Clade: Systellommatophora Super family: Onchidioidea Family: Onchidiidae	-

Haliotis diversicolor diversicolor	AY146397	Clade: Vetigastropoda Super family: Haliotoidea Family: Haliotidae	-

Results and Discussions:

A total of 972 characters obtained after multiple sequence alignment of which 88 characters are constant without gaps. ML & NJ trees were constructed and shown in figures 2 & 3 respectively. Cut-off value for consensus tree was set at 50%. Indicated on the nodes are the bootstrap values. *Achatina fulica* sequenced in this study clustered with remaining *Achatina fulica* retrieved from the NCBI GenBank. Considering the topology of ML & NJ trees, *Achatina fulica* belonging to the family Achatinidae under clade Stylommatophora formed a single group (Group 1). The node connecting the *Achatina* sps (Group 1) with other species was bootstrap supported in both ML & NJ trees. However, the internal nodes with in this group (Group 1) were not bootstrap supported (<50). ML & NJ trees were rooted at group 2 which is strongly bootstrap supported. Group 2 consisted of two species *Orcula zilchi* belonging to clade Stylommatophora, family Orculidae and *Platevindex cf. mortoni* under clade Systellommatophora. *Achatina fulica* (group 1) and *Orcula zilchi* (species present in group 2) fall under the Clade Stylommatophora. However, they were not clustered together. Instead, *Orcula zilchi* was grouped with *Platevindex cf. mortoni* belonging to Systellommatophora. It is observed that group 1 and group 2 falls under Pulmonates and their positions are intact. In the present study, *Haliotis diversicolor diversicolor* belonging to the clade Vetigastropoda was included to consider it as an out group. However, it has emerged with in the two groups (bootstrap not supported), which need to be further investigated. A plausible solution for this is to include more number of species or another species as an out group as indicated by Wade & Mordan, 2000.

Figure 1: *Achatina fulica* Bowdich, 1882 (Giant African Snail) collected from the state of Odisha, India

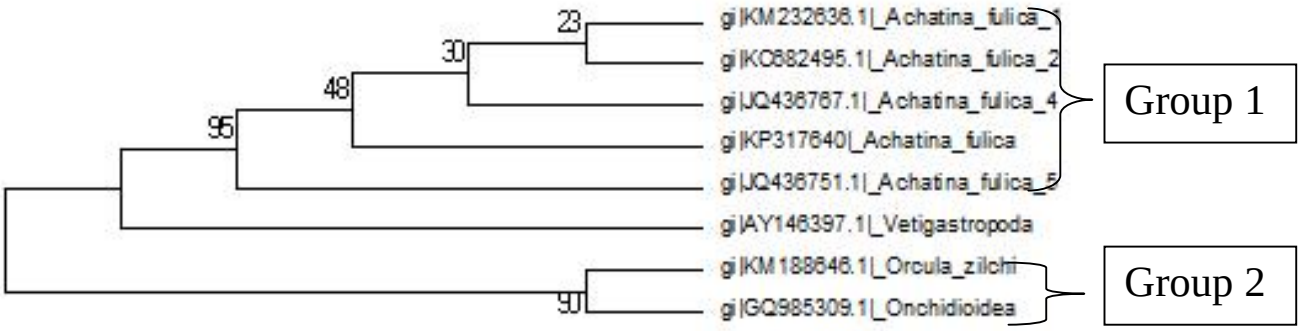

Figure 2: Maximum Likelihood Tree

Phylogenetic reconstruction of the taxa by ML method. Values indicated above the nodes are the bootstrap values.

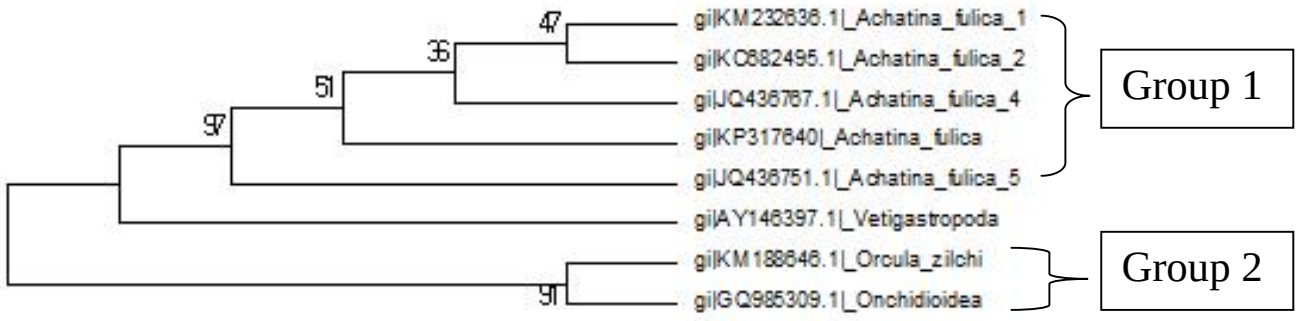

Figure 3: Neighbour Joining Tree

Phylogenetic reconstruction of the taxa by NJ method. Values indicated above the nodes are the bootstrap values.

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Yoga – A holistic treatment modality for Neurological diseases

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Abstract:

Yoga, an integrated and holistic healing modality of Indian subcontinent is gaining prominence worldwide. Since the conventional treatment of neurological diseases by using pharmaceutical drugs is proved to have side effects in many cases, the necessity of a complementary and alternative treatment method has arise. The scientific studies shows that the three components of yoga i.e. Pranayama, asnanas, and dhyana, have proved to be effective in treating the neurological diseases such as Alzheimer's disease, Schizophrenia, Parkinson's disease, Autism etc. The breathing techniques such as Bhastrika, Kapalabhati and Anulom & viloma can reduce the stress levels which is the basic cause for several neurological disorders. the consistent practice of asanas (postures) such as Sarwangasana, Sirshasana, Pincha mayurasana, and Halasana can stimulate production of Oxytocin, a feel good hormone. Moreover, it is very striking that there is nothing in western culture which can teach us how to master our own physiology but yoga can does the same as it unites the body and mind.

Introduction:

Yoga, the ancient Indian science of healing and healthy living has recently become an alternative and complementary means of therapy. The remaining world particularly western countries have started recognizing the potential of yoga as a therapeutic modality since the World Health Organization (WHO) began promoting yoga for developing countries in 1978. Though, yoga was primarily intended for personal enlightenment, now the focus has been shifted towards a holistic treatment modality for various neurological disorders.

Yoga started roughly 5000 years ago in the Indian sub continent as a part of healing science. It is a feather in the cap of Hindu culture and has its origin in Vedas. The word yoga comes from the Sanskrit word "*yukti"* meaning "*union"*, aiming to unify spirit (consciousness) with the super spirit (God). The ancient yogis recognized that to accomplish

this highest state of yoga, a healthy body is essential. Yoga is spiritual practice that utilizes mind (meditation) and body (exercise) to balance our system. It explores the mind's abilities to affect the senses and the body. Yoga consists of three primary components: *asanas* (postures), *pranayama* (breathing exercise) and *dhyana* (meditation)**.**[1] Yoga is thought to treat symptoms of certain neurological and psychiatric disorders through a variety of biological mechanism related to either the aerobic components of yoga (the changing sequence of asanas) or the breathing and meditative components of yoga (pranayama and dhyana). The aerobic components of yoga enhance mental health via a variety of mechanisms, which may include stimulating the nervous system by the release of endorphins, monoamines, and the brain derived neurotrphic factor (BDNF) in the hippocampus.[2]

As many scientific studies revealed, Yoga has profound healing effect on many neurological disorders such as Alzheimer's,[3] Parkinson,[4] schizophrenia,[5] traumatic brain injury,[6] autism,[7] addiction,[8] and manic depression. The allopathic medicines for these diseases proved to have side effects in many cases. Therefore, there is a need of an alternative;side effect free mode of treatment arises. The holistic healing technique yoga can effectively serve the purpose.

Discussion:

Alzheimer's disease (AD) is a degenerative disease; it implies a progressive lost of nervous cells. At a neuropsychological level, it affects different cognitive processes as memory, language, praxis, gnosis and executive functions. Neurological testing, can report signs as frontal reflexes liberation, smell disorders, agraphestesia, walking disorders, shaking, extra pyramidal signs and convulsions. Hatha yoga, one of the main classes of yoga proved to be particularly useful in treating AD. The consistent practice of various Hatha yoga postures[3] could improve the cognitive abilities such as working and procedural memories. The regular practice of yoga could also increase the circulation of blood in the brain which can decrease the risk of cognitive damage. Also, patients presented slow movements, equilibrium problems, poor instructions monitoring and limited skills to develop positions at the beginning of the program. However, the final report indicates improved their positions and movement skills. Besides, patients improved instructions understanding and became more confident and prepared to their work.

Parkinson's disease (PD) is a progressive neurological pathology that causes significant functional limitations, such as impaired gait and balance eventually leading to profound disability. Specifically, impaired balance is a major problem for people with PD. People with

PD have delayed onset time and decreased amplitude of anticipatory postural adjustments, which can be reversed by deep brain simulation. A shuffling gait pattern is the most prominent feature of gait seen in person with PD with reduced stride length, decreased walking speed and longer double stance time. People with PD also suffer from weak muscle strength, which makes difficult even to rise from a chair. The regular practice of yoga by PD victims for a particular period resulted in a significant improvement in overall motor function[4] as measured using the motor examination section of the UPDRS and the BBS. The motor section of the UPDRS targets multiple areas of function that are typically impaired by PD including balance, coordination, posture, muscle tone and presence of abnormal movements and gait.

Schizophrenia is one of the psychotic mental disorders and is affecting individual's thoughts, behaviors, and social functioning. Symptoms of schizophrenia may include delusions, hallucinations, catatonia, negative symptoms, and disorganized speech or behavior. The other symptoms are inhibition of facial expressions, poor grooming and hygiene, self neglect and lack of motivation. The consistent practice of pranayama and asana by the patients suffering from schizophrenia was reported to improve the oxytocin hormone[5] levels which in turn diminish depression. Improvement in cognition, increased quality of life was also observed in those patients.

Autism is a common developmental life-long brain disorder that is normally diagnosed in early childhood. People with autism have difficulties in communicating, forming relationships with others and find it hard to make sense of the world around them. The autism patients may also have unusual patterns of language development, narrow interests and engage in repetitive and sometime challenging behaviors. The consistent practice of *super brain yoga*[7] autism affected children could show improvements in sensory processing, visual perception, visual motor, communication and social behaviors.

Conclusions:

The present day yoga is a part of the ancient Hindu mythological texts "Vedas" and it was practiced by Indians primarily to attain enlightenment. But since the allopathic medicines used for neurological diseases proved to have side effects such as migraine, weight gain, and sleep disturbances, yoga has become a boon to the people affected by neurological diseases. Moreover, hardly any side effects with yoga treatment are known.

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SEASONAL STRESS ON THE TOTAL PROTEIN CONTENT OF COMMON CARP FISHES

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Abstract

Total protein and amino acid content has been studied in common carp in its non osmotic and osmotic tissues of common carp during summer (30c), rainy season (20c) and winter season (15c).

During summer season the total protein content in common carp decreases and the amino acid content increases. the carp fish has to face the stress so its has tom perform strenuous activity to counter the summer stress. During rainy season the amino acid content decreases and the total protein content increases the tissues of common carp. It is the favorable season so more amount of protein synthesis from amino acids there is no stress on the fish in rainy season.

During winter season at cold stress the protein content somewhat decreases the amino acid content comparatively increases because the fish had to tide over cold stress it converts proteins to amino acids which can be utilized to counteract cold stress. Has rainy season is most suitable for the growth of fish has compare to summer and winter.

Keywords: cold stress, seasons, Total proteins, common carp.

Introduction

The stepping up in soluble structural and total protein level was most probably due to decrease in the levels of free amino acids in the tissues in (table 6-A – 6C figVI-A – VI-G). The raise in protein levels in the tissues could be attributed to decrease in proteolysis and the good increase of protein synthesis.

Among the food stuffs required by the animals proteins are inevitable and without these metabolites, the animal cannot operate its life activities. Further it has also been reported that proteins could be taken an index of stress conditions (Vijay Joseph 1989). Proteins being the most important organic constituents of organisms, their role in the compensatory mechanisms of an animal can be expected during rainy stress conditions (Singaraju etal., 1991). Shifts in protein may ultimately led to alteration in the entire protein metabolism of animals (De Smet H, Blust R 2007). Proteins the basic units of life usually account for 68—85% of the dry matter of any growing animal (Joseph etal., 1992). The survival ability of an animal to seasonal stress majorly depends on its protein synthetic potentials. Hence change in protein metabolism can be considered as one of the important diagnostic tools in evaluating the seasonal stress. Teleost fishes are well suited as experimental animals for the study of protein metabolism because these animals largely derive energy even by the catabolism of proteins (Warde, 1981).

Discussion

During summer season – (30°C - control) severe decrement in total protein content was noticed in common carp fishes. During summer season water become more acidic, with high temperature and low oxygen content. Due to acidic stress proteins break down occurs in common carp fishes. Generally, breakdown of proteins dominates over synthesis under enhanced proteolytic activity (Harper etal., 1979 Hayat, S., M. Javed and S. Razzaq, 2007). Due to increase in protease activity protein content decreases. the increase in protease activity could be due to the entry of protons in to the tissues of fishes from blood, since the blood of such fishes shows an acidic shift in lower pH (Bhaskar etal., 1982). The maintenance of proteins in a highly organized state requires an active and continuous supply of energy. The impairment of it also result in the dissolution of structural and dynamic proteins leading to tissue disorganization (Daye and Garside, 1976).

Protease hydrolyse proteins and peptides resulting in the production of amino acids as end products. A drastic decrease in the soluble, structural and total protein content occurs, which elevates the amino acid levels, their mobilization into TCA cycle for energy releasing purpose to meet the high activity of fish during summer. The decrease also helps in lowering

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the pH of the body fluid of common carp fishes. The increased production of dicarboxylic acids of TCA cycle in tissues of summer seasonal fish may increase the proteolytic activity in proportion to increased activity. Depletion of Liver glycogen during thermal stress might have triggered utilization of proteins to meet the energy demands during thermal stress. Proteins are degraded to amino acids which are fed in to TCA cycle through transferase system. Roger and Fellow; 1980 suggests that low protein levels may be due to the inhibition of amino acids incorporation in to them. To meet the high energy demands during thermal stress the decrease in proteins can be envisaged as a consequence of liver glycogen Increased protease activity in the tissues of summer exposed fish might be due to increased susceptible protein content. The increased amino acids may be usefull for the synthesis of new proteins and enzymes to face the stress imparted by temperature (James et al., 1979). The amino acid pool size also found to be largest in worm adapted gold fish gill and muscle (Das 1965). The stress influences and leads to an increased protein catalystic activity as also reported by Kunneman and Precht., 1975.

As for as tissue variation is considered there is a stepping down of total proteins, structural proteins, soluble proteins is higher in non osmotic tissues when compared to that of osmotic tissues. The breakdown of protein is higher in non osmotic tissues than that of osmotic tissues due to steep elevation of protease activity and free amino acid level of the fish. Similar explanation was also given by Bhaskar govindappa (1985) for the decrease of total proteins in the muscle of fish Tilapia mosambica exposed to acidic water. Kllasson (1991) reported that the depletion of protein suggests increased proteolysis and possible utilization of the products of their degradation for metabolic purposes.

The function of protein degradation is to remove abnormal proteins needed for intracellular proteins degradation and the digestion of the proteins taken in to the cell. (Segal etal., 1976). Protease specific for pyridoxal enzymes show cleavage of the apoenzyme (Kominami and katnuma, 1975). Increase in proteolytic activity. May also be due to the destruction of the organ, systems and there by disturbing the biochemical functioning of cellular activities (Karel and Sexena, 1975). As for as species variation is considered the total protein content follows the following descending order.

Catla catla > Cyprinus carpio > Labeo rohita.

During rainy season - (22°C-cold stress) at low salinity moderate temperature and high oxygen content the total proteins significantly increased in all the three species off common carp. Protein synthesis dominates over proteolytic activity causing increase in soluble Protein structural Protein and total protein contents. This indicates the domination of

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protein synthesis over break down during cold stress. The increase in the structural protein content may he help full to the animal to fortify its organs for development. The increase in soluble protein fraction may be helpful for the synthesis of enzymes necessary for development (Coombs and George, 1978). As for as tissue variation is considered the total proteins structural proteins and soluble proteins are more in osmotic tissues and less in non-osmotic tissues. During cold stress the osmotic tissues are more active and respond greater than that of non osmotic tissues. As for as species variation is considered total proteins and structural proteins occur in the following descending order.

Cyprinus carpio > Labeo rohita > Catla catla.

Whereas the soluble protein occurs in the following descending order.

Labeo rohita > catla catla > Cyprinus carpio.

In case of total proteins and structural protein as Cyprinus carpio lives in the bottom and undergo highest stress due to minimal exposer to environmental factors, where as Catla catla is highly exposed to environmental factors. The enzymatic activity in the case of carp fishes occurs in the above descending order for soluble proteins.

During winter season - (15°C-cold adaptation) the total proteins, structural proteins and soluble proteins reaches nearer to the control values. It is due to the increase of proteolysis causeing degredation of proteins and increase of amino acid pool in common carp fishes. Due to degredation of proteins there is a decrease in protein synthesis. Total proteins structural proteins and soluble protein decreases without causing any burden on metabolism of the animal. The increase in amino acid level may be partly helpful for the production of energy (Solinkka etal., 1983). And also for the synthesis of required proteins. Further the free amino acids may act as osmotic and ionic effector (Cola, 1977; Jwiss, 1980). Bringing electrolytic equilibrium between external medium and ions of the blood there by regulating the ionic and osmotic changes (Schmidt-Nelson 1971). Hence there will be a constant mobilization of these biomolecules which contribute to various metabolic pathways as well as in the regulation of protein synthesis in common carp in non osmotic tissues and osmotic tissues, percent recovery is greater than that of percent change. Percent recovery is grater in non osmotic tissues compared to osmotic tissues. In case of species variation is considered percent recovery is greater than that of percent change. Percent change occurs in the following descending order.

Labeo rohita > Cyprinus carpio > Catla caltla.

Percent recovery also occurs in the following descending order

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Cyprinus carpio > Catla catla > Labeo rohita.

In case of structural protein percent change is greater than percent recovery because in structural protein recovery will take long time for structural synthesis. In non osmotic tissues percent change and percent recovery is greater than that of osmotic tissues. Non osmotic tissues are affected more like muscle and liver than osmotic tissues. In case of soluble proteins it is grater in osmotic tissues than that of non osmotic tissues Percent change is greater than percent recovery. Percent change occurs in the following descending order.

Labeo rohita > Cyprinus carpio > Catla caltla.

In common carp fishes at cold stress in non osmotic tissues and osmotic tissues the total protein levels are statistically significant at ($P < 0.05$). Adaptation is insignificant in Labeo rohita but stress and adaptations both are significant in catla catla. In Cyprinus carpio stress is only significant and adaptation is not significant.

In case of structural proteins in common carp fishes stress and adaptation both are significant. In case of Labeo rohita stress and adaptation both are significant but in catla catla stress is significant but adaptation is not significant. Where as in Cyprinus carpio stress and adaptation both is not significant.

For soluble proteins in case of tissue variations and species variations stress and adaptation both are significant in common carp fishes, as for as soluble proteins are more affected than other.

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Immunological implications in mammalian semi-allogeneic pregnancy

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Abstract

The mammalian conceptus is a semi-allograft. The maternal tolerance towards semi-allograft is primed by a battery of cytokines and resident decidual cells. Invasion of embryonic trophoblast, decidual vascular remodeling and unresponsive incipient 'Triple-negative' T-cells of foetus promote normal pregnancy. On the other hand, the maternal cytokine profile shift towards Th1 response is shown to enhance the risk during pregnancy. The periodical examination of maternal serum levels of Th1 and Th2 response mediated cytokines constitutes prognostic biomarkers to initiate therapeutic regimens.

Keywords: Semi-allograft, Pregnancy, Immune cells, Cytokines, Biomarkers
Running Title: Immunological implications in mammalian semi-allogeneic pregnancy

Introduction

The zygote, implants in parous woman that further grows as foetus, is found to be unique being tolerated by the maternal immune system. Whether the foetus is immune-privileged or the parous woman is immunosuppressive or both cleverly adopt a transient immune compromise or the feto-maternal interface protects the foetus, need to be understood in a wider perspective of the evolution of viviparity. Importantly, targeting 'non-self' is an attribute of adaptive immune system[1]. In contrast, viviparous organisms are tolerating 'semi-self' conceptus. The same must have been an evolutionary adaptation for the race to continue[2]. Though there is a wide array of immune parameters

namely hormones[3], HLA[4], cytokines[5], receptors[6], ligands[7], defense cells[8&9], antigen presentation[10&11] and antibodies participation[12] in the pregnant women, yet she maintains a perfect balance in accepting foetus despite being characterized by having desperate paternal antigens. The interplay among immune activation, biased immunity, immune suppression and immune regulation needs to be looked to unfold the disorders of pregnancy.

In the present mini review, it is envisaged to elaborate on the pregnancy-induced immune alterations and the potential biomarkers to avert risks during pregnancy.

Immune components of primiparous women: Rejection of an allograft is one of the inbuilt mechanisms of cell mediated immunity[1] primarily the allograft is inevitably characterized by individual specific peptides namely MHC Class I and II. Between these two antigens, MHC class I is expressed on all nucleated somatic cells which must have been the primary cause to abort fetuses with somatic cell nuclear transfer shown by Davies[2]. The foetus being a semi-allograft is sharing only one half of the MHC expression in common with the maternal tissues.

Immune cells: The foetal development through the progressive pregnancy during penultimate second and ultimate third trimesters coincides with an increase in acquiring immune privilege by the foetus and in the process takes the lead by recruiting a number of decidual immune cells through the interaction of stromal cells and a battery of cytokines. Among the immune cells, uterine natural killer cells (uNK)[13], T-cells[14 & 15], dendritic cells and macrophages[16 & 17] are the significant contributors due to their mucosal infiltration during luteal phase and also in the first trimester. Both leukocytes and lymphocytes are shown to aggregate in the uterus[18]. Soon after implantation, predominantly NK cells and macrophages appear at the site of invasion[19]. The prevailing hormonal environment in uterine endometrium influences immune cells to bind to the resident decidual stromal cells utilizing the intercellular adhesive molecules[20].

NK cells are endowed with differential potential to perform. Peripheral NK cells are of two subsets namely dim and bright due to the difference in the density in the expression of CD56 surface receptors. Uniquely, uNK cells are characterized by having surface receptors CD 56^{bright}, $CD16^{-}$ and $cyCD3^{+}$ and they are found to be immunoregulatory involving the cytokine production[21]. In contrast, peripheral NK cells are dim ($CD56^{+dim}$ $CD16^{+}$, $cyCD3^{-}$) and they are highly cytolytic. Normally, NK cells exhibit discrete specificity through their conventional activating and inhibitory receptors and lyse the worn out /abnormal/foreign cells that either least or fail to express 'self' HLA I. Further, this HLA I group is classified as (a) classical which consists of HLA-A, HLA-B, and HLA-C and among them the first two are highly polymorphic and (b) non-classical antigens comprising of HLA-E, HLA-F and HLA-G with least polymorphism. It is shown that both these classical and non-classical HLA molecules interact in decidua and participate in spiral artery remodeling[22] to provide nutrition to the growing foetus. The polymorphic classical HLA molecules of the semi-allograft especially HLA-A and HLA-B are least expressed, whereas the less polymorphic HLA-C and non-classical HLA-G are more pronounced in the trophoblast which must have been a possible motivation from the foetus to prompt maternal immune tolerance and also effectively inhibit maternal immune cells. Furthermore, it has been reported that there is no evidence that NK cells are aggressive during pregnancy[21] instead they facilitate in angiogenesis in the interface of uterus and trophoblast. Reproductive hormone, progesterone also alters cytokine balance and inhibits foetal tissue lysis mediated by NK and CD8 T cells. In addition, during pregnancy maternal immune system gears more towards humoral immunity and keeps the cell mediated immunity under check to avert foetal tissue damage. Therefore, uNK cells play a profound role in remodeling tissue at the site of implantation and building the placenta.

Macrophages do play active part in both innate and adaptive immunity due to their potential role in processing pathogens and tissue remodeling. Immunomodulatory roles of deciduous macrophages and their involvement in normal and pathological pregnancies have been reviewed by Takeshi Nagamatsu[22] and elucidated that decidual macrophages behave as an

immunosuppressive phenotype by producing IL-10 and indoleamine 2, 3-dioxygenase activity to promote maternal immune tolerance and decidual vascular remodeling during pregnancy. During implantation, decidual tissue remodeling and vascularisation are imperative and the resultant apoptotic cells are to be scavenged by decidual macrophages. Otherwise, a few of embryonic apoptotic cells possibly dwell in the maternal circulation and elicit immune response against the semi-allogenic foetus. Further, it is also shown that the malfunctions due to the aberrant decidual macrophages resulted into the abnormal pregnancies such as pre-eclampsia and preterm delivery[17].

In addition to NK cells and macrophages, T-regulatory cells also contribute toward building maternal tolerance. It is reported that the maternal immune environment during pregnancy induces the generation of well defined T regulatory cells and their subsets namely $CD4^+$, $CD25^+$ and $FoxP3^+$ to prevent rejection of the foetus[24].

Cytokines: Cell to cell communication is invariably facilitated by small molecules secreted into the interstitial fluids. They are variously named as cytokines/lymphokines/interleukins due to their specific involvement. Interleukins are having multiple and redundant functions in promoting the elicitation of immune response[25]. Progesterone is an effective inducer of the production of Th2-type cytokines viz., LIF (leukemia inhibitory factor) and M-CSF (macrophage colony stimulating factor). *In vitro* studies have shown that the interaction between the trophoblast and uNK cells induced the production of several cytokines and growth factors related to placental development including the following: 1) tumour necrosis factor (TNF-α); 2) interferon gamma (IFN-γ); 3) granulocyte macrophage colony-stimulating factor (GM-CSF); and 4) macrophage inhibitory factor (MIF)[25-27]. In furtherance of the maternal hormonal influence, the decidua, trophoblast and the embryo take the lead in the regulation of cytokine interplay. Ponzio et al.,[26] administered murine IL-2 to pregnant mice during mid-gestation, analyzed their offspring (IL-2 pups) and compared the offspring of pregnant mice injected with placebo, phosphate buffered saline (PBS pups). They also reported that considerable levels of IL-2 were noticed in amniotic fluid and

tissues of embryos, confirming that the injected IL-2 crossed the placenta and entered fetuses. Further, IL-2 pups witnessed the accelerated T cell development, with an orientation toward TH1 cell differentiation. The growth factors produced by endometrial NK cells were found to inhibit TNF-β and IFN-γ and the participation of both Th2 and Th1 cells were reported in pregnancy[27]. *In vitro* behavior of PBMCs obtained from normal and recurrent spontaneous abortion (RSA) women co-cultured with PHA (phytohemagglutinin) was evaluated in relation to their secretions[28]. Interestingly, PBMCs of normal first trimester pregnant women were reported to secrete high concentrations of Th2 mediated interleukins in culture medium namely IL-4, IL-6 and IL-10, whereas IL-2, IFN-γ, TNF-α and TNF-β were found higher in the cultures of PBMCs obtained from the RSA group suggesting that there exists Th2 bias in normal pregnancy as against Th1 bias. While the Th2-type cytokines (IL-4, IL-5, IL-10) were also reported to inhibit the Th1 responses so as to promote allograft tolerance and therefore improve fetal survival[28]. In addition, IL-5 was found to stimulate uNK cells whose role in the augmentation of trophoblastic and cytotrophoblastic invasion were well acknowledged[5&9]. Piccinni[29] reported that T cells' LIF, M-CSF, IL-4 and IL-10 production at the fetomaternal interface contribute to the maintenance of pregnancy. In an experimental design to evaluate the lapses due to Th1 interleukins during pregnancy, it is shown in mouse models that an increase in maternal levels of IL-2 during pregnancy induced long-lasting vulnerability to neurobehavioural abnormalities associated with autism among offspring[26]. The most interesting contribution from the trophoblast is IL-15 which mediates cell to cell interaction between uNK cells and decidual cells to develop decidual integrity and spiral artery at the site of implantation. Therefore, Th2 mediated immune surveillance predominates not only during pregnancy but also influence the offspring for a better survival.

Trophoblast as an incipient mediator: The zygote through multiple cleavages reaches a stage namely blatocyst that freely floats in uterus and, develops communion and adhesive interaction with the uterine endometrium. Importantly, the outer layer (outer mass of cells) of blastocyst namely trophoblast initiates for the invasion as the first step for implantation,

primarily to secure nutrition to the growing inner mass of cells. To ensure the same, the blastocyst in the uterus relays on the unique specialized cell type known as functional trophoblast derived from the trophoecotoderm of the balstocyst. The functional trophoblast differentiates into villous and extravillous cytotrophoblast, each having a distinct function. The former is multinucleated syncytiotrophoblast which promotes foetal and maternal exchanges and the latter invades the endometrium and spiral arteries. One of the causes of implantation failures is found to be due to the disturbances in the functional components of trophoblast which leads to an early pregnancy loss that is estimated to be nearly 40%. Therefore, the implantation of blastocyst in the uterus depends upon the competence and receptivity of blastocyst and uterine endometrium respectively. The process of implantation includes three phases: (1) apposition, (2) attachment and (3) penetration in a specific time frame. The blastocyst in its free floating sojourn settles on the uterine endometrium and further invasion authenticates the competence of the embryo. Once the invasion is ensured, the endometrial stromal cells undergo decidualization, a response of receptivity from maternal side. Each one of these process involves the role of several cytokines such as LIF, IL-6 and IL-11. The deficiency of these cytokines leads to decrease in viable implantation sites[18]. LIF, a pleiotropic cytokine, induce macrophages proliferation and onsite remodeling of uterine endometrium leading to decidualization[30&31] whereas LIF mutant mice is defective and fails to initiate implantation. In addition, integrins, matrix metalloprotienases (MMP) and tissue inhibitors of MMP mediate matrix disintegration to the desired extent required for blastocyst implantation and decidualization. All these factors involved in the implantation stage of blastocyst are progesterone dependent. Indian Hedgehog and Wnt signaling pathways are crucial for blastocyst–uterus communication and also for the onset of implantation[32]. Thus, the implantation is a highly coordinated process wherein blastocyst initiates both receptive signals and uterine tissue remodeling.

Foetal Immune system: Foetus is under the protection of maternal immune system. Also, the responsiveness of foetus towards infection is reasonably low. It does not mean that foetus is free from immune components. The

mammalian foetus gradually builds its immune system in a captive sterile uterine environment. To begin with foetus develops tolerance towards the self 'antigens' and maternal antigens. Initially, it modulates to coexist with the maternal immune system and this must have been made plausible due to the incipient T-cells that are 'Triple-negative' ($CD3^-$, $CD4^-$ and $CD8^-$)[14&15], possibly promotes unresponsiveness towards the maternal antigens. Parallel to this, lymphoid cells start forming from the yolk sac, later shift to foetal liver and ultimately to the bone marrow. Further, lymphoid cells also colonize in the primordial thymus derived from the third visceral arches around six weeks of gestation. Parallel to this, the secondary lymphoid organs provide a temporal space for the naïve immune cells to mature. In addition, mucosal associated lymphoid tissue in gastrointestinal tract and nasal region is at its infancy and soon after parturition mucosal tissues get activated due to the influx of lymphocytes[12]. The most important aspect of suppressive immune response in the foetus would be primarily due to T regulatory lymphocytes[12].

Trophoblast of foetus plays a pivotal role in resisting the maternal aggressiveness towards paternal antigens. Among the cell surface antigens derived from the HLA complex, trophoblast predominantly expresses HLA-G, a non-classical MHC class I, and its isoforms in contrast to maternal tissues. HLA-G is gaining importance because of its role as a specific ligand to multiple receptors of maternal leukocytes and lymphocytes. Evolutionarily, the alleles of HLA-G must have been conserved to support the semi-allograft foetus among viviparous mammals ie, HLA-G initiates maternal tolerance towards the foetus. Interestingly, unlike HLA- A and B, HLA-G is least polymorphic and restricted mostly to embryonic tissue. In the recent past several reviews [7, 9 & 22] and scientific reports [25 & 33] elucidated the role of HLA-G in maternal-foetal interface in pregnant viviparous mammals. HLA-G receptors are distributed on maternal immune cells namely NK cells, CD4 and CD8 T cells, B-cell, macrophages and dendritic cells. These receptors are KIR 2DL4, ILT2, ILT4, CD8 and CD160. The interaction of HLA-G ligand with these receptors generates angiogenic factors to build spiral arteries in placenta and immune tolerance against paternal antigens. Therefore, the non-classical HLA-G ligand in mammalian foetus has a major role in promoting to

acquire immune privilege and to induce immune tolerance under the canopy of a well sophisticated maternal immune surveillance.

Biomarkers to avert risks in pregnancies: Decidual vascular remodeling is one of the main requirements during implantation of the early embryo. During this time, as indicated in the previous sections, several immunological challenges have to be encountered by both the maternal immune cells and also embryonic trophoblast. The functions of immune cells are always under the influence of a variety of cytokines. The imbalance in cytokines in a pregnant woman leads to risks such as preeclampsia, pre-term birth etc. The cytokines such as IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8 and IL-10, TNF-α, INF-γ do interplay and contribute in the safe delivery of foetus (Table 1). The relationship between psychological factors and biomarkers among 49 African American pregnant woman was studied by Carmen Giurgescu, et al.,[34] and reported that the levels of cytokines were found to be altered among hypertensive disorders of pregnancy[35]. The shift in the regulation of immune system towards Th1 cytokine profile was also noticed in preeclampsia, a hypertensive risk during pregnancy due to heterogeneous origin[36]. The evaluation of maternal serum pro-inflammatory cytokine levels and Th1/Th2 response yields prognostic approach to avert the risk during pregnancy.

Table 1: A handful of Interleukins to evaluate the risk during pregnancy.

Interleukins	**Destined roles**
IL -1 β	Promote the synthesis of monocyte chemotactic factor in decidual cells
IL -2	Promote decidual NK and T cells proliferation for building spiral arteries
IL -4	Promote Th2 response that is predominant in 2nd and 3rd trimesters
IL -5	Promote Th2 response
IL -6	Potent pro-angiogenic factor
IL -8	Chemoattractant and contribute for spiral arteries formation
IL -10	Protect allo-foetus from rejection during gestation. Also, activates HLA-G expression in embryonic trophoblast cells.
TNF- α	Promote the synthesis of monocyte chemotactic factor in decidual cells to induce decidual vascularization.
INF- γ	Trophoblast is insensitive to INF- γ and hence takes a lead in uninterrupted decidual invasion

Conclusion: The uterus is a pouch destined for the semi-self foetus to get conditioned and to withstand the maternal immunological challenges. Soon after the process of implantation initiated and mediated by the embryonic trophoblast, the embryo invades the decidua of pregnant uterus successfully and induces maternal immunological tolerance through several mechanisms i.e., expression of HLA-G and 'Triple-negative' T-cells and also promotes on the maternal side the shift towards Th2 cytokine response. However, the occasional maternal intolerance would be primarily because of the inherent nature of shift towards Th1 cytokine profile possibly culminated to abnormal miscarriages and the same could be maneuvered through the frequent evaluation of maternal serum pro inflammatory cytokines.

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Effects, Characteristics and Causes of Cancer disease in the present scenario: Its prevention

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ABSTRACT

Mans life is free gift given by god. Thus we are living in the lap of the nature. But fortunately or unfortunately man becomes week both in physically and mentally. Now a days everyone should afraid of the incurable diseases. It is very needed in the present scenario, to aware the public free from the dangerous and terrible diseases like cancer. So far much research has been done, it is required to aware and enlighten the pupil more and more. A variety of psychosocial and cultural barriers and believes impact Indians as well as Americans and how they might perceive the issues of cancer in their lives. Health care professionals carrying for a native people with cancer and their family members need to be apprised of the issues, which may affect the rapport between the provider and patient.

The dangerous one of cancer is a malignant tumor, which spreads into surrounding tissues and also may be disseminated through the lymphatic and circulatory system. When the viral DNA begins its characteristics rapid reproduction, it also stimulates usually rapid expression of the oncogene next to it. Some forms of cancer occur because of sequence of genetic abnormalities involving both oncogene activation and tumor suppressor inactivation as well as possible environmental influences. P53 the inactivation of one such gene a tumor suppressor can causes the colane, liver, as well as Brest and brain. Yet until recently few culturally acceptable materials were available and there continuous to be a need to develop resources materials for those who interested in reducing cancer.

Key Words: Malignant tumor, abnormal nuclei, adopting behaviors, metastasis.

Introduction

Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected.

Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems, and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign.

More dangerous, or malignant, tumors form when two things occur:

1. a cancerous cell manages to move throughout the body using the blood or lymph systems, destroying healthy tissue in a process called invasion
2. that cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis.

When a tumor successfully spreads to other parts of the body and grows, invading and destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis, and the result is a serious condition that is very difficult to treat.

Characteristics of cancer cells:-

1. Cancer cells form tumors:

A cancer cell in the body divides to form an abnormal mass of cells called a tumor, which invades and destroys neighboring tissue. This new growth , called neoplasia, is made up of cells that are disorganized , a condition termed anaplasia. A benign tumer is disorganized , usually encapsulated , mass that does not invade adjacent tissue. Normal cells achor themselves to substratum end/or adhere to their neighbors. The exbit contact inhibution-when the come in cantact with a neighbour, they stop dividing. In culture , normal cells form a single layer. Thar covers the bottom of periadish. Cancer cells have lost all restraint; they pile on the top of one another grow in multiple layers. They have a reduced need for growth factors, such as epidermal growth factor. Epidermal growth factor can stimulate the growth of many types of cells., growth factors are hormones needed by normal cells to grow.

2. Cancer cells have abnormal nuclei: : The nuclei of cancer cells are enlarged and there may be an abnormal number of chromosomes. The chromosomes have mutated; some parts

may be duplicated and some may be deleted. In addition, gene amplification is seen much more frequently than in normal cells.

3. Cancer cells lack differentiation : cancer cells are non-specialized and do not contribute to the functioning of a body part. A cancer cell does not look like a differentiated epithelial, muscle, nervous or connective tissue cell and instead has a shape and form that is distinctly abnormal. Normal cells can enter the cell cycle for about 50-60 times, and then they die. Cancer cells can enter the cell cycle repeatedly, and in this way they are immortal. In cell tissue culture , they die only because they run out of nutrients or are killed by their own toxic waste products.

4. Cancer cells undergo angiogenesis and metastasis- Angiogenesis: - the formation of new blood vessels, is required to bring nutrients and oxygen to a cancerous tumor whose growth is not contained within a capsule. Cancer cells release a growth factor that causes neighbouring blood vessels to branch into the cancerous tissue.

The first report of the association between alcohol and an increased risk of esophageal cancer was published in 1910 (1). Since then, a number of studies have revealed that chronic alcohol consumption is a risk factor for cancers of the upper aerodigestive tract, including cancers of the oral cavity, pharynx, hypopharynx, larynx, and esophagus (2), as well as for cancers of the liver, pancreas, mouth, and breast (3). Williams and Horn (4), for example, reported an increased risk of breast cancer due to alcohol. In addition, a collaborative group who studied hormonal factors in breast cancer published their findings from a reanalysis of more than 80% of individual epidemiological studies that had been conducted worldwide on the association between alcohol and breast cancer risk in women. Their analysis showed a 7.1% increase in relative risk of breast cancer for each additional 10 g/day intake of alcohol (5). In another study, Longnecker et al., (6) showed that 4% of all newly diagnosed cases of breast cancer in the USA are due to alcohol use. In addition to it being a risk factor for breast cancer, heavy intake of alcohol (more than 50–70 g/day) is a well-established risk factor for liver (7) and colorectal cancers. Thus anti-inflammatory agents may be effective for the treatment of alcohol-induced toxicity.In the upper aerodigestive tract, 25–68% of cancers are attributable to alcohol, and up to 80% of these tumors can be prevented by abstaining from alcohol and smoking (8). Globally, the attributable fraction of cancer deaths due to alcohol drinking is reported to be 3.5% (9). The number of deaths from cancers known to be related to alcohol consumption in the USA could be as low as 6% (as in Utah) or as high as 28% (as in Puerto Rico). These numbers vary from country to country, and in France have approached 20% in males (10).

Hence, we propose a unifying hypothesis that all lifestyle factors that cause cancer (carcinogenic agents) and all agents that prevent cancer. The fact that chronic inflammation is closely linked to the tumorigenic pathway is evident from numerous lines of evidence.

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MODULATION OF LIPID PEROXIDATION AND Na^+/K^+, Mg^{2+}, AND Ca^{2+} ATPase ACTIVITY IN RAT BRAIN DURING STZ- INDUCED DIABETES AND PROTECTIVE ROLE OF PIMPINELLA TIRUPATIENSIS

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Abstract

The objective of the present study was to investigate the role of Pimpinella tirupatiensis aqueous extract on brain lipid peroxidation and membrane bound ATP ases in Streptozotocin (STZ) induced diabetic rats. Adult male wistar strain rats were divided into five groups such as NC (normal control), Pt. (Pimpinella treatment), DC (Diabetic control), D+Pt (Diabetic+ Pimpinella treatment), D+Gli (Diabetic+Glibenclamide treatment). Treatment was given as per the experimental protocol. Xonthine oxidase(XOD) activity, MDA levels and the membrane bound ATP ases like Na^+/K^+, Mg^{2+} and Ca^{2+} ATP ases, were assayed in the brain tissue. In diabetic rats, we observed that Na^+/K^+, Mg^{2+} and Ca^{2+} ATPase activities are depleted and XOD activity, MDA levels are up regulated. However with the Pimpinella tirupatiensis treatment XOD and MDA levels and Na^+/K^+, Mg^{2+} and Ca^{2+} ATPases activities were came back to normalcy. Our results suggest the ability of Pimpinella tirupatiensis extract to modulate XOD ,Na^+/K^+, Mg^{2+} and Ca^{2+} - ATP ase activities, and lipid peroxidation in STZ - induced diabetes and thus offers effective management in the treatment of diabetes.

Keywords: Diabetes, Pimpinella tirupatiensis, Streptozotocin (STZ) , Na^+/K^+. Mg^{2+} and Ca^{2+} -ATPase.

Introduction

Diabetes is a metabolic disorder that produces various dysfunctions in the body, including vascular dysfunction, retinopathy, nephropathy, peripheral neuropathy, and central nervous system (CNS) dysfunction (Mooradian, 1997; Bhardwaj et al., 1999). Diabetes is also considered to be a risk factor for Alzheimer's disease and other neurodegenerative diseases (Ott et al., 1999; Arvanitakis et al., 2004; Ristow, 2004). Hyperglycemia associated with diabetes increases the glucose autoxidation and protein glycation and the subsequent oxidative degradation of glycated proteins leads to enhanced production of reactive oxygen species (ROS) (Limaye and Sivakami, 2003). The neurological consequences of diabetes mellitus in the central nervous system (CNS) are now receiving greater attention. A variety of structural changes have been described in the CNS of diabetic patients and animals (Kirsch and Koehler, 1991; Tilton et al., 1995) in which glucose utilization could decrease in brain tissue leading to acute potential mechanism for increased vulnerability to acute pathological events during diabetes (McCall,1992). About 150 million people suffering from diabetes worldwide and this number may probably double by the year 2030 (King et al., 1998; Pradeepa et al., 2002). India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes will affect approximately 57 million people by the year 2025 in India.

Na^+/K^+-ATPase a membrane linked enzyme that catalyzes the hydrolysis of ATP and couples it to the transport of Na and K across cell membrane there by generating the trans membranous Na+/K+- gradient (Hernandez et. al., 1992). Na^+/K^+-ATPase is responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the CNS necessary to maintain neuronal excitability. Na+/K+-ATPase is present at high concentrations in brain, consuming about 40-50% of the ATP generated in this organ (Erecinska and Silver, 1994). Na+/K+-ATPase is implicated in metabolic energy production as well as in the uptake, storage, and metabolism of catecholamines, serotonin, and glutamate (Carageorgiou et al. 2007). Ca^{2+}-ATPase activity is associated with neuronal excitability, cellular depolarization and fine tuning of Ca^{2+}-channel activity (Lees, 1991). Mg^{2+}-ATPase activity associated with mitochondrial

membrane bound enzyme which is involved in turnover of ATP synthesis in conjugation with oxidative phosphorylation.

Pimpinella tirupatiensis (Balakrishnan and Subramanyam, 1960) is an herbaceous medicinal plant, distributed on Tirumala hills (1000m above MSL) of chittoor district, Andhra Pradesh (Mahadeva Chetty and Rao, 1990). It is endemic species of Umbellifereae and seasonal occurrence with underground tubers root system (Rangacharyulu et al., 1995). It is used for as a aantifertility anti ulcer and aphrodisiac agent (Vedavathy and Mrudala, 1997). Pimpinella tirupatiensis is used to treat cough, stomach, asthma, ulcer (Madhava Chetty et al., 2008). Though there is no scientific evidence to support the antidiabetic property of Pimpinella tirupatiensis tribal's of Tirumala region continue to use it in the management of diabetes.

Keeping in view of the importance of ATPases in the neuronal metabolism, the present study was carried out with Pimpinella tirupatienesis to lipid peroxidation and ATPases activities in rat brain.

Material methods

Procurement of Chemicals

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Plant material collection:

Tuberous roots of Pimpinella tirupatiensis (Pt) were collected from Tirumala hills, (chittoor district, Andhra Pradesh, India) during the raining season and identified by the taxonomist of the herbarium, department of botany, SV University, Tirupati. A voucher specimen (AECBT-05/2007-2008) was deposited in the department of botany, SV University, Tirupati.

Preparation of plant extract

Pimpinella tirupatiensis tubers were dried at room temperature and tubers were powdered in an electrical grinder then stored at 5^0C until further use. Tubers powder 500g was extracted with distilled water 1L for a period of over 24 hours. After filtration, the residue obtained was

given resuspended in equal volume of distilled water for 48 hours and filtered again. The above two filtrates were mixed and the solvent was evaporated in a Rota Vapor (Model No- HS-2005V) at 50-65^{0}C under reduced pressure and then lyophilized to get a powder and the same was used for the study.

Animals and treatment

Male albino wistar strain rats, aged 3-4 months (200±250 g) were used for the present study. The rats were maintained on standard pellet diet ((M/s Hindustan Lever Ltd., Mumbai) and provided access to water ad libitum. They were housed in clean, dry polypropylene cages and maintained in a well ventilated animal house with 12 h light-12 h dark cycle. All the experiments were carried out between 8 am to 10 am in order to avoid circadian rhythm induced changes. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee(RegdNo.438/01/a/CPCSEA/dt.17.07.2001) in its resolution number 09 (iii)/a/CPSCA/IAEC/07-08/SVU/Zool/KSR-DVNK/dated 26/6/08.

Induction of diabetes

The animals are fasted over night and diabetes was induced by single intra peritoneal injection with a freshly prepared STZ (40 mg/kg b.w) dissolved in ice cold 0.1M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12-15 hr as per the method followed by Rakieten et al., (1963). 8 hr after STZ administration the rats were kept for next 24 hr on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of producing fatal hypoglycemia due to destruction of β cells which in turn results in to massive pancreatic insulin release. Diabetes was assessed by determining the fasting blood glucose after 48 hr of injection of STZ. The blood glucose levels in STZ rats were increased to markedly higher levels than normal. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (blood glucose level ≥250 g/dl) were selected. Pimpinella tirupatiensis aqueous extract given to the diabetic rats for 30 days.

Experimental design

The rats were divided into 5 groups, six rats in each group and treated as follows:

1. Group I- Normal control (NC) : Six rats were received the 0.9%Nacl / kg bodyweight via orogastric tube for a period of one month.
2. Group II -Diabetic control (DC) : Six rats were used as diabetic control rats by the injection of STZ (40 mg / kg b.w.) intraperitonially to the fasted rats.
3. Group III - (Pt) : Normal animals were treated orally with 750 mg/kg b.w/day of Pimpinella aqueous extract for 30 days
4. Group IV - (D+Pt) : Diabetic animals were treated orally with 750 mg/kg b.w/day of Pimpinella aqueous extract for 30 days,

5. Group V (D+Glb) : Diabetic animals were treated with 20 mg/kg/day of glibenclamide for 30 days.

After completion of 30 days treatment, the animals are sacrificed by cervical dislocation and the brain tissues were excised at 4^0C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80^0C for further biochemical analysis.

Analytical procedures

The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product MDA by using the method of Ohkawa et al. (1979). Xanthine oxidase activity was assayed by the dye reduction method of Srikanthan and Krishnamurthy, (1955). The activities of Na+/K+, Mg2+ and Ca2+ ATP ases in the brain are estimated by the method of Desaiah and Ho (1979). The enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin (BSA) as a standard.

Statistical analysis

The data are expressed as Mean values with their SD. In order to carry out statistical analysis, Ms Excel and SPSS 11.5 Version statistical packages are used. In my study the comparison is with respective groups, hence one way analysis of variance technique is applied to observe the significance between the groups. The post – Hoc test Duncan's multiple range test is also

performed to know the significant difference among the groups. Entire statistical analysis is carried out at 0.01 levels.

Results

Blood glucose and body weight changes. The STZ-induced diabetic rats had shown significant increase of blood glucose levels in comparison to normal control rats, which further increased during the experimental period. Oral administration of Pimpinella tirupatiensis aqueous extract significantly decreases the blood glucose levels in comparison to diabetic group. However, glibenclamide treatment also decreased the blood glucose levels in a significant manner when compared to diabetic group. The body weight of diabetic rats was also lower than the control group. However, Pimpinella tirupatiensis aqueous extract and glibenclamide treatments significantly improved the body weight and brought down towards near normal level. In the present study MDA levels, XOD activity increased and Na^+/K^+-ATPase Ca^{2+}- ATPase activities were decreased significantly ($P<0.01$) in diabetic control rats when compared to normal animals. After treating with Pimpinella tirupatiensis aqueous extract MDA levels, XOD activity decreased and Na^+/K^+-ATPase, Ca^{2+}- ATP ase activity was significantly increased. The activities of Na^+/K^+-ATPase and Ca^{2+}-ATPase in diabetic rats treated with Pimpinella tirupatiensis are recovered to normal levels. No significant changes were observed in the normal rats treated with Pimpinella tirupatiensis aqueous extract. But there was no significant change Mg^{2+}-ATPase in diabetic rats when compared to normal control rats.

DISCUSSION

Diabetes mellitus is a common metabolic disorder that affects the peripheral as well as the central nervous system. Neuropathy is quite common and undoubtly, the major health problem among diabetic patients (Tarsy et al., 1994). It was suggested that the possible sources of oxidative stress in diabetes include increased generation of ROS by glucose auto-oxidation, decreased tissue glutathione concentration, and impaired antioxidant enzymes.

In the current study, we observed significant increase in blood glucose levels in diabetic rats. This may be due to the deterioration of pancreatic β cells due to oxidative stress (kaneto et al., 2001). The elevation of glucose in STZ-treated rats was due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA (Yamamoto, Uchigata, & Okamoto, 1981). We have registered a decrease in body weight in STZ diabetic rats. The characteristic loss of body weight associated with STZ-induced diabetes is due to increased

muscle wasting in diabetes (Ravi, Ramachandran, & Subramanian, 2004). When Pimpinella tirupatiensis was administered to diabetic rats, the weights seemed to be increased, as was the ability to reduce hyperglycaemia. However, it could not normalise the body weight completely. This study showed that administration of Pimpinella tirupatiensis improved the body weight in diabetic rats, which could be attributed to its antidiabetic and antihyperlipidemic role. The administration of Pimpinella tirupatiensis to STZ diabetic rats reduced blood glucose levels, in accordance with earlier reports (Dhanabal et al., 2006). In the present study, the blood glucose data clearly showed that dietary Pimpinella tirupatiensis restrained the level of hyperglycaemia resulting from the experimental destruction of beta pancreatic cells induced by STZ. The hypoglycaemic effect of Pimpinella tirupatiensis increased gradually and was observed to be maximum at the end of the study period, i.e. 30 days. Our findings are similar to reported previously for ginger (Shanmugham et al., 2011). The decrease in blood glucose levels was due to the antidiabetic compounds of Pimpinella tirupatiensis. Our results are supporting its use as folklore medicine for the treatment of diabetes.

In the present study the formation of TBARS, a product of lipid peroxidation reaction, was significantly increased in diabetic brain tissues. Our results were also supported by studies of (Gamila S.M. El-Saeed et al., 2013). TBARS and hydroperoxides showed high lipid peroxidation. This may be cause the brain contains relatively high concentration of easily peroxidizable fatty acids (Carney et al., 1991). The elevated lipid peroxidation is responsible for the formation of lipid hydroperoxides in membrane and would result in damage of the membrane structure and inactivation of membrane bound enzymes. The accumulation of lipid peroxides adds hydrophilic moieties into the hydrophobic phase and thereby brings about changes in the membrane permeability and cell functions (Pascoe and Redd, 1989). This increased content of MDA was triggered by Pimpinella tirupatiensis tuberous root aqueous extract. Similar reports were found in the brain regions of diabetic rats, the elevated level of MDA was significantly decreased in animals fed with ginger (Shanmgham et al., 2010). Safinaz &. Ibrahim., (2008) have reported MDA levels were decreased in brain after supplementation of hesperidin. The anti-oxidant compounds and other pharmacological compounds of Pimpinella tirupatiensis extract may inhibit the production of free radicals, and reduced the products of lipid peroxidation

In the current study Xanthine oxidase was increased in diabetic rat brain. This result provides support for the previously reported diabetes-induced brain oxidative stress (Traverso et al., 1997). Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid and generates O_2 •− Hydrogen peroxide formed from O_2 •− could be converted into highly reactive •OH leading to oxidative stress (Singh and Pushpa, 2005). After treatment with glibanclamide and Pimpinella tirupatiensis aqueous extract to the diabetic animals the activity of Xanthine oxidase was down regulated. This could be due to the decreased degeneration of ATP, down regulation of purine metabolism that leads to the low profile of xanthine, hypoxanthine levels which are necessary forhigh activity of XOD. Similar results have been obtained by the treatment with etimode in the diabetic rat CNS (Ates et al., 2006).

Na^+/K^+-ATPase play an important role in the functional activity of nervous cells. The present study has shown that diabetes decreased Na^+/K^+-ATPase activity in brain. This is in agreement with the earlier published data (Franzon et al., 2005). Hyperglycemia has been shown to generate free radicals from auto-oxidation of glucose, formation of advance glycated end products (AGEs) and increased polyl pathway, with concomitant increase in cellular lipid peroxidation and damage of membrane in diabetes (El-Missiry et al., 2004). This increased lipid peroxides formation during diabetes disturbes the anatomical integrity of the membrane, leading to the inhibition of several membrane bound enzymes. Previously it has been reported that the inhibition of mouse cerebral Na^+/K^+-ATPase activity by ultraviolet C generated OH^- and a proxyl ($ROO^{-)}$ radical is mediated via lipid peroxidation induced disruption of membrane integrity (Jamme et al., 1995). The reduction in the activity of Na^+/K^+-ATPase observed in diabetic tissue may be due to the membrane peroxidative damage induced by increased lipid peroxidative status.

Na^+- K^+ ATPase are a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It catalyzes the hydrolysis of ATP and couples it to transport of Na^+ and K^+ across the cell membrane, thereby generating the trans membranous Na^+- K^+ gradient (Erecinsk et. Ai., 1994). The inhibition of such enzyme provokes an increased uptake of Na + and cytosolic free Ca 2+, releasing of acetylcholine and decreasing the membrane potential of synaptosomes from cerebral cortex (Satoh et al., 1992). Decreased Na^+-K^+ ATPase activity leads to neuron-selective lesion in the brain (Lees et al., 1990) suggesting that inhibition of this

enzyme may be used as useful indicator of brain neurodegenerative pathophysiology related to memory and cognitive disorders of diabetic state. In the present study, treatment with Pimpinella tirupatiensis aqueous extract significantly increased the Na+/K+, ATPase activity in the brain of induced diabetic rats. Mohammad Rizwan Siddiqui (2005) also reported the Na^+/K^+-ATPase decreased in diabetic rat brain by treatment of Trigonella seeds. Treatment of the diabetic animals with Trigonella, Vandate and combined therapy of Trigonella and Vanadate restored the decreased activity of Na^+/K^+-ATPase increased lipid peroxides and altered membrane fluidity after 21 days of treatments. It also has antioxidant properties (Genet et al., 2002). A reduction in the production of free radical and lipid peroxides formation by restoring the antioxidant enzymes can beneficially prevent the decreased of activity Na^+/K^+-ATPase enzyme. Administration of Pimpinella tirupatiensis aqueous extract increased the Na^+/K^+-ATPase enzyme activity and may help to control free radical generated. In the current study, Mg^{2+}-ATPase activity was not significant change in diabetic rats when compared to normal control rats. Previous studies have suggested that Mg^{2+}-ATPase activity was not significant change in diabetic rats (Liapi et al., 2009).

In the present study a decrease in the brain Ca $^{2+}$ATPase activity was noticed. Diabetes-related ATPase activity changes in cerebral microvessels may depend on altered blood-brain barrier functions (Mooradian et al., 1994). Moreover the decrease in Ca^{2+}-ATPase activity was related to protein glycosylation and lipid peroxidation. Ca^{2+}-ATPase is sensitive to its phospholipids milieu and to polyunsaturated fatty acids. The content of these lipids may change in diabetes and may cause alterations in enzyme activity (Das et al., 2004). The reversal of Ca^{2+}-ATPase activity in Pimpinella tirupatiensis aqueous extract treated and glibenclamide treated diabetic rats towards normal level shows the normal functioning of Ca^{2+}-ATPase. This is in agreement with the earlier published data (Anupama et al., 2012). Administrations of Erythrina variegata plant extract prevents the inhibition of Ca^{2+}-ATPase activity of diabetic rat brains and consequently would attenuate the resultant neurotoxicity. Treatment of diabetic animals with a mixture of certain antioxidants compounds, such as beta-carotene, vitamine E or its analog trolox C, prevented the development of diabetes-induced defects, such as enhanced lipid peroxidation (Kowlru et al., 2000). Previous studies have suggested that some lippholic and water soluble antioxidants, including buthylated hydroxytoluene and vitamine E, are able to prevent the effects of oxidative

stress on Ca^{2+}-ATPase activity (Tappia et al., 2001). Recent studies established that Ca^{2+}-ATPase became subnormal in the hippocampus in hyperglycemic rats and administration of centella asiatica has prevented the diabetes–induced decreases in enzyme activity (Kowlru et al., 1999). Administration of Pimpinella tirupatiensis aqueous extract increased the Ca^{2+}-ATPase enzyme activity and may help to control free radical generated.

To conclude the present findings reveals that one month treatment with selected intensity that was adopted is beneficial in countering the alterations in lipid peroxidation and ATPases in wistar strain rats. The antioxidant defense system which plays a major role in countering the free radicals in diabetic rats were reversed back to normal levels when Pimpinella tirupatiensis is given. The changes in markers of oxidative stress which include MDA content and antioxidant enzymes indicating efficient adaptative machinery of oxygen species that was operated in the brain tissue in detoxification of oxygen species that are produced due to diabetes. This study drawn a conclusion stating that Pimpinella tirupatiensis treatment to diabetic rats may be beneficial to improve the metabolic efficiency and thereby improve the health status. Thus Pimpinella tirupatiensis may be used in the formulation of herbal drugs which can be used in the treatment of diabetes. Since Pimpinella tirupatiensis exhibited antioxidant and ant diabetic activity, it might be clinically useful in the control of human diabetes. Thus we conclude that successive studies are mandatory to establish the precise nature of Pimpinella tirupatiensis active constituents as well as their mechanism of action.

Fig.1: MDA content in the brain Normal Control (NC), Diabetic control (DC), Diabetic rats treated with Pt Aqueous extract (DC+PtAq.e), Control rats treated with Pt Aqueous extract (PtAq.e), Diabetic rats treated with Glibenclamide. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.

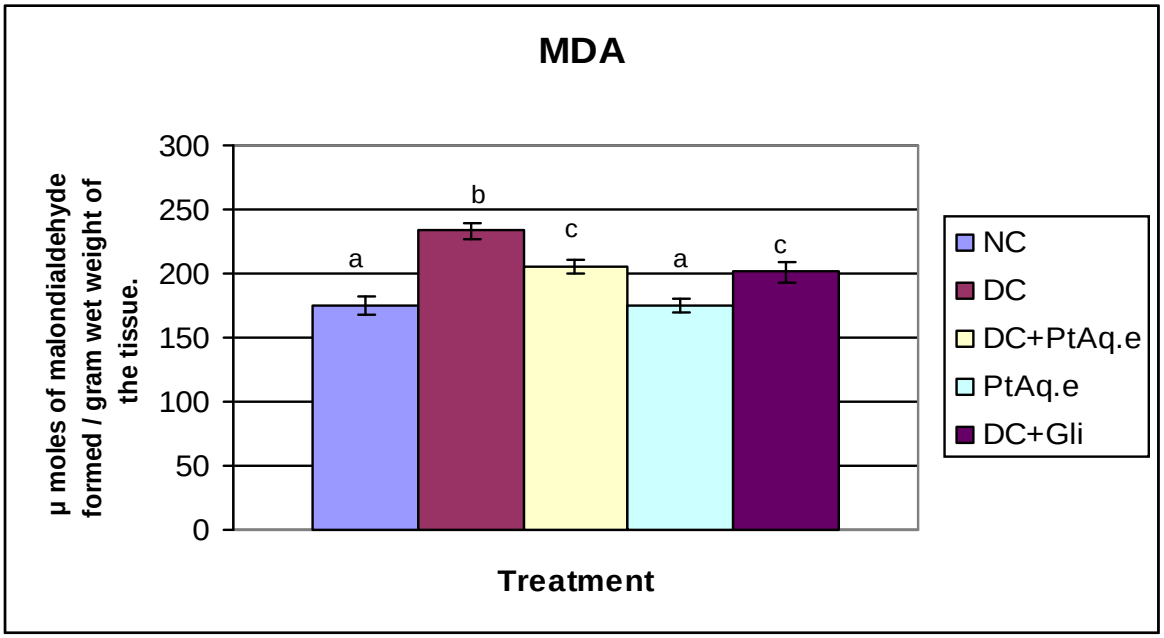


Fig.2:Changes in XOD activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with Pt Aqueous extract (DC+PtAq.e), Control rats treated with Pt Aqueous extract (PtAq.e), Diabetic rats treated with Glibenclamide. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.

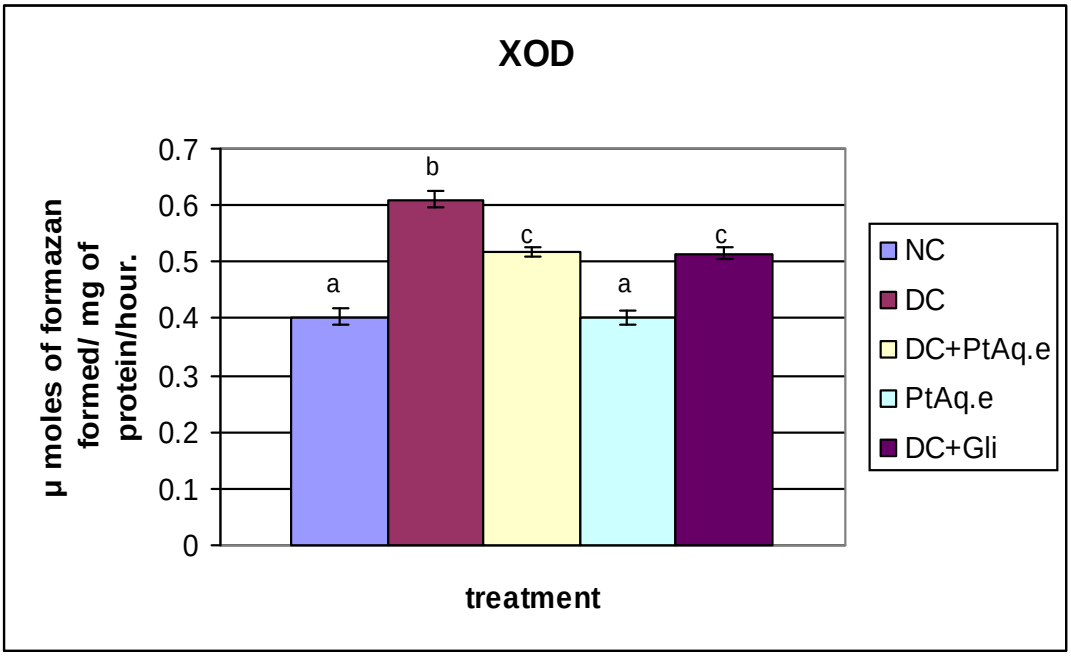


Fig.3:Changes in Na^+/K^+-ATPase activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with Pt Aqueous extract (DC+PtAq.e), Control rats treated with Pt Aqueous extract (PtAq.e), Diabetic rats treated with Glibenclamide (DC+Gli). Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.

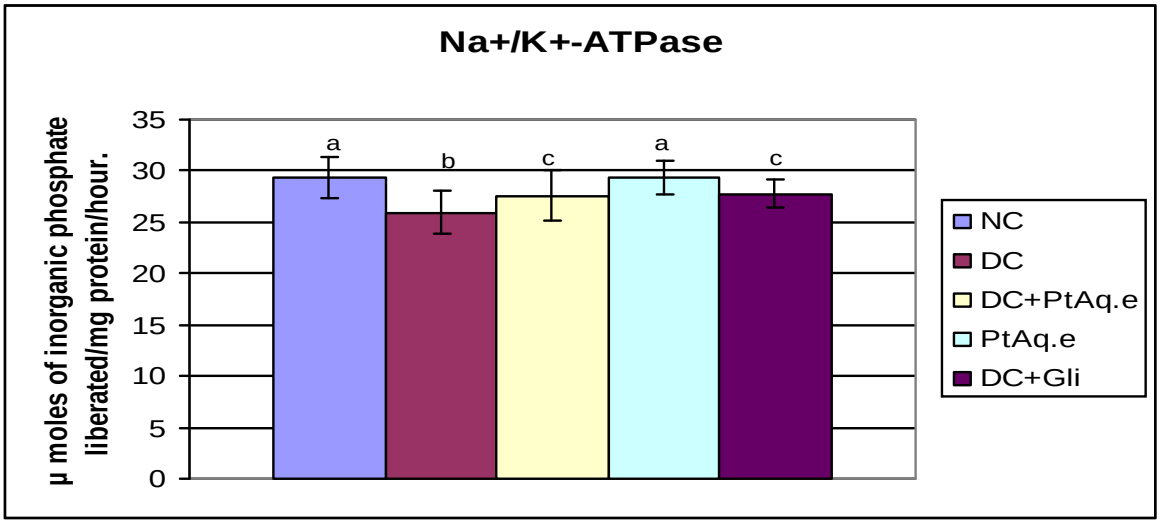


Fig.4: Changes in Ca^{2+} activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with Pt Aqueous extract (DC+PtAq.e), Control rats treated with Pt Aqueous extract (PtAq.e), Diabetic rats treated with Glibenclamide (DC+Gli). Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.

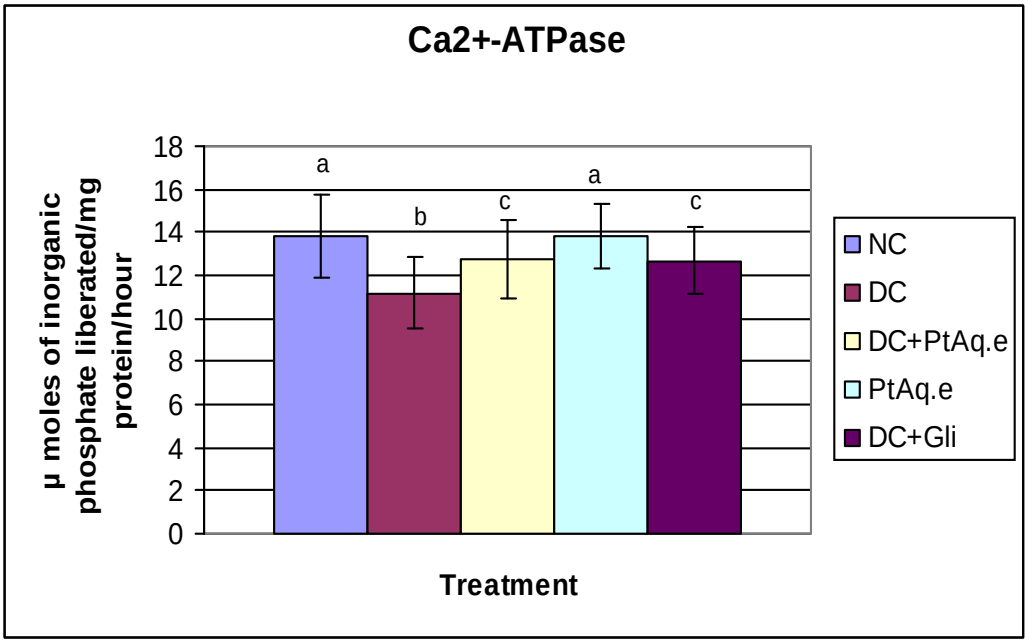
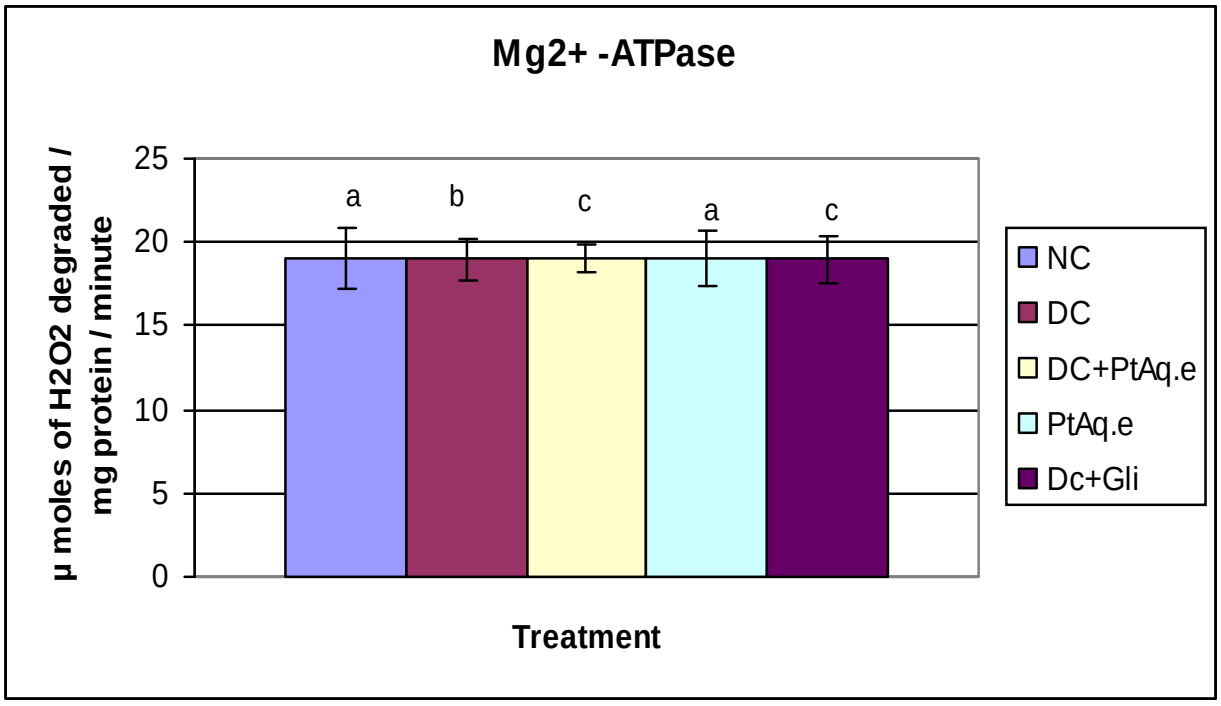


Fig.5: Changes in Mg^{2+}-ATPase activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with Pt Aqueous extract (DC+PtAq.e), Control rats treated with Pt Aqueous extract (PtAq.e), Diabetic rats treated with Glibenclamide(DC+Gli). Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.



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Nelli Giribabu,[1] Nelli Srinivasarao,[2] Somesula Swapna Rekha,[3] Sekaran Muniandy,[4] and Naguib Salleh[1] Centella asiatica Attenuates Diabetes Induced Hippocampal Changes in Experimental Diabetic Rats. Evidence-Based Complementary and Alternative Medicine 2014

Overview of Parkinson's disease: its neurotransmitters

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Parkinson's disease

Parkinson's disease (PD) is a degenerative disorder of the central nervous system. It was first described in 1817 by James Parkinson, a British physician who published a paper on what he called "the shaking palsy." In this paper, he set forth the major symptoms of the disease that was later bear his name. It occurs when nerve cells, or neurons, in an area of the brain known as the substantia nigra die or become impaired. Normally, these neurons produce an important brain chemical known as dopamine. Dopamine is a chemical messenger responsible for transmitting signals between the substantia nigra and the next "relay station" of the brain, the corpus striatum, to produce smooth, purposeful movement. Loss of dopamine results in abnormal nerve firing patterns within the brain that cause impaired movement. Studies have shown that most Parkinson's patients have lost 60 to 80 percent or more of the dopamine-producing cells in the substantia nigra by the time symptoms appear. In addition to the degeneration of the dopaminergic pathways in the brain of Parkinson's patients, there is also a presence of eosinophilic intracytoplasmic inclusions or Lewy bodies in residual dopaminergic neurons. The average age of onset for a patient with Parkinson's disease is 64.2 years and 65.5 years of age when they are first diagnosed with this idiopathic disease. It is important to recognize the fact that as Parkinson's patients age and reach the later stages of the disease they may also experience other non-motor symptoms that can be as debilitating as the motor dysfunctions found at the onset (Chen, 2007). Psychological problems are frequently present in the later stages of the disease and manifest themselves in conjunction with motor symptoms. Depression and anxiety are the most often diagnosed disorders found in Parkinson's patients. In addition, dementia also plays a significant role in the cognitive and emotional decline of this particular population. Parkinson's disease (PD) is characterized by motor and non motor symptoms.

The main motor features involved in Parkinson's disease are combination of symptoms as (TRAP): Tremor, Rigidity Akinesia or bradykinesia, and Postural instability.

The main non - motor features involved in Parkinson's disease are combination of symptoms such as Depression, Anxiety, Emotional changes, Difficulty with swallowing and chewing, Speech changes, Urinary problems or constipation, Skin problems, Sleep problems, Dementia or other cognitive problems, Orthostatic hypotension.

PD is uncommon in patients less than 40 years of age. It is found in about 1% of those greater than 50 and 3% of those aged 95 or greater. As the second most common neurological disorder in adults, the personal, economic, and societal costs of Parkinson's disease are enormous.

The genetics of Parkinson's disease is being studied worldwide there may be a genetic component. A genetic abnormality in one gene may cause mutation. This mutation is the absence of a gene called "Parkin" gene, and it has been associated with a number of early on-set Parkinson's disease cases. A genetic basis for Parkinson's disease could lead to earlier detection, which is crucial since 70 to 90 percent of the dopamine producing cells of the substantia nigra die before symptoms develop. Parkinson's disease is currently diagnosed based on physical symptoms. Mutations in the Alpha-synuclein, DJ-, PINK-1 and LRRK2genes cause Parkinson disease. Genetic studies have demonstrated several inheritable gene abnormalities in certain families, but the vast majority of cases of Parkinson's occur sporadically. It is believed that heredity factors may render some individuals more vulnerable to environmental factors such as pesticides.

Alpha-synuclein: On Chromosome 4, this gene due to triplication causes excess of protein and excess of alpha synuclein.

Parkin gene: Parkin gene is translated into a protein which helps cell breakdown and recycle protein.

DJ-1: Regulate gene activity and protect cell from oxidative stress.

PINK-1: Codes for a protein active in mitochondria, mutations may cause increase susceptibility to cellular stress. It also causes rare onset of Parkinson's disease symptoms.

LRRK2: It translates a protein called Dardarin, which causes sporadic Parkinson's disease.

At present, there is no cure for PD. But medications or surgery can sometimes provide dramatic relief from the symptoms.

Medications for PD fall into three categories. The first category includes drugs that work directly or indirectly to increase the level of dopamine in the brain. The most common drugs for PD are dopamine precursors – substances such as levodopa that cross the blood-brain barrier and are then changed into dopamine. Other drugs mimic dopamine or prevent or slow its breakdown.

The second category of PD drugs affects other neurotransmitters in the body in order to ease some of the symptoms of the disease. For example, anticholinergic drugs interfere with production or uptake of the neurotransmitter acetylcholine. These drugs help to reduce tremors and muscle stiffness, which can result from having more acetylcholine than dopamine.

The third category of drugs prescribed for PD includes medications that help control the non-motor symptoms of the disease, that is, the symptoms that don't affect movement. For example, people with PD-related depression may be prescribed antidepressants.

Other troubling and distressing problems may occur with long-term levodopa use. Patients may begin to notice more pronounced symptoms before their first dose of medication in the morning, and they may develop muscle spasms or other problems when each dose begins to wear off. The period of effectiveness after each dose may begin to shorten, called the wearing-off effect. Another potential problem is referred to as the on-off effect — sudden, unpredictable changes in movement, from normal to parkinsonian movement and back again. These effects probably indicate that the patient's response to the drug is changing or that the disease is progressing.

One approach to alleviating these side effects is to take levodopa more often and in smaller amounts. People with PD should never stop taking levodopa without their physician's knowledge or consent because rapidly withdrawing the drug can have potentially serious side effects, such as immobility or difficulty breathing.

Cholinergic system:

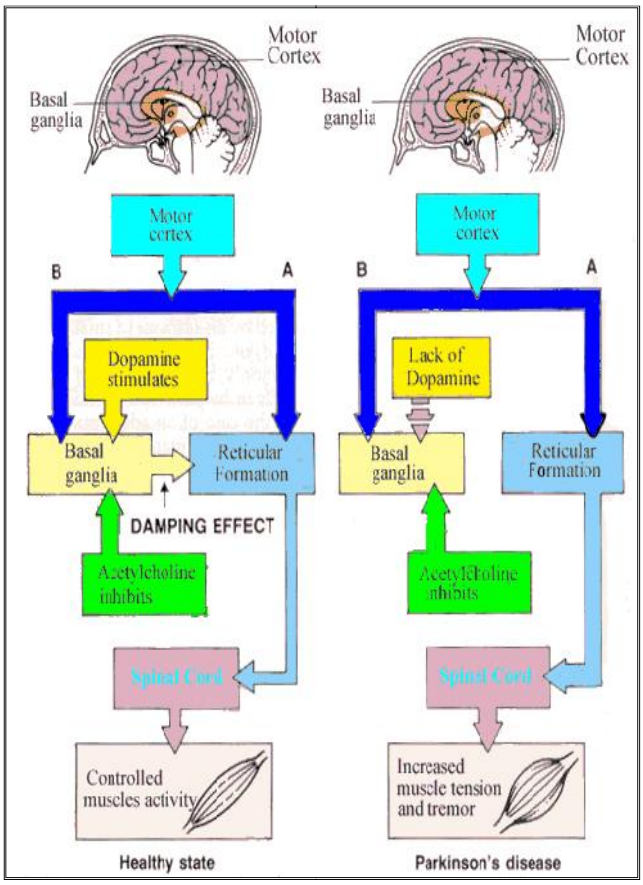

Fig: Imbalance of cholinergic system in PD

Cholinergic nervous systems are demonstrated to innervate virtually all tissues of the mammalian body. In the periphery, the major cholinergic system is the parasympathetic branch of the autonomic nervous system. Overall effect of Acetylcholine is to stimulate muscle contraction and Dopamine (DA) is to inhibit muscle contraction. Parkinson's disease consequently occurs when the effect of dopamine is less than that of acetylcholine. Due to imbalance of ACh and DA, the muscles exhibit uncoordinated contraction leading to tremors which further lead to the symptoms of muscle fatigue, a characteristic feature of PD. Cholinergic fibres, which originate from the brainstem and the basal forebrain, is impaired in dementia associated with Lewy bodies which can be a consequence of Parkinson's disease (Perry et al., 1999). Although a neural basis for cognitive dysfunctions in PD remains unknown, pathological and functional neuroimaging studies suggest that the cholinergic system arising from the basal forebrain has an important role in cognitive functions of PD patients (Caviness et al., 2007). Recent in vivo cholinergic functional imaging data have also demonstrated that degeneration of the basal forebrain cholinergic system appears in early in PD, prior to the occurrence of dementia, and deteriorates further in dementia (Hilker et al., 2005; Shimada et al., 2009). Additionally, Aarsland et al., (1994) reported that cholinergic blockade disturbed language functions, which are the major symptoms of progression of PD.

Overall, PD is associated with general cognition as well as performance in individual cognitive subsets, which further support the role of the cholinergic system in cognitive performance of PD patients. Moreover, the loss of functioning in the dopaminergic system of the substantia nigra and the loss of cells in the cholinergic pathways in the nucleus basalis are responsible for the most significant cognitive deficits in Parkinson's disease (Ferreri et al., 2006). Rotenone toxicity depended on a direct interaction with complex I inhibition (Sherer et al., 2003). The effects of rotenone observed in the system (modest ATP depletion,

oxidative damage, and cell death) can be entirely explained by the inhibition of complex I, which enhances ROS production (Cassarino et al., 1997; Kushnareva et al., 2002).

The augmentation of cholinergic activity as evidenced by increased ACh content and decreased AChE activity during induced PD, may be due to the upsurge in the generation of reactive oxygen species that occur due to enhanced DA oxidation (Graham et al., 1978). Earlier investigations have demonstrated the decreased AChE activity in PD patients (Hartikainen et al., 1992). This may be due to the interaction between molecules of DA and AChE which resulted not only in modification of catecholamine oxidation, but also caused the inactivation of AChE catalytic activity (Klegeris et al., 1995). A direct interaction of DA and AChE molecules, as suggested here, could have crucial implications in regulating the availability of DA in normal and pathological brain. Complex I, the rotenone-binding site, is a site of electron leakage that produces ROS (Hensley et al., 1998; Kushnareva et al., 2002). As yet, however, any extrapolation from the cell-free system described here and a true physiological situation should be made with caution. The inactivation of AChE during DA autoxidation occurred mainly due to the direct interaction of a quinone or semiquinone oxidation products with the enzyme. Reactive oxygen species (ROS) that are generated during dopamine metabolism and by mitochondrial respiration, which are shown to cause protein damage, (Guptasarma and Balasubramanian, 1992) including AChE activity.

Biogenic amines

Amine neurotransmitters are small molecules sharing an amine group (-NH2). There are five main amine neurotransmitters in mammals: dopamine, norepinephrine, epinephrine, serotonin, and histamine. Dopamine, norepinephrine, and epinephrine share a catechol moiety and are called catecholamines or monoamines.

The major problem encounted with Parkinson's is the degeneration of the dopaminergic system, leading to lowered dopamine levels. Dopamine is a neurotransmitter that plays a key role in body movements and motor control. Thus dopamine levels, when abnormally low, due to degeneration of the dopamine system in the brain, leads to the loss of motor control and other conditions associated with Parkinson's disease. Dopamine, along with the neurotransmitters norepinephrine and epinephrine, forms part of a system called the catecholamine system. Since these three monoamines play a pivatol role in motor control, so enhancing the catecholaminergic can be considered as one of the preferred method to reduce

the Parkinson's symptoms and to improve overall health. Parkinson's disease is affected by the disturbance of many neurotransmitter systems including the dopaminergic, serotonergic, and noradrenergic pathways. All of these neurotransmitter pathways play a crucial role in the emotional well-being of an individual given their linkages to neurobehavioral processes as emotion, mood and cognation (Aarsland, 2006).

Glutamate metabolism

Parkinson's disease (PD) is associated with degeneration of the pigmented dopaminergic neurons. Although the mechanisms by which these neurons degenerate in PD are poorly understood, indirect evidence suggests involvement of glutamatergic mechanisms in the pathogenesis of this disorder. Glutamate, the major excitatory transmitter in the mammalian central nervous system, is known to be neurotoxic when present in excess at the synapses. There is evidence that the severe loss of dopaminergic innervations of the striatum in PD is associated with over-activity of glutamate (Bladini, et al., 1996; Hornykiewicz, 2001). There is evidence that the decreased dopaminergic innervations of the striatum in PD are associated with over-activity of glutamate (Bladini, et al., 1996). The enzymes participated in Glutamate metabolism are GDH which catalyzes the glutamate to 2-oxoglutarate (Brusilow and Horwich, 1995), GS plays a vital role in the ω - amidation of glutamate to form glutamine and Glutaminase strongly favors glutamate formation rather than glutamine synthesis. Glutamate is a major cause of neuronal cell death in a number of different neurodegenerative diseases (Lees, 1993). Two pathways for glutamate toxicity have been described: excitotoxicity, which occurs through the activation of glutamatergic receptors (Choi, 1988; Michaels and Rothman 1990), and oxidative glutamate toxicity, which is mediated via a series of disturbances to the redox homeostasis of the cell (Murphy et al., 1989). These pathways are incompletely characterized, but both result in the production of free radicals (Choi, 1992). Glutamatergic neuronal stimulation may be a common final pathway in several brain conditions in which oxidative stress and ensuing excitotoxicity plays a role. A contributing factor in many such conditions is excessive glutamate release, and subsequent glutamatergic neuronal stimulation, that causes increased production of reactive oxygen species (ROS), oxidative stress, excitotoxicity and neuronal damage (Savolainen et al., 1998). Loss of dopamine and stimulation of dopamine receptors on the glutamatergic terminals result in an increased level of glutamate in substantia nigra (SN) (Morari et al., 1998) may cause excitotoxicity in PD. Glutamate excitotoxicity causes ROS production

which inducing oxidative stress which may be an important factor in several pathological brain conditions of PD.

Electroencephalography (EEG)

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Abstract

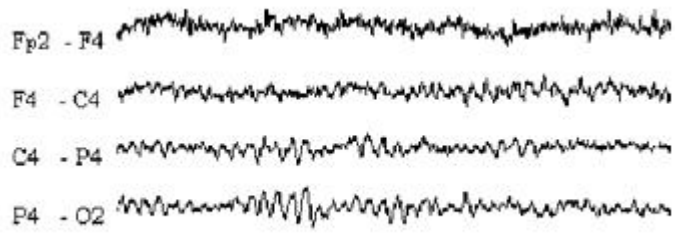
EEG is detailed by Barbara E. Schwartz in Electroencephalography and Clinical Neurophysiology. Simultaneous EEG recordings and fMRI scans have been obtained successfully, though successful simultaneous recording requires that several technical difficulties be overcome, such as the presence of ballisto cardiographic artifact, MRI pulse artifact and the induction of electrical currents in EEG wires that move within the strong magnetic fields of the MRI. While challenging, these have been successfully overcome in a number of studies. MRI's produce detailed images created by generating strong magnetic fields that may induce potentially harmful displacement force and torque. These fields produce potentially harmful radio frequency heating and create image artifacts rendering images useless. Due to these potential risks, only certain medical devices can be used in an MR environment. Similarly, simultaneous recordings with MEG and EEG have also been conducted, which has several advantages over using either technique alone: EEG requires accurate information about certain aspects of the skull that can only be estimated, such as skull radius, and conductivities of various skull locations. MEG does not have this issue, and a simultaneous analysis allows this to be corrected for MEG and EEG both detect activity below the surface of the cortex very poorly, and like EEG, the level of error increases with the depth below the surface of the cortex one attempts to examine. However, the errors are very different between the techniques, and combining them thus allows for correction of some of this noise.

MEG has access to virtually no sources of brain activity below a few centimeters under the cortex. EEG, on the other hand, can receive signals from greater depth, albeit with a high degree of noise. Combining the two makes it easier to determine what in the EEG signal comes from the surface (since MEG is very accurate in examining signals from the surface of the brain), and what comes from deeper in the brain, thus allowing for analysis of deeper brain signals than either EEG or MEG on its own. EEG has also been combined with positron emission tomography. This provides the advantage of allowing researchers to see what EEG signals are associated with different drug actions in the brain.

Introduction

The electroencephalogram (EEG) is a recording of the electrical activity of the brain from the scalp. The first recordings were made by Hans Berger in 1929 although similar studies had been carried out in animals as early as 1870.

The waveforms recorded are thought to reflect the activity of the surface of the brain, the cortex. This activity is influenced by the electrical activity from the brain structures underneath the cortex.



EEG traces

The nerve cells in the brain produce signals that are called action potentials. These action potentials move from one cell to another across a gap called the synapse. Special chemicals called neurotransmitters help the signals to move across the gap. There are two types of neurotransmitters, one will help the action potential to move to the next cell, the other will stop it moving to another nerve cell.

The brain normally works hard to keep an equal amount of each of these neurotransmitters in the brain.

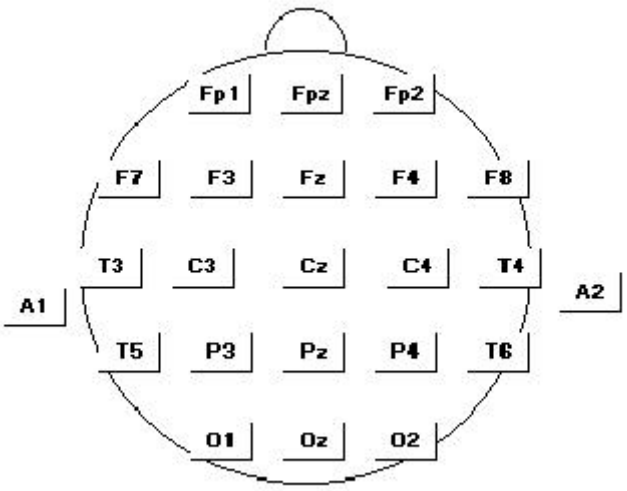
EEG activity is quite small, measured in microvolts (µV) with the main frequencies of interest up to approximately 30 Hertz (Hz).

Electrodes

Small metal discs called electrodes are placed on the scalp in special positions. These positions are identified by the recordist who measures the head using the International

10/20 System. This relies on taking measurements between certain fixed points on the head. The electrodes are then placed at points that are 10% and 20% of these distances.

Each electrode site is labelled with a letter and a number. The letter refers to the area of brain underlying the electrode e.g. F - Frontal lobe and T - Temporal lobe. Even numbers denote the right side of the head and odd numbers the left side of the head.



10/20 System of electrode placement

There is a great variety of electrodes that can be used. The majority are small discs of stainless steel, tin, gold or silver covered with a silver chloride coating. These normally have a lead attached. Alternative methods consist of a cap in which the electrodes are already imbedded.

EEG Applications

One of the major roles of EEG is as an aid to diagnose epilepsy. Abnormal patterns such as spikes, sharp waves and/or spike and wave complexes can be seen. The type of activity and the area of the brain that it is recorded from will assist the physician in prescribing the correct medication for that type of epilepsy.

Patients with epilepsy that cannot be controlled by medication will often have surgery in order to remove the damaged tissue. The EEG plays an important role in localising this tissue. Special electrodes can be inserted through the cortex or alternatively a grid of electrodes placed directly on the surface of the cortex. These recordings, often called Long

Term Monitoring for Epilepsy (LTME), can be carried out for periods ranging from 24 hours to 1 week. The EEG recorded will indicate which areas of the brain should be surgically removed.

EEG studies can also be used in patients who are deeply unconscious, to distinguish between brain death and possible reversible conditions.

Electrocerebral inactivity (ECI) or electrocerebral silence (ECS) is defined as no EEG activity over 2 μ V in amplitude when recording from electrodes on the scalp, that are 10 cm or more apart.

Sleep studies

The EEG is frequently used in the investigation of sleep disorders especially sleep apnoea. EEG activity together with other physiological signals such as heart rate, airflow, respiration, oxygen saturation and limb movement are measured simultaneously. These recordings are usually carried out overnight although some sleep studies can be carried out in the department during the day under strictly controlled conditions.

The EEG record can be broken down into epochs which are normally of 30 seconds duration. Using the EEG activity, each epoch is classified into one of 5 sleep stages. This is displayed visually as a Sleep Histogram.

Respiration and airflow are used to look for periods of apnoea which occur when the patient stops breathing. These are then correlated with the sleep stage in which they occurred and the level the oxygen saturation fell to during the apnoea.

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Protective role of some medicinal plants against different Neurological Disorders

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Abstract

Medicinal plants have contributed immensely to health care in India. This is due in part to the recognition of the value of traditional medical systems, particularly in Asian origin, and the identification of medicinal plant from indigenous pharmacopoeias, which have significant healing power.

Among all families of the plant kingdom, members of the Apiaceae (Umbelliferae) have been used for centuries in folk medicine. *Centella asiatica*, commonly known as "gotukola" is naturally used in the treatment of different diseases, for example migraine. It also showed effect against herpes simplex viruses , *Mycobacterium leprae* and *Mycobacterium tuberculosis* and antidepressant activity in rats. *Centella asiatica* is referred as one of the great multipurpose miracle herbs of oriental medicine. It is considered as one of the most powerful rejuvenating herbs in Indian Ayurvedic medicine. It has been used in ayurvedic preparation either in the fresh or in the extract form. CA has been subjected to extensive experimental and clinical investigation. The active constituents of *Centella* include triterpenoid glycosides (asiatic acid, asiaticoside, madecassic acid, madecassoside, oxyasiaticoside, and centelloside); saponin glycosides (1.4-3.4%) (brahmiside, brahminoside); flavonol glycosides (quercetin-3-glycoside and Kampferol-3-glycoside); flavonoids viz., naringin, quercitin, rutin, cathecin, kampeferol and apigenin; phytosterols such as ß-sitosterol, stigmasterol and camposterol and a volatile oil consisting of vallerin, camphor, cineole and terpene acetate that comprises 35% of the total oil content. CA also contains naturally occurring vitamins A, B, C, G, K, tannins (24.5%); essential oils (0.8-1%); monoterpenes (α-pinene, ß-pinene, myrcene, α-terpineol, borneol); sesquiterpenes (α-copanene, ß-elemene, ß-caryophyllene, trans-ß-farnesene, germacrene, bicyloelemene); several aminoacids (lysine, alanine, phenylalanine, serine, aspartic acid, glutamic acid); fatty acids (palmitic, oleic and linoleic acids); resin (8.9%);

an alkaloid named hydrocotyline and elements Calcium, Magnesium and Sodium. CA appears to be non-toxic but mild allergic causes contact dermatitis in sensitive individuals (WHO, 1999).

This study was designed to evaluate the active constituents of different extracts of *Centella asiatica* and to determine the presence of secondary metabolites.

Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium.

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more

widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US$2.2 billion. In the Indian system of medicine the following medicinal plants have shownpromising activity in neuropsychopharmacology: *Bacopa monnierae, Centella asiatica, Celastrus paniculatus, Nicotiana tabaccum, Withania somnifera, Ricinus communis, Salvia officinalis, Ginkgo biloba, Huperiza serrata, Angelica sinensis, Uncaria tomentosa, Hypericum perforatum, Physostigma venosum, Acorus calmus, Curcuma longa, Terminalia chebula, Crocus sativus, Enhydra fluctuans, Valeriana wallichii, Glycyrrhiza glabra* etc. In Chinese medicine, numerous plants have been used to treat stroke, and some of the them are: *Ledebouriella divaricata, Scutellaria baicalensis, Angelica pubescens, Morus alba, Salvia miltiorrhiza, Uncaria rhynchophylla, and Ligusticum chuanxiong*.[1]. Herbal medicine has long been used to treat neural symptoms. Although the precise mechanisms of action of herbal drugs have yet to be determined, some of them have been shown to exert anti-inflammatory and anti-inflammatory herbal medicine and its constituents are being proved to be a potent neuroprotector against various brain pathologies[2]. Structural diversity of medicinal herbs makes them a valuable source of novel lead compounds against therapeutic targets that are newly discovered by screening techniques [3]. This review will highlight the importance of phytochemicals on neuroprotective function and neurological disorders [4]. A number of pharmaceutical compounds are in the market which have been used for their neuroprotective property. Drugs to improve neurofunction generally work by altering the balance of particular chemicals (neurotransmitters) in the brain [5]. Number of medicines are derived from the medicinal plants and have shown memory enhancing properties of their bioactive phytochemical constituents. One of the mechanisms suggested to epilepsy is decreased cholinergic activity in brain[6]. Numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease (AD) and other memory related disorders.

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Neuroprotective effect of different extracts of *Centella asiatica* during Pentylenetetrazole (PTZ)-induced epilepsy with reference to protein profiles

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Abstract

Centella asiatica (CA) is being used in traditional medicine in the treatment of several neurological disorders including epilepsy. The present study is carried out to investigate the anticonvulsant effect of different extracts of CA with particular reference to protein and amino acid metabolism in different regions of rat brain (Cerebellum, Cerebral Cortex, Hippocampus and Pons-medulla) during Pentylenetetrazole (PTZ)- induced epilepsy. The rats were randomly divided into 8 groups having 6 in each group: 1. Control group received Saline, 2. PTZ-induced epileptic group (60 mg/kg b.w./ i.p/ 1 day) 3.Epileptic group pretreated with n-Hexane extract (n-HE), 4. Epileptic group pretreated with Chloroform extract (CE), 5. Epileptic group pretreated with n-butanol extract (n-BE), 6. Epileptic group pretreated with Ethyl acetate (EAE) extract, 7. Epileptic group pretreated with Aqueous(AE) extract and 8. Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/i.p). The CA extracts were administered at the dose of 200 mg/kg body weight orally for one week. Selected parameters representing protein (total, soluble and structural proteins) different regions of brain during induced epilepsy and on pre-treatment with different extracts of CA. PTZ treatment in a convulsive dose of 60 mg/kg significantly reduced total and soluble proteins content in all the brain regions compared to controls. Treatment with different extracts of CA reversed the alterations that have occurred during PTZ-induced epilepsy. Hence, it is evident that the different bioactive factors of CA offered protection against PTZ-induced epilepsy.

Key words: Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole

Introduction

Proteins are the most abundant bio-chemical compounds of the living organisms, which have a pivotal role to play in cellular metabolism. They constitute about one fifth of an animal body on wet weight basis (1). Proteins have important activities including catalysis of metabolic reactions and transport of vitamins, minerals, oxygen and fuels. Functionally proteins exhibit a great diversity and constitute heterogeneous group having diverse physiological functions as structural elements, in contractile systems, for nutrient storage, as vehicles of transport, as hormones, as catalysts, as toxins and as protective agents (2).

Hence, an important goal of molecular medicine is the identification of proteins whose presence, absence, or deficiency is associated with specific physiologic states or diseases (3). Proteins play a dual role as a building material and as a source of energy for the organism. It provides the organism with energy liberated through its breakdown and utilized in life processes (4). In view of the importance of protein, the present study is taken up to study the alterations in protein profiles and their turnover in different regions of brain during PTZ-induced epilepsy and on antiepileptic treatment using different extracts of *Centellaasiatica.*

Material and methods

Procurement and Maintenance of Experimental Animals

Male adult Wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±2^{0}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt:17.07.2006 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2006.

Drugs and Chemicals

Pentylenetetrazole and diazepam were obtained from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were analytical grade.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688).

The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of Plant Extracts

The active principles of the leaves of plant were extracted into different solvents, Methanol, Water, n-Hexane, Chloroform, Ethyl acetate and n-Butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants (5, 6). Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for further studies.

Induction of Epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) in saline (7,8).

Administration of Test substance

Each fraction of CA extract (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ (9). A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA . The volume of administration was kept at 1ml to the animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Experimental design for screening of plant extracts for anticonvulsant activity

The rats were divided into 8 groups i.e., Group1-Normal saline treated control rats (SC), Group 2-Rats treated with PTZ (Epileptic group), Group 3-Epileptic rats pretreated with n-Hexane Extract (nHE+PTZ), Group 4-Epileptic rats pretreated with Chloroform Extract (CE+PTZ), Group 5-Epileptic rats pretreated with Ethyl acetate Extract (EAE+PTZ), Group 6-Epileptic rats pretreated with n-Butanol Extract (nBE+PTZ), Group 7-Epileptic rats pretreated with Aqueous Extract (AE+PTZ) and Group 8-Epileptic rats pretreated with

Diazepam (DP+PTZ). Each group consisted of 6 rats and used for studying the effects of different fractions/extracts of plant, *Centella asiatica*.

Isolation of Tissues

After stipulated duration, the animals were sacrificed by cervical dislocation and different brain regions such as Cerebral Cortex (CC), Cerebellum (CB), Pons Medulla (PM) and Hippocampus (HC)) were immediately isolated, frozen in liquid nitrogen and were stored at -80^0C until analysis.

Biochemical Analysis

The total, soluble and structural protein content was estimated by the method of Lowry et al.(10)

Statistical Analysis

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

Results

Proteins

Total and Soluble proteins were decreased with non-significant changes in structural proteins in different regions of rat brain during PTZ-induced epilepsy. An increase in the levels of different protein fractions were recorded during treatment with different CA extracts. During PTZ-induced epilepsy Cerebral cortex (CC) recorded highest depletion in total protein content (-40.34) followed by Cerebellum (CB) (-24.33), Pons medulla (PM) (-20.35) and Hippocampus (HC) (-11.65). Whereas the total protein content was increased in all the brain regions of epileptic rats pre-treated with different extracts of CA and diazepam (Table 1.1).

Table 1.1: Changes in Total protein content in different regions of rat brain during PTZ- induced epilepsy and on pre-treatment with different extracts of *Centella asiatica.*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+DP
CC	79.205	47.235*	99.222	111.099*	96.434	109.751*	107.675*	117.083*
	±1.775	±2.935	±11.705	±21.250	±3.628	±10.016	±5.968	±2.137
		(-40.36)	(25.27)	(40.26)	(21.75)	(38.56)	(35.94)	(47.82)
CB	84.850	64.206*	87.428	107.675*	116.712*	93.850*	115.623*	113.364*
	±1.775	±4.224	±4.899	±2.252	±2.249	±4.352	±2.874	±2.001
		(-24.33)	(3.03)	(26.90)	(37.55)	(10.60)	(36.26)	(33.60)
HC	91.874	81.162*	97.668*	115.66*	106.685*	101.874*	119.448*	122.981*
	+1.775	+2.754	+2.250	+1.038	+1.457	+2.354	+3.233	+2.752
		(-11.65)	(6.30)	(25.89)	(16.12)	(10.88)	(30.01)	(33.85)
PM	67.840	54.034*	76.947*	81.328*	89.516*	81.781*	91.478*	91.985*
	±1.775	±1.405	±2.048	±1.972	±4.736	±0.773	±3.885	±2.849
		(-20.35)	(13.42)	(19.88)	(31.95)	(20.55)	(34.84)	(35.59)

All the values are mean, ±SE of six individual observations.
Values in '( )'parentheses are % change over saline control
*Values are significant at P < 0.05 in Scheffe test.
(Values are expressed in mg/g wet wt of the tissue)

During PTZ-induced epilepsy, Pons medulla (PM) recorded highest depletion in soluble protein content (-48.88) followed by Cerebral cortex (CC) (-37), Hippo campus (HC) (-20.35) and Cerebellum (CB) (-16.64). Whereas, the soluble protein content was increased in all the brain regions in the epileptic animals pre-treated with different extracts of CA and diazepam (Table 1.2).

Table 1.2: Changes in Soluble protein content in different regions of rat brain during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+DP
CC	46.705	29.424*	52.118*	54.506*	57.326*	49.873	60.352*	60.001*
	±1.383	±0.965	±1.296	±1.567	±1.104	±1.455	±3.275	±1.478
		(-37.00)	(11.59)	(16.70)	(22.74)	(6.78)	(29.22)	(28.46)
CB	51.935	43.292*	54.507	57.903*	63.656*	68.466*	69.112*	73.987*
	±1.175	±1.890	±3.244	±0.808	±0.834	±1.178	±1.283	±1.502
		(-16.64)	(4.95)	(11.49)	(22.56)	(31.83)	(33.07)	(42.46)
HC	52.406	43.207*	54.901	57.552*	62.863*	67.827*	69.781*	71.160*
	±1.821	±1.648	±1.754	±0.152	±1.244	±2.092	±1.313	±1.610
		(-17.55)	(4.76)	(9.82)	(19.95)	(29.42)	(33.15)	(35.78)
PM	35.351	18.070*	40.764*	43.152*	45.972*	38.519	48.598*	48.647*
	±1.383	±0.965	±1.296	±1.567	±1.104	±1.455	±3.275	±1.478
		(-48.88)	(15.31)	(22.06)	(30.04)	(8.96)	(38.60)	(37.61)

All the values are mean, ±SE of six individual observations.
Values in '( )'parentheses are % change over saline control
*Values are significant at P < 0.05 in Scheffe test.
(Values are expressed in mg/g wet wt of the tissue)

During the PTZ-induced epilepsy Pons medulla (PM) recorded highest depletion in structural protein content (-19.87) followed by Cerebral cortex (CC) (-18.13), Hippo campus (HC) (-17.74) and Cerebellum (CB) (-13.85). Whereas, pre-treatment with CA extracts and diazepam caused an increase in the structural protein content in all the brain regions (Table 1.3).

Table 1.3: Changes in Structural protein content in different regions of rat brain during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*.

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+DP
CC	48.835	39.978*	53.213	58.069*	58.288*	54.892	56.608	55.506
	+1.381	+0.204	+0.248	+1.746	+2.297	+1.297	+2.456	+0.765
		(-18.13)	(8.96)	(18.90)	(19.35)	(12.40)	(15.91)	(13.66)
CB	56.447	48.627	59.883	63.323	62.762	65.637	61.014	63.851
	+0.527	+0.555	+0.389	+1.285	+1.208	+1.228	+1.584	+1.406
		(-13.85)	(6.08)	(12.18)	(11.18)	(16.28)	(8.09)	(13.11)
HC	65.684	54.030	67.806	70.678	72.230	75.123	70.533	77.951*
	±1.715	±1.121	±1.787	±1.812	±1.433	±0.738	±0.942	±1.312
		(-17.74)	(3.23)	(7.60)	(9.96)	(14.37)	(7.38)	(18.67)
PM	49.399	39.579*	51.920	56.087	57.447	55.764	57.139	55.199
	±2.683	±2.134	±0.523	±0.512	±1.032	±1.578	±1.103	±0.670
		(-19.87)	(5.10)	(13.53)	(16.29)	(12.88)	(15.66)	(11.74)

All the values are mean, ±SE of six individual observations.
Values in '( )'parentheses are % change over saline control
*Values are significant at P < 0.05 in Scheffe test.
(Values are expressed in mg/g wet wt of the tissue)

Discussion

In the present investigation the total and soluble proteins were decreased significantly in all the brain regions during PTZ-induced epilepsy. Among all the protein fractions, the soluble protein content was found to be decreased more than that of insoluble protein.

Pretreatment with different extracts of CA caused a marked elevation of total and soluble proteins with moderate elevation in insoluble or structural protein content. The present findings are in coherence with the observation of Oliveira *et al.* (11) and Fighera *et al.* (12) who reported the reversal of PTZ-induced alterations by *Centella asiatica*. Keeping in view of neuroprotective role of CA, it is expected that different extracts of CA might have prevented the cellular damage by inhibiting the proteolytic activity as evidenced by elevation in protein content. it is presumed that the bioactive factors present in different extracts of CA possibly modulate the different pathways related to protein metabolism thus reducing

the endogenous production and accumulation of glutamate as one of the facets of antiepileptic treatment.

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Effect of Neuro Protective Drugs on Brain Cancer

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Abstract

Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected.

Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign.

More dangerous, or malignant, tumors form when two things occur:

1. a cancerous cell manages to move throughout the body using the blood or lymph systems, destroying healthy tissue in a process called invasion
2. That cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis.

When a tumor successfully spreads to other parts of the body and grows, invading and destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis, and the result is a serious condition that is very difficult to treat.

Introduction

Cancer, also called malignancy, is an abnormal growth of cells. There are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. Symptoms vary depending on the type. Cancer treatment may include chemotherapy, radiation, and/or surgery.

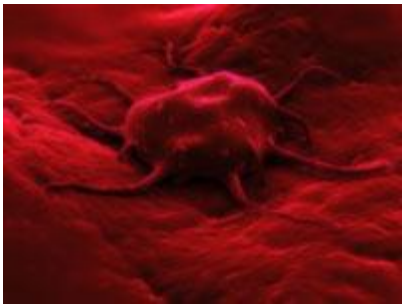
Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected.

Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems, and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign 1-10.

More dangerous, or malignant, tumors form when two things occur:

1. a cancerous cell manages to move throughout the body using the blood or lymph systems, destroying healthy tissue in a process called invasion
2. that cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis.

When a tumor successfully spreads to other parts of the body and grows, invading and destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis, and the result is a serious condition that is very difficult to treat.



Certain molecular interactions between cells and the scaffolding that holds them in place (extracellular matrix) cause them to become unstuck at the original tumor site, they become dislodged, move on and then reattach themselves at a new site.

The researchers say this discovery is important because cancer mortality is mainly due to metastatic tumors, those that grow from cells that have traveled from their original site to another part of the body. Only 10% of cancer deaths are caused by the primary tumors.

The scientists, from the Massachusetts Institute of Technology, say that finding a way to stop cancer cells from sticking to new sites could interfere with metastatic disease, and halt the growth of secondary tumors. In 2007, cancer claimed the lives of about 7.6 million people in the world. Physicians and researchers who specialize in the study, diagnosis, treatment, and prevention of cancer are called oncologists.

Malignant cells are more agile than non-malignant ones. Malignant cells can pass more easily through smaller gaps, as well as applying a much greater force on their environment compared to other cells.

Professor Robert Austin and team created a new catalogue of the physical and chemical features of cancerous cells with over 100 scientists from 20 different centers across the United States.

The authors believe their catalogue will help oncologists detect cancerous cells in patients early on, thus preventing the spread of the disease to other parts of the body.

Prof. Austin said "By bringing together different types of experimental expertise to systematically compare metastatic and non-metastatic cells, we have advanced our knowledge of how metastasis occurs."

Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division, and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not experience programmatic death and instead continue to grow and divide. This leads to a mass of abnormal cells that grows out of control.

Cancer Prevention

According to the American Cancer Society, there is strong evidence that an individual's risk of developing cancer can be substantially reduced by healthy behavior:

- don't use tobacco
- get sufficient physical activity
- eat healthy foods in moderation
- participate in cancer screenings according to recommended guidelines

The National Cancer Institute estimates that in 2014 about 1,665,540 people will be diagnosed with cancer, and of that number 585,720 people are estimated to die from cancer related causes. 224,210 of those diagnosed will be related to tobacco use alone

Anyone can get cancer

Anyone can get cancer. One of the biggest factors that can make a person more likely to get cancer is age: 3 out of 4 cancers are found in people aged 55 or older. But there are many other factors that affect cancer risk and some of them can be changed. It is only natural that people are looking for more ways to prevent cancer.

Sometimes cancer can be prevented. Looking at the whole country, it is quite possible that more than half of cancer deaths could be prevented -- if no one used tobacco and if everyone took steps to improve their health. Of course, that is a big "if."

But is there a way to guarantee that you or your loved ones won't get cancer? So far, nothing has been found that is proven to prevent every case of cancer. Right now we know there are ways to prevent many cases of cancer in large groups of people. And there are things you can do that might help reduce your personal chance of getting cancer.

Lifestyle Choices May Prevent Cancer

For people who do not use tobacco, the most important cancer risk factors that can be changed are body weight, diet, and physical activity. One-third of all cancer deaths in the United States each year are linked to diet and physical activity, including being overweight or obese, while another third is caused by tobacco products.

Some diet and exercise recommendations developed by the American Cancer Society's Nutrition and Physical Activity Guidelines Advisory Committee and approved by the American Cancer Society National Board of Directors are:

- Achieve and maintain a healthy weight throughout life
- Be physically active
  - **Adults:**

    Get at least 150 minutes of moderate intensity or 75 minutes of vigorous intensity activity each day (or a combination of these), preferably spread throughout the week.
  - **Children and teens:**

    Get at least 1 hour of moderate or vigorous intensity activity each day, with at least 2.5 hours of moderate intensity aerobic activity each week.
- Limit sedentary behavior such as sitting, lying down, watching TV, and other forms of screen-based entertainment.
- Doing some physical activity above usual activities, no matter what one's level of activity, can have many health benefits.
- Eat a healthy diet, with an emphasis on plant foods
- Get Routine Medical Care
  - Mammogram

    Women age 40 and older should have a screening mammogram every year and should continue to do so for as long as they are in good health, or up to age 70 if there are no other risk factors.
  - Colonoscopy

    Beginning at age 50, both men and women at average risk for developing colorectal cancer should use one of the screening tests:
    - Flexible sigmoidoscopy every 5 years

- Colonoscopy every 10 years
- Double-contrast barium enema every 5 years
- CT colonography (virtual colonoscopy) every 5 years
- Pap smear

All women should begin cervical cancer testing (screening) at age 21. Women aged 21 to 29, should have a Pap test every 3 years. HPV testing should not be used for screening in this age group (although it may be used as a part of follow-up for an abnormal Pap test). Beginning at age 30, the preferred way to screen is with a Pap test combined with an HPV test every 5 years. This is called co-testing and should continue until age 65. Another reasonable option for women 30 to 65 is to get tested every 3 years with just the Pap test.

- Other Health Tips
  - Individuals should check their skin for moles that are new, large, or irregular; contain more than one color; or change color.
  - An open dialogue with a family doctor supports important preventive measures on a timely basis, and if any tests suggest possible cancer, the result can be further explored quickly.
  - Women in their 20s and 30s should have a clinical breast exam (CBE) as part of a periodic (regular) health exam by a health professional, at least every 3 years. After age 40, women should have a breast exam by a health professional every year.

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Docking and Molecular Modelling of the Target - Penicillin Binding Protein -1A of *Haemophilus influenzae*

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Abstract

Penicillin binding protein-1A (PBP-1A; IPR011816) plays a pivotal role in the biogenesis of cell-wall and biosynthesis of peptidoglycan in bacteria. It is considered to be a novel target for pneumonia. The present study is to deduce the structure of the PBP-1A using ICM Molsoft and to validate the same with Ramachandran plot. These bioinformatics tools have been used to identify a lead molecule against PBP-1A of *Haemophilus influenza*. The proposed model structure is further explored for *in silico* docking studies with suitable inhibitors. The docking scores indicated that the ampicillin could be a better inhibitor among the seven antibiotics chosen in the present investigation.

Key words: *Haemophilus influenzae* strain 86-028NP, PBP protein, ICM Molsoft, Argus lab 4.0.1, Gold.

Introduction

Pneumonia is an inflammation of lungs, usually caused by a pathogen. Three common causative groups of pathogens that cause pneumonia are bacteria, viruses and fungi (1). The people at risk are older than 65 or younger than 2 years of age, or those with impaired immunity. Some of the gram-negative bacteria that cause pneumonia include *Haemophilus influenzae* (HI), *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Moraxella catarrhalis*.

HI is a non-motile gram-negative bacterium belonging to the family *Pasteurellaceae*. Pneumonia caused by HI seems to occur exclusively among humans. HI was first isolated by Pfeiffer during the influenza pandemic of 1890. This pathogen is present in the nasopharynx of approximately 75 percent of healthy children and adults. It is rarely encountered in the oral cavity and has not been detected in any other animal species (2). HI was the first pathogenic organism to have its entire genome sequenced during the year 1995 (3).

Bacterial cell wall and PBPs are the targets for drugs of selective toxicity because the related metabolic pathways and enzymes are unique to prokaryotes (4). Whereas, mitogen-activated protein kinase and trypanothione reductase are considered as targets in a eukaryotic protozoan parasite viz., *Leishmania infantum* (5, 6). However, in bacterial system, PBPs have been shown to catalyse a variety of reactions involved in the process of synthesizing cross-linked peptidoglycan from lipid intermediates and mediate the removal of D-alanine from the precursor of peptidoglycan (7). PBP-1A in HI strain 86-028np is coded by mrcA gene. This possesses two domains *viz.,* penicillin-insensitive transglycosylase EC=2.4.2.- and penicillin-

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sensitive transpeptidase EC=3.4.-.- Its location is in the inner cell membrane as type II membrane protein and plays a key role in the biogenesis of cell wall and peptidoglycan (4). The present article aims at the screening of a few chosen antibiotics against PBP-1A with the intention of identifying a lead molecule.

Materials and Methods

The amino acid sequence of PBP-1A of HI strain 86-028NP was extracted from SWISS Prot database with ID: Q4QNA5. Later, submitted to NCBI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST), searched against protein data bank (http://www.rcsb.org/pdb/), extracted suitable templates for the query sequence and found one of the best templates *viz*., peptidoglycan glycosyltransferase (PDB ID:2OQOa) with 52 % identities (8). The length of PBP-1A was found to be 864 amino acids and the source of the template is *Aquifex aeolicus* (http://www.rcsb.org/pdb/). The primary properties of protein sequence was analysed by using Bioedit and the protein secondary structure prediction was carried out using SOPMA (Self Optimized Prediction Method). Both the sequences were aligned using ICM Molsoft and the same was employed in the construction of three dimensional structure of PBP-1A. The alignment of the sequence to be modeled was done with known related structure in PDB format. ICM Molsoft further generated a model structure by minimizing the side chains. To correct the geometric inaccuracies the theoretical model was subjected to Procheck. The program, Procheck concentrates on the parameters such as bond length, bond angle, main chain and side chains parameters, residue properties, rms distance from planarity and distorted geometry plots. The Protein ligand interaction was performed using Argus lab engine with flexible docking (9). Docking analysis was carried out using on-line GOLD programme.

Results

The *in silico* tools as indicated in the previous section, *viz*., homology modelling, active site analysis and docking were employed in the present study for screening the chosen drugs to bind to the active site of PBP-1A.

The homology modelling of PBP-1A from HI was obtained from the protein data bank with ICM Molsoft software. The final model was evaluated using Procheck. Further validation analysis employing Ramachandran plot, revealed that the PBP-1A model showed 92.0% of residues lie in the most favoured regions and the remaining 6.3% in the additional allowed regions. Thus, 98.3% residues are in the allowed portions of the plot. Hence, PBP-1A is a potential target to exploit for drug designing, energy minimization and molecular dynamic simulations by means of refining loops and rotomers, checking bonds and adding hydrogen atoms. The refined model structure thus obtained after energy minimization and molecular

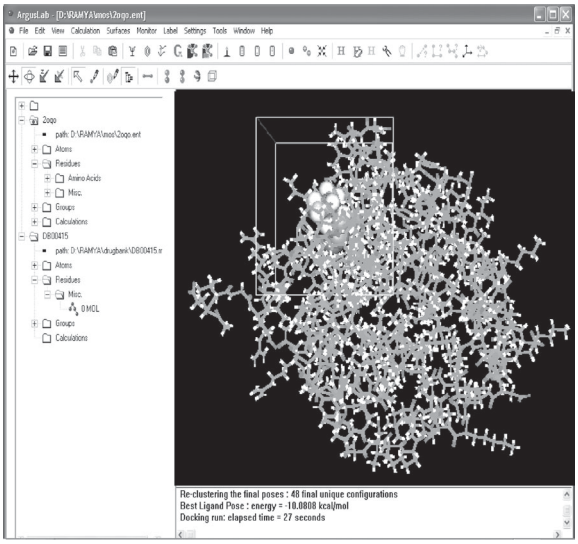

Fig. 1. Docking in Argus Lab. Docking studies with Argus lab engine showed that the active site residue is at 159 LEU in PBP-1A. This made a strong hydrogen bond interaction with ampicillin and yielded least energy -10.2333.

dynamic simulations was subjected for docking studies.

Docking studies with Argus lab engine gave an insight into the binding modes of the various inhibitors. Out of the seven drugs that were selected in the present study for screening, it was found that the active site residue *viz*., 159 LEU of PBP-1A forms a strong hydrogen bond interaction with ampicillin and yielded least energy -10.2333 (Table-1; Fig.1). GOLD program uses a genetic algorithm to explore the full range of ligand conformational and the rotational flexibilities of selected receptor hydrogen atoms. The mechanism for ligand placement is based on fitting points. The GOLD score fitness was found to be -16.596 (Fig.2). Amino acid primary structure analysis was done

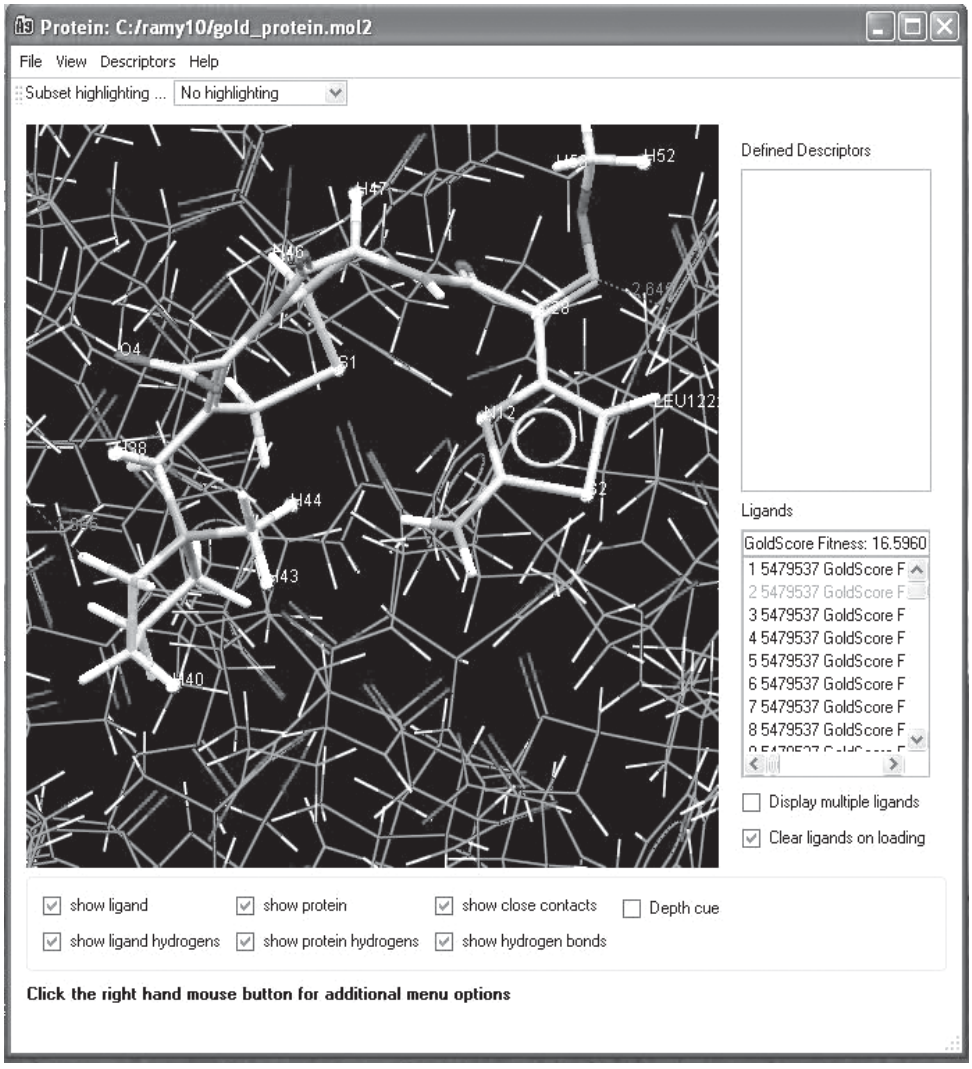

Fig. 2. Protein-Ligand interactions in GOLD**.** The mechanism for ligand placement is based on fitting points. The GOLD score fitness was found to be -16.569.

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by using Bioedit (Fig.3). Pre-ADME properties envisaged that among the chosen ligand molecules, ampicillin fulfilled the requirements for the drug solubility, distribution and elimination (Table- 2).

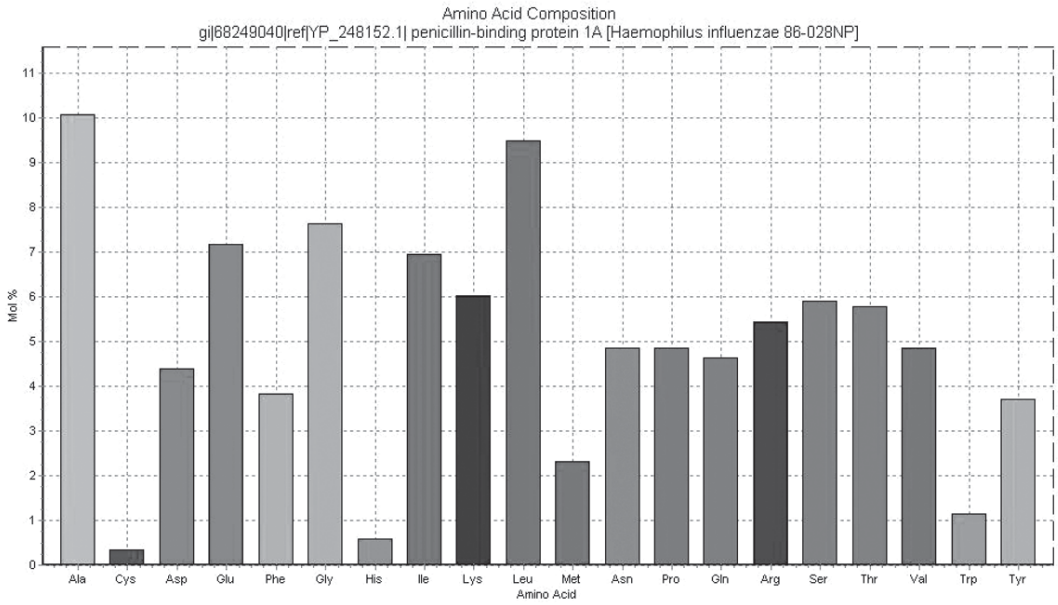

Fig. 3. Amino Acid Composition by Bioedit**.** Protein: gi|68249040|ref|YP_248152.1| penicillin-binding protein 1A [Haemophilus influenzae 86-028NP]. Length = 864 amino acids. Molecular Weight = 95630.89 Daltons

Discussion

The proteomic analysis of PBP-1A revealed its primary properties and secondary structural information and they are essential to understand its structure, function and nature of interaction. The stereochemistry evaluation of predicted 3D structure of the drug target suggested that the proposed model is of a good quality. The most common goal of protein-drug (small molecule) docking relates to drug design (5, 10). Moreover, the interaction between the target and the ligand proposed in this study (Fig.2) is useful for understanding the potential mechanism of enzyme and the substrate binding (11). Hydrogen bonds play important role for the structure and function of biological molecules, especially for the enzyme catalysis (12). In the present study, it was found that the active site residue of PBP-1A *viz.*, LEU at the position 159 yielded least energy viz., -10.233 (Table-1), and formed a strong hydrogen bond interaction with ampicillin upon comparison with the remaining six ligands. GOLD, binding site–dependent software, showed energy minimization and re-ranking of the top N poses. The GOLD score fitness was found to be -16.5690.

PBPs have been shown to catalyse a number of reactions involved in the process of synthesising cross-linked peptidoglycan from lipid intermediates and mediate the removal of D-alanine from the precursor of peptidoglycan (13). PBPs are reported to have a penicillin-insensitive transglycosylase N-terminal domain involved in the formation of linear glycan strands and a penicillin-sensitive transpeptidase C-terminal domain involved in cross-linking of the peptide subunits and the serine at the active site

Table 1. Docking score obtained through GOLD programme

Aminoacid	Residue No	*Ampicillin*	*Meropenem*	*Calvulanic acid*	*Ceftriaxone*	*Cefepime*	*Amoxicillin*	*Cefotaxime*
LEU	159	-10.2333	-7.3479	-5.5314	-7.6496	-6.4803	-9.3322	-7.4691
ASN	162	-8.8721	-7.9781	-5.4862	-7.5311	-7.7525	-9.1216	-6.8802
LYS	163	-9.55741	-7.3903	-5.5597	-7.7845	-7.4571	-8.6496	
TYR	171	-8.93631		-5.4171	-6.8628		-8.7681	
PHE	126	-9.78251	-8.0307	-5.5429	-7.4848	-6.8116	-8.9479	
PHE	127	-8.8829	-7.9319	-5.1586	-6.7787	-6.9444	-9.405	-8.0038
LEU	128	-8.6724	-7.5348	-5.3248	-7.1164	-6.0453	-7.8047	-7.681
THR	129	-8.2623	-7.3276	-5.3279	-7.4413		-7.3466	
LYS	132	-7.0311	-6.6989	-5.2153	-6.161	-6.411	-7.1245	
ARG	136	-9.0483	-7.0893	-5.2048	-6.3208	-6.2259	-8.4738	
GLU	140	-9.6544	-7.9182	-5.4616	-6.7677	-7.3372	-7.4998	
GLN	61	-8.3597	-7.4121	-5.4106	-7.6063	-7.0948	-8.3251	
ARG	62	-9.3446	-7.1034	-5.4239	-7.3805	-7.1783	-8.9781	
ARG	63	-7.8562	-6.2306	-5.0896	-6.3539	-6.0002	-6.702	
ILE	64	-7.2376	-6.7924	-5.0286	-7.0368	-5.9156	-7.1915	-6.0871
LEU	122	-9.9338	-7.1636	-5.2466	-6.8293	-7.7662	-8.2897	
ARG	124	-9.1766	-7.3872	-5.6417	-7.3239	-7.849	-9.3103	-7.3105
ASN	125	-9.3158	-7.7364	-5.7181	-7.3453	-7.9354	-8.9336	

Table 2. Pre-ADME properties derived through Accelrys discovery studio 2.1.

Ligand Molecule	Absorption level	solubility	Solubility level	Hepato-toxicity	CYP 2D6	CYP 2D6 Probability	Ppb level	A log P98	PSA-2D
Ampicillin	0	-2.502	3	0	0	0.495	0	0.182	115.42
Meropenem	1	-0.929	4	0	0	0.386	0	-0.932	113.04
Ceftriaxone	3	-4.547	2	1	0	0.267	0	-0.576	209.02
Cefepime	2	-2.194	3	0	0	0.396	0	-1.125	146.93
Amoxicillin	1	-2.523	3	0	0	0.445	0	-0.06	136.23
Cefotaxime	3	-3.124	3	1	0	0.425	0	-0.53	173.16

is conserved in all members of the PBP family (14, 15).

Both bacterial and protozoan parasites are wide spread around the world and more so in the tropical region causing an impediment in the health of humans and livestock. In the recent years, there is an upsurge in the biotechnological and *in silico* approaches to identify the possible pathogen-directed targets (5, 6). Interestingly, the biochemical mechanisms such as cell membrane receptors, metabolic pathways, cellular protein synthetic machinery and growth and

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differentiation of cellular systems of parasites which are nonetheless unique for their survival and hence they are considered as avenues for drug/ligand targets. Furthermore, the software tools for homology modelling, simulations and structure-based virtual screening and on-line databases are in turn enhancing the rapid developments in building the repertoire of the receptor and ligand libraries. Therefore, in the present study, the chosen inhibitors *viz.*, ampicillin, amoxicillin, ceftriaxone, cefotaxime, cefepime, meropenem, and calvulanic acid were made to bind in the active site of PBP-1A. Of which, ampicillin has a good docking score (Table-1) and thus, ampicillin is considered as the best inhibitor for PBP-1A of HI (Francis et al., 2003). The Pre-ADME properties of ampicillin (Table-2) are coinciding with the results of Sarah et al., (16).

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Effect of *Centella asiatica* (Gotu kola) on the antioxidant enzyme activities and glutathione levels in different regions of rat brain during pentylenetetrazole-induced epilepsy

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Abstract

Epilepsy is a convulsive episode and is the most frequent neurodegenerative disorder affecting about 50 million people world-wide. *Centella asiatica* (CA) has been used as a medicine in the Ayurvedic tradition of India for thousands of years. The purpose of the present study is to determine the antiepileptic activity from a medicinal plant CA. The fractionated extracts are found to be active against the pentylenetetrazole (PTZ) induced epileptic rats. The enzymic and non-enzymic antioxidants i.e. reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) superoxide dismutase (SOD), and catalase (CAT) were evaluated in control and experimental groups. The results showed that the CA extracts significantly suppress the (PTZ)-induced epileptic seizures in rats.

Key-Words: *Centella asiatica*, epilepsy, pentylenetetrazole, antioxidants

Introduction

Epilepsy is a common and heterogenous neurological disorder characterized by recurrent spontaneous seizures. It is well known that the epileptic seizures result from excessive discharge in a population of hyperexcitable neurons[1]. Despite the multiple molecular mechanisms have been proposed in generating and spreading epileptic discharges, it has been well established that impaired GABA ergic activity and/ or exaggerated glutamatergic neurotransmission primarily contribute to the various types of epilepsies[2]. Excitotoxicity, the process of neuronal death evolved by glutamate receptor activation, has been hypothesized in both acute and chronic degenerative disorders including epilepsy. It has also been postulated that massive influx of glutamate secreted during epilepsy, activates various free radical generating systems resulting in marked production of oxygen free radicals[3]. Considering the multifactorial neurochemical and neurophysiological malfunctions consequent to epileptic seizures, a few antiepileptic drugs (AEDs) are designed, to mitigate the debilitating aspects of epilepsy[4].

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AED regimens employed in ameliorating seizures generally met with partial success and suffer from substantial problems such as pharmacoresistance and neurotoxic effects[5]. During the past few years, considerable progress has been made towards identifying active factors from indigenous medicinal plants for different human ailments including neurodegenerative disorders such as Alzheimer's disease, parkinsonism etc. Keeping in view of this the present investigation is aimed at studying the modulations of antioxidant metabolism during PTZ-induced epilepsy and antiepileptic treatment with CA.

Material and Methods

Experimental animals

The rats were procured from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±2^{0}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were maintained according to the Animal Ethics Committee approval and welfare bearing the CPCSEA 438/01/a/cpcsea/dt:17.07.2006 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2006.

Epilepsy induced drug

Pentylenetetrazole (PTZ), a convulsing drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of Plant Extracts

The active principles of the leaves of plant were extracted into different solvents, methanol, water, n-hexane, chloroform, ethyl acetate and n-butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants[6, 7]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for our studies.

Administration of tested substance

Each fraction of CA extract (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ at the dose of 60mg/kg body weight[8]. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA[7]. The volume of administration was kept at 1ml to the animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/kg bw i.p.) for one week to the experimental animals (Reference control).

Experimental setup

The rats were divided into 8 groups, each group consisted of 6 rats, Group1-Normal saline treated control rats (SC), Group 2-Rats treated with PTZ (Epileptic group), Group 3-Epileptic rats pretreated with n-Hexane extract (nHE+PTZ), Group 4-Epileptic rats pretreated with Chloroform extract (CE+PTZ), Group 5-Epileptic rats pretreated with Ethyl acetate extract (EAE+PTZ), Group 6-Epileptic rats pretreated with n-Butanol extract (nBE+PTZ), Group 7-Epileptic rats pretreated with Aqueous extract (AE+PTZ) and Group 8-Epileptic rats pretreated with Diazepam (DP+PTZ).

Isolation of Tissues

After stipulated duration, the animals were sacrificed by cervical dislocation and different brain regions such as Cerebral Cortex (CC), Cerebellum (CB), Pons, Medulla (PM) and Hippocampus (HC) were immediately isolated, frozen in liquid nitrogen and were stored at -80^0C until analysis.

Biochemical analysis

Glutathione content was determined according to the method of Theodorus et al[9] (1981). Se-Dependant Glutathione Peroxidase (Se-GSH-Px) was determined by a modified version of Flohe and Gunzler[10] (1984). Glutathione reductase activity was determined by a slightly modified method of Carlberg and Manervik[11] (1985). Glutathione-S- transferase activity was measured with its conventional substrate, 1-chloro 2, 4-Dinitro Benzene (CDNB) at 340 nm as per the method of Habig et al[12] (1974). Superoxide dismutase activity was determined according to the method of Misra and Fridovich[13] (1972). Catalase activity was measured by a slightly modified version of Aebi[14] (1984).

Statistical analysis

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software (11.5 version) for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

Results and Discussion

GSH content

When compared with saline control, PTZ-induced animals had significantly decreased the GSH content in all the brain regions, with highest decrease noted in the cerebral cortex (CC). Pretreatment with CA extracts i.e. n-HE, CE, EAE, n-BE, AE and diazepam (Reference control) were resulted in significantly increased GSH content in all the brain regions (Table 1).

Effect of CA extracts on Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione s-transferase (GST), Superoxide dismutase (SOD) and Catalase (CAT) in different regions of the brain tissue of control and experimental groups

The activities of enzymatic antioxidants in different regions of the brain tissue of control and experimental rats are shown in Tables 2-6. The GPx, GR, GST, SOD

and CAT were significantly reduced in all the brain regions, with the highest decrease were noted in the hippocampus (HC) during PTZ-induced epilepsy (Group II). In the CA extracts treated animals (Groups III -VII) and the diazepam (DP) treated animals (Group VIII); the activities of GPx, GR, GST, SOD and CAT were significantly increased when compared to PTZ bearing animals (Group II).

Oxidants are formed as a normal product of aerobic metabolism and antioxidant defense involves several strategies both enzymatic and non-enzymatic[15]. Chronic oxidative stress has numerous pathological consequences including cancer, arthritis and neurodenerative diseases[16]. Glutathione and associated metabolism is a major mechanism for cellular protection against agents which generate oxidative stress. Glutathione participates in detoxification at several different levels and may scavenge free radicals, reduce peroxides or be conjugated with electrophilic compounds. Thus glutathione provides the cell with multiple defences not only against Reactive oxygen species (ROS) but also against their toxic products.

It is well established that reduced glutathione (GSH) protects against chemically induced injury[17, 18]. The GSH content and the activity levels of Glutathione peroxidase (GPx), Glutathione reductase (GR) and Glutathione s-transferase (GST) were decreased in different regions of brain during PTZ-induced epilepsy. The reduced glutathione metabolism is in agreement with the earlier reports of different models of epilepsy[19]. De Freitas et al[20] have reported similar decrease in GSH levels in rat hippocampus during pilocarpine-induced epilepsy. A wide spread impairment of glutathione system was reported by Mullar et al[21] who have demonstrated decreased glutathione peroxidase in plasma and brain of PTZ-induced epileptic rats. Similar reduction in Glutathione peroxidase activity was also reported in rats treated with PTZ. The depleted levels of Glutathione and subsequent occurrence of oxidative stress have also been demonstrated in *in vitro* glutamate toxicity in neuronal cell line[22]. Reduced microsomal glutathione levels in PTZ-induced epilepsy[23], reduced antioxidant enzyme activities and glutathione levels[24], a generalized diminished antioxidant activity in PTZ-induced epilepsy[25], significant decrease in GSH, Glutathione disulfide (GSSG) in the cerebral cortex of mouse after PTZ-induced seizure have also been reported which are in agreement with the present findings.

The reduced glutathione levels suggest an impairment of cell defence against toxic insult caused due to PTZ and thus result in increased levels of superoxide and hydroxyl radicals. The reduced SOD activity observed in the present study might have lowered the chance of converting oxidized form of glutathione to reduced form of GSH. On par with the diazepam treatment, pre-treatment with different extracts of CA caused reversal of changes in the glutathione metabolism that was impaired during PTZ-induced seizures and exerted their antiepileptic effect by modulating the antioxidant metabolism in different regions of brain. It has also been reported that supplementation of CA significantly protects against arsenic-induced oxidative stress by restoring the blood GSH levels[26]. Gupta et al[27] have also reported that the administration of CA extracts elicited a marked improvement in the learning deficit induced by PTZ kindling as evidenced by decreased seizure score and restoration of oxidative stress parameters. Lee et al[28] have reported that the asiatic acid, the major constituent of CA, exerted significant neuroprotective effects on cultured cortical cells by their potentiation of the cellular oxidative defence mechanism. Asiaticoside, another component of CA enhanced induction of antioxidant levels such as SOD, CAT and GPx at an initial stage of wound healing and thus may be regarded as important contributing factors in the healing properties of this substance. The present findings coupled with the earlier reports suggest that the extracts of CA improve the antioxidant ability and offers neuroprotective effect against PTZ-induced epilepsy.

The decreased SOD activity in different regions of brain during PTZ-induced epilepsy suggests failure of dismutation of superoxide anions generated by xanthine oxidase activity. The significant inhibition of SOD activity coupled with increased lipid peroxidation and purine catabolism as observed in the present study suggest the occurrence of oxidative damage during PTZ-induced epilepsy. Similar inhibition of SOD has also been reported in different models of seizures. Wilhelm et al[29] have reported inhibition of Na+, K+ -ATP ases and SOD activities in brains of rats on pilocarpine model of seizures. Obay et al[24] have demonstrated increased lipid peroxidation and decline in antioxidant enzyme activities during PTZ-induced epilepsy. It is speculated that the copious production of glutamate receptors (NMDA and non-NMDA) might have been responsible for spurt in the ROS production and inhibition of SOD activity[30].

In rats treated with different extracts of CA, the SOD activity was enhanced in all the brain regions which suggest the possible involvement of SOD is quenching superoxide anion radical. Consistent with these observations, Shukla et al[31] have reported that Asiaticoside, the anticonvulsant product derived from

Centella asiatica enhanced enzymatic and non-enzymatic antioxidants such as SOD, Catalase, GPx , Vitamin-E and ascorbic acid in induced wound-healing. Gupta and Flora[26], in another study, have concluded that supplementation of *Centella asiatica* significantly protected arsenic-induced oxidative stress. The findings of the present study coupled with the above reports, it can be speculated that the bioactive factors present in different extracts of CA may modulate the pro oxidant / antioxidant balance and pre-treatment with these extracts has a beneficial role is mitigating the debilitating effects of induced epilepsy.

The catalase activity was induced in different regions of rat brain in PTZ-induced epileptic rats after pre-treatment with different extracts of CA. Similar induction of different antioxidant enzymes including catalase has been reported by Jayashree et al[32]. It has also been reported that Asiaticoside, a major constituent of CA, promoted wound-healing by reducing lipid peroxide levels in wounds while it increased enzymatic (SOD, CAT, GPx) and non-enzymatic (Vitamin-E and Ascorbic) antioxidant levels[33]. Significant increase in catalase activity has also been reported after oral treatment with the crude methanol extracts of CA in lymphoma-bearing mice[32]. Decreased lipid peroxidation and increased catalase activity have been recorded in erythrocytes of CA treated rats during H_2O_2-induced oxidative stress[34]. Improved catalase and SOD activity levels have also been demonstrated in monosodium glutamate treated rats after pre-treatment with chloroform, methanolic extract of CA[35].

From the present findings coupled with the earlier reports, it is obvious that the bioactive factors present in *Centella asiatica* stimulated the antioxidant enzymes such as GPx, GR, GST, SOD and CAT in order to neutralize the oxidant radicals and lipid peroxides generated during induced epilepsy. Further more, extracts of CA significantly attenuated the excitotoxic effects of glutamate a major abundant excitatory neurotransmitter that is produced in excess during epileptogenesis. The present data also suggest that the CA extracts modulate the pro oxidant / antioxidant balance and reduce the seizure manifestations and accompanying biochemical changes and highlights the possible role of antioxidant therapy as adjuncts to antiepileptic drugs for better seizure control.

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Table 1: Alterations in the content of Glutathione (GSH) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	2.023	0.829*	3.660*	3.346*	2.643*	3.306*	2.840*	3.548*
	±0.011	±0.007	±0.869	±1.554	±0.023	±0.017	±0.010	±0 .012
		(-59.01)	(80.91)	(65.39)	(30.63)	(63.43)	(40.40)	(75.39)
CB	5.071	3.457*	7.333*	7.639*	6.393	6.036	6.901*	6.486
	±0.037	±0.017	±0.025	±0.024	±0.020	±0.028	±0.036	±0 .023
		(-31.83)	(44.60)	(50.63)	(26.07)	(19.02)	(36.09)	(27.90)
HC	3.648	1.595*	4.536	5.710*	5.364*	6.420*	4.352	5.547*
	±0.017	±0.016	±0.012	±0.989	±0.016	±0.022	±0.016	±0 .022
		(-56.35)	(24.14)	(58.45)	(46.80)	(75.69)	(19.11)	(51.79)
PM	3.423	2.094*	6.048*	4.332	5.949*	4.902*	4.613*	5.154*
	±0.032	±0.018	±0.018	±0.021	±0.019	±0.023	±0.014	±0 .015
		(-38.83)	(76.69)	(26.56)	(73.80)	(43.19)	(34.76)	(50.57)

All the values are mean, ±SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as nano moles of GSH formed/ gm wet wt. of tissue
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 2: Alterations in the activity of Glutathione peroxidase (GPx) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	1.315	1.237	1.543*	1.510*	1.451*	1.403	1.363	1.553*
	±0.017	±0.024	±0.023	±0.016	±0.024	±0.011	±0.028	±0.017
		(-5.95)	(17.36)	(14.79)	(10.36)	(6.68)	(3.63)	(18.12)
CB	1.504	1.405	1.703*	1.664*	1.651	1.591	1.541	1.724*
	±0.033	±0.007	±0.015	±0.020	±0.009	±0.029	±0.007	±0.021
		(-6.59)	(13.26)	(10.65)	(9.80)	(5.78)	(2.48)	(14.65)
HC	1.252	1.141	1.440*	1.332	1.366	1.351	1.342	1.444*
	±0.021	±0.011	±0.012	±0.007	±0.022	±0.020	±0.029	±0.016
		(-8.84)	(15.03)	(6.40)	(9.07)	(7.89)	(7.18)	(15.36)
PM	1.043	0.942	1.294*	1.222*	1.188*	1.143	1.093	1.239*
	±0.023	±0.016	±0.030	±0.017	±0.019	±0.015	±0.015	±0.019
		(-9.67)	(24.01)	(17.12)	(13.88)	(9.56)	(4.78)	(18.77)

All the values are mean, ±SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of NADPH oxidized/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 3: Alterations in the activity of Glutathione reductase (GR) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	1.759	1.102*	2.296*	2.387*	2.349*	2.348*	2.312*	2.412*
	±0.013	±0.247	±0.205	±0.203	±0.107	±0.245	±0.241	±0.121
		(-37.34)	(30.53)	(35.71)	(33.54)	(33.50)	(31.44)	(37.10)
CB	5.357	3.715*	7.520*	6.659*	7.603*	6.852*	6.984*	7.175*
	±0.018	±0.146	±0.179	±0.210	±0.128	±0.230	±0.121	±0.245
		(-30.47)	(40.74)	(24.63)	(42.29)	(28.25)	(30.71)	(34.29)
HC	2.930	1.574*	4.132*	3.878*	4.222*	4.084*	4.076*	4.331*
	±0.020	±0.247	±0.145	±0.128	±0.249	±0.120	±0.246	±0.121
		(-46.29)	(41.02)	(32.36)	(44.10)	(39.39)	(39.13)	(47.82)
PM	3.695	2.366*	4.604*	4.743*	4.842*	4.804*	4.793*	4.885*
	±0.032	±0.124	±0.012	±0.069	±0.078	±0.089	±0.099	±0.240
		(-35.97)	(24.60)	(28.36)	(31.04)	(30.01)	(29.72)	(32.20)

All the values are mean, ±SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of NADPH oxidized/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 4: Alterations in the activity of Glutathione s-transferase (GST) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	4.320	2.865	5.718	5.592	5.935	5.285	5.642	6.150
	±0.018	±0.027	±0.028	±0.021	±0.077	±0.047	±0.038	±0 .016
		(-33.68)	(32.36)	(29.44)	(37.39)	(22.33)	(30.60)	(42.37)
CB	1.907	1.294	2.450	2.415	2.387	2.426	2.515	2.648
	±0.020	±0.102	±0.060	±0.086	±0.083	±0.120	±0.059	±0 .060
		(-32.13)	(28.45)	(26.62)	(25.15)	(27.21)	(31.86)	(38.81)
HC	0.731	0.461	0.988	1.009	1.065	1.021	1.088	1.066
	±0.011	±0.060	±0.056	±0.029	±0.058	±0.064	±0.027	±0 .075
		(-36.88)	(35.16)	(38.07)	(45.65)	(39.64)	(48.86)	(45.88)
PM	2.672	1.831	3.427	3.581	3.825	3.616	3.862	3.862
	±0.032	±0.037	±0.068	±0.056	±0.031	±0.047	±0.074	±0 .080
		(-31.44)	(28.28)	(34.05)	(43.16)	(35.36)	(44.57)	(44.55)

Values in ‘ ( ) ‘ parenthesis are % change over saline control
* Values All the values are mean, ± SE of six individual observations.
are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of thioether formed/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 5: Alterations in the activity of superoxide dismutase (SOD) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	13.242	8.621*	15.963*	15.915*	16.003*	16.161*	16.066*	16.038*
	±0.398	±0.175	±0.539	±0.554	±0.335	±0.385	±0.217	±0.261
		(-34.89)	(20.54)	(20.18)	(20.85)	(22.04)	(21.32)	(21.11)
CB	18.258	10.579*	23.27*	23.072*	23.523*	24.082*	23.744*	24.127*
	±0.418	±0.304	±0.611	±0.303	±0.304	±0.143	±0.435	±0.356
		(-42.05)	(27.47)	(26.36)	(28.83)	(31.89)	(30.04)	(32.14)
HC	30.967	15.946*	42.647*	40.076*	43.109*	43.176*	41.697*	41.548*
	±0.322	±0.323	±0.260	±0.524	±0.237	±0.317	±0.588	±0.250
		(-48.50)	(37.71)	(29.41)	(39.20)	(39.42)	(34.64)	(34.16)
PM	23.838	13.592*	31.668*	29.014*	31.406*	32.223*	29.251*	31.875*
	±0.497	±0.223	±0.212	±0.364	±0.112	±0.231	±0.137	±0.329
		(-42.98)	(32.84)	(21.71)	(31.74)	(35.17)	(22.70)	(33.71)

All the values are mean, ± SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of epinephrine oxidized/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 6: Alterations in the activity of Catalase (CAT) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	0.425	0.286*	0.527*	0.564*	0.562*	0.622*	0.615*	0.615*
	±0.104	±0.071	±0.125	±0.146	±0.131	±0.103	±0.149	±0.103
		(-39.21)	(35.60)	(30.37)	(29.99)	(32.63)	(39.71)	(34.52)
CB	0.528	0.369*	0.775*	0.797*	0.624*	0.723*	0.678*	0.601*
	±0.139	±0.108	±0.097	±0.111	±0.078	±0.055	±0.080	±0.124
		(-31.73)	(34.80)	(40.84)	(26.24)	(29.18)	(36.28)	(28.08)
HC	0.626	0.292*	0.771*	0.831*	0.857*	0.844*	0.846*	0.838*
	±0.059	±0.070	±0.031	±0.092	±0.085	±0.062	±0.152	±0.099
		(-46.28)	(44.34)	(51.05)	(47.04)	(45.98)	(49.30)	(52.04)
PM	0.716	0.375*	0.815*	0.847*	0.922*	0.831*	0.849*	0.974*
	±0.112	±0.124	±0.085	±0.080	±0.066	±0.046	±0.063	±0.152
		(-38.81)	(36.74)	(26.59)	(43.85)	(43.23)	(34.79)	(50.62)

All the values are mean, ± SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles H_2O_2 hydrolyzed/mg protein/min.
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

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ALTERATIONS IN THE MUSCLE CARBOHYDRATE METABOLISM DURING PENTYLENETETRAZOLE-INDUCED EPILEPSY: PROTECTIVE ROLE OF CENTELLA ASIATICA

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ABSTRACT

The aim of this study is to investigate the anticonvulsant effect of different extracts of *Centella asiatica* (CA) in functionally different muscles with reference to carbohydrate metabolism during pentylenetetrazole (PTZ) induced epilepsy and also during pre-treatment with different CA extracts. The rats were randomly divided into 7 groups having 6 in each group: (1) Control group received saline, (2) PTZ-induced epileptic group (60 mg/Kg, i.p.), (3) Epileptic group pretreated with n-hexane extract (n-HE), (4) Epileptic group pretreated with chloroform extract (CE), (5) Epileptic group pretreated with ethyl acetate extract (EAE), (6) Epileptic group pretreated with n-butanol extract (n-BE) and (7) Epileptic group pretreated with aqueous extract (AE). PTZ-induced epilepsy increased the glycogen, glucose and lactate contents and decreased the levels of total carbohydrates (TC) and pyruvate (PYR), Lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities in all the muscles (White vastus, Red vastus, Soleus and Gastrocnemius). Pre-treatment with different CA extracts showed a conspicuous recovery in the levels of glycogen, glucose and lactate contents and LDH, ICDH, SDH and MDH activity levels. From the results, it is presumed that the bioactive factors present in different extracts of CA offered protection against PTZ- induced alterations occurred in different muscles.

Key words: Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, Carbohydrates.

INTRODUCTION

Epilepsy is the most frequent neurodegenerative disorder affecting more than 50 million people world wide[1]. It is well established that impaired GABAergic activity and exaggerated glutamatergic activity are thought to contribute to the various types of epilepsy[2]. The epileptic seizures occur via alterations in the behavior of neural networks in the brain that induce synchronized bursting interspersed by periods of normal electrical activity[3]. The abnormal activity of the brain is transmitted to the rest of the body as incorrect signals, resulting in abnormal muscular activity or convulsions (Convulsions-ecure-com.html). On the basis of multiple neurophysiological mechanisms exhibited during epilepsy, a few antiepileptic drugs have been emerged with different target specifities. However, many antiepileptic drugs (AEDs) show very narrow therapeutic window and showed either limited efficacy or severe adverse effects. From the survey of literature, it is obvious that screening of phytochemicals with particular reference to anticonvulsant/

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antiepileptic activity was performed by number of workers for the past few years from other countries and much is awaited from our country, which is endowed with rich heritage of flora and fauna. *Centella asiatica* (CA), one of the multipurpose miracle herbs of oriental medicines, has been used in ayurvedic preparations in the treatment of mental fatigue and anxiety[4]. The extracts of CA also showed antidepressant activity[5], improving learning deficits[6] and protection against convulsions induced by pentylenetetrazole and Strychnine[7]. Even though much work has been done on the anticonvulsant effects of this medicinal plant as reported in the foregoing account, no systematic investigation was carried out on the neurobiological role of *Centella asiatica*, with particular reference to anticonvulsant and neuroprotective activity. In addition to the neuropathological abnormalities associated with the epilepsy, it has been well documented that seizure are characterized by repetitive, rhythmic jerking of limbs resulting from involuntary muscle twitching and loss of muscle tone (Convulsions-ecure-com.html). The main overt symptoms of epileptic patient include stiffening of muscles for 30 seconds to one minute (Tonic phase) followed by the phase of muscle jerking convulsions (Clonic phase). Although many reports are available on the neurochemical and neurophysiological abnormalities during epilepsy, not much is known on the influence of epilepsy on muscle metabolism. Hence, the present study is undertaken to examine the anticonvulsant effect of different fractions of *Centella asiatica* on selected biochemical parameters in functionally different types of rat skeletal muscle with particular reference to carbohydrate metabolism.

EXPERIMENTAL

Materials and methods

Experimental animals

Male adult wistar rats weighing 150 ± 25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28 ± 2^{o}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution No: 9/IAEC/SVU/2007/dt 04.03.2007.

Selection of drug

Pentylenetetrazole (PTZ), a convulsing drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma Chemicals, USA.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and identified by a botanist, Department of Botany, S.V. University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V. University, Tirupati (Voucher No. 1688). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of plant extracts

The active principles of the leaves of plant were extracted into different solvents, methanol, water, n-hexane, chloroform, ethyl acetate and n-butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants[8,9]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times

until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-hexane, ending with the more hydrophilic n-butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for further studies.

Induction of epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of pentylenetetrazole (60 mg/Kg body weight)[10-14].

Administration of the tested substance

Each fraction of CA extract (200 mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ[13]. A gavage tube was used to deliver the substance by the oral route, which is the clinically 5 expected route of administration of CA[9]. The volume of administration was kept at 1 mL/Kg b.w. to the animal. The rats were divided into 7 groups, each consisted of 6 rats and used for studying the effects of different fractions/ext racts of plant, *Centella asiatica*.

Group 1 - Normal saline treated control rats (SC)

Group 2 - Rats treated with PTZ (PTZ)

Group 3 - Epileptic rats pretreated with n-hexane extract (n-He + PTZ)

Group 4 - Epileptic rats pretreated with chloroform extract (CE + PTZ)

Group 5 - Epileptic rats pretreated with ethyl acetate extract (EAE + PTZ)

Group 6 - Epileptic rats pretreated with n-butanol extract (n-Be + PTZ)

Group 7 - Epileptic rats pretreated with aqueous extract (AE + PTZ)

Isolation of tissues

The animals were sacrificed after the treatment by cervical dislocation. Functionally different muscles such as white vastus, red vastus, soleus and gastrocnemius muscles were separated and frozen in liquid nitrogen (-180^{o}C) and stored at -40^{o}C until further use. At the time of analyses, the tissues were thawed and selected parameters were estimated by employing standard methods.

Procurement of chemicals

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Biochemical analyses

The total carbohydrate content was estimated by the method of Carroll *et al.*[15]. Glycogen was estimated by the method of Kemp and Van Hejnigen[16]. Glucose was estimated by the method of Mendal *et al.*[17]. Lactic acid in the muscle was estimated by the method of Barker and Summerson[18] as modified by Huckabee[19]. Pyruvate content of the muscle was estimated by the method of Friedmann and Hangen[20]. The activity levels of lactate (LDH), succinate (SDH), and malate (MDH) dehydrogenases were estimated by the method of Nachlas *et al.*[21] with slight modifications as described by Prameelamma and Swami[22].

Isocitrate dehydrogenase activity was assayed by the method of Korenberg and Pricer[23] as modified by Mastanaiah *et al*.[24].

Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and analysis of variance (ANOVA) were done using SPSS statistical software for different parameters. Difference between control and experimental assays was considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Different parameters of glycolytic and oxidative pathways were estimated in different muscles of rat during PTZ-induced epilepsy and during pre-treatment with different CA extracts. Total carbohydrates were decreased in all the muscles during PTZ-induced epilepsy which were elevated in the muscles of epileptic animals pre-treated with different CA extracts. Glycogen and glucose levels were elevated in all the muscles during epilepsy which were recovered to normalcy in the epileptic rats pre-treated with different extracts of CA. Increased lactate and decreased pyruvate levels were recorded in all the muscles during PTZ-induced epilepsy. Where as lactate levels were decreased and pyruvate levels were increased in some muscles during treatment with different CA extracts. Although 7 significant changes were not observed in the lactate and pyruvate levels in some muscles, the levels were recovered to normalcy during pre-treatment with different extracts of CA. Decreased lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities were recorded in some muscles during epilepsy, which were elevated or reached to normalcy in the epileptic animals pre-treated with different extracts of CA (Tables 1-4).

Table 1: Alterations in the carbohydrate metabolism in white vastus of wistar male albino rat during PTZ-induced epilepsy and on pre-treatment with different extracts of centella asiatica

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

WV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	5.092	4.440*	5.661*	6.864*	6.252*	5.921*	5.575*
TC							
	± 0.010	± 0.007	± 0.018	± 0.013	± 0.022	± 0.021	± 0.009
		(-12.81)	(11.18)	(34.8)	(22.79)	(16.29)	(9.48)
	0.945	1.243*	0.894*	0.887*	0.866*	0.888*	0.914*
GLY							
	± 0.024	± 0.012	± 0.008	± 0.012	± 0.011	± 0.009	± 0.011
		(31.56)	(-5.37)	(-6.11)	(-8.39)	(-6.03)	(-3.29)
	0.911	1.218*	0.889	0.888	0.882	0.900	0.891
GLU							
	± 0.054	± 0.049	± 0.052	± 0.057	± 0.055	± 0.062	± 0.048
		(33.71)	(-2.41)	(-2.486)	(-3.23)	(-1.22)	(-2.17)

Cont…

WV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	1.322	1.768*	1.216*	1.035*	1.293	1.164*	1.266
LAC							
	± 0.059	± 0.042	± 0.067	± 0.012	± 0.068	± 0.026	± 0.048
		(33.77)	(-8.04)	(-21.73)	(-2.192)	(-11.97)	(-4.22)
	86.4	48.159*	89.761	104.233*	93.511	114.333*	92.733
PYR							
	± 3.069	± 3.664	± 7.070	± 10.023	± 5.956	± 4.780	± 4.79
		(-44.26)	(3.89)	(20.64)	(8.23)	(32.33)	(7.33)
	2.307	1.365*	3.273*	2.906*	2.832*	2.689*	3.074*
LDH							
	± 0.272	± 0.059	± 0.128	± 0.115	± 0.006	± 0.073	± 0.137
		(-40.83)	(41.85)	(25.96)	(22.74)	(16.57)	(33.23)
	1.043	0.853*	1.087	1.207*	1.069	1.183*	1.112
ICDH							
	± 0.021	± 0.039	± 0.037	± 0.041	± 0.047	± 0.041	± 0.026
		(-18.17)	(4.24)	(15.68)	(2.49)	(13.45)	(6.64)
	2.048	1.311*	3.014*	2.647*	2.573*	2.430*	2.682*
SDH							
	± 0.272	± 0.059	± 0.128	± 0.115	± 0.006	± 0.073	± 0.137
		(-35.99)	(47.18)	(29.25)	(25.62)	(18.66)	(30.96)
	1.219	1.110*	1.869*	1.803*	1.816*	1.704*	1.569*
MDH							
	± 0.043	± 0.029	± 0.017	± 0.026	± 0.021	± 0.023	± 0.020
		(-8.96)	(53.36)	(47.92)	(49.01)	(39.8)	(28.75)

All the values are mean, ± SE of six individual observations.

Values in '( )'parentheses are % change over saline control.

*Values are significant at $P < 0.05$ in Scheffe test.

Table 2: Alterations in the carbohydrate metabolism in red vastus of wistar male albino rat during PTZ-induced epilepsy and on pre-treatment with different extracts of centella asiatica

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

RV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	8.681	7.067*	8.960	10.131*	9.645*	9.086	9.568*
TC							
	± 0.017	± 0.016	± 0.013	± 0.031	± 0.028	± 0.015	± 0.023
		(-18.59)	(3.21)	(16.7)	(11.11)	(4.66)	(10.22)

Cont…

RV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
GLY	0.957	1.072*	0.934	0.933	0.934	0.955	0.935
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(12)	(-2.42)	(-2.5)	(-2.36)	(-0.17)	(-2.263)
GLU	0.766	0.842*	0.743	0.742	0.724	0.764	0.744
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(9.98)	(-3.02)	(-3.13)	(-5.44)	(-0.21)	(-2.82)
LAC	1.430	1.558	1.287	1.335	1.284*	1.413	1.498
	± 0.038	± 0.077	± 0.029	± 0.005	± 0.073	± 0.031	± 0.003
		(8.92)	(-9.99)	(-6.65)	(-10.18)	(-1.16)	(4.74)
PYR	92.940	64.389*	97.894	107.401*	110.180*	118.777*	101.658*
	± 7.268	± 5.559	± 2.126	± 4.931	± 3.069	± 6.91	± 4.14
		(-30.72)	(5.33)	(15.56)	(18.55)	(27.8)	(9.38)
LDH	1.519*	1.213	1.542	1.543	1.637	1.563	1.541
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(-20.16)	(1.524)	(1.579)	(7.789)	(2.9)	(1.42)
ICDH	0.754	0.604*	0.864*	0.947	0.912*	0.807	0.787
	± 0.029	± 0.016	± 0.026	± 0.037	± 0.021	± 0.071	± 0.026
		(-19.88)	(14.53)	(25.58)	(21)	(6.98)	(4.37)
SDH	1.260	0.954*	1.283	1.284	1.378*	1.262	1.282
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(-24.3)	(1.838)	(1.904)	(9.39)	(0.132)	(1.715)
MDH	2.612	1.884*	2.808	3.435*	2.866*	2.836*	2.829*
	± 0.040	± 0.036	± 0.031	± 0.025	± 0.047	± 0.021	± 0.032
		(-27.86)	(7.52)	(31.51)	(9.70)	(8.57)	(8.309)

All the values are mean, ± SE of six individual observations.

Values in '( )'parentheses are % change over saline control.

*Values are significant at $P < 0.05$ in Scheffe test

Table 3: Alterations in the carbohydrate metabolism in Soleus muscle of wistar male albino rat during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

SOL	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	6.269	5.206*	7.282*	9.158*	8.147*	8.222*	6.720
TC							
	± 0.020	± 0.016	± 0.017	± 0.016	± 0.011	± 0.020	± 0.016
		(-16.96)	(16.16)	(46.08)	(29.95)	(31.15)	(7.2)
	0.927	1.232*	0.922	0.784	0.807	0.793	0.920
GLY							
	± 0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(32.95)	(-0.593)	(-15.4)	(-12.92)	(-14.47)	(-0.755)
	0.736	1.041*	0.731	0.593*	0.616*	0.602*	0.729
GLU							
	± 0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(41.5)	(-0.747)	(-19.4)	(-16.28)	(-18.22)	(-0.951)
	1.269	1.703*	1.252	0.977*	1.074*	0.897	1.249
LAC							
	±0.029	± 0.011	± 0.020	± 0.026	± 0.02	± 0.005	± 0.024
		(34.17)	(-1.36)	(-23.02)	(-15.39)	(-29.31)	(-1.6)
	82.041	55.493*	86.988	89.564*	100.467*	103.363*	84.018
PYR							
	±8.606	± 3.069	± 2.427	± 2.455	± 2.577	± 1.904	± 5.50
		(-32.36)	(6.03)	(9.17)	(22.46)	(25.99)	(2.41)
	1.489	1.184*	1.494	1.632*	1.609*	1.623*	1.496
LDH							
	±0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(-20.51)	(0.36)	(9.592)	(8.047)	(9.01)	(0.47)
	1.06	0.973*	1.243*	1.229*	1.197*	1.390*	1.060
ICDH							
	±0.030	± 0.016	± 0.012	± 0.040	± 0.029	± 0.034	± 0.026
		(-8.23)	(17.25)	(15.96)	(12.91)	(31.14)	(0.031)
	1.23	0.925*	1.235	1.373*	1.350*	1.364*	1.237
SDH							
	±0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(-24.83)	(0.447)	(11.61)	(9.74)	(10.9)	(0.56)

Cont…

SOL	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	3.243	3.134	3.894*	4.074*	4.450*	4.460*	3.594*
MDH							
	±0.043	± 0.029	± 0.017	± 0.026	± 0.021	± 0.023	± 0.020
		(-3.37)	(20.06)	(25.61)	(37.22)	(37.52)	(10.81)

All the values are mean, ± SE of six individual observations.

Values in '( )'parentheses are % change over saline control.

*Values are significant at $P < 0.05$ in Scheffe test.

Table 4: Alterations in the carbohydrate metabolism in Gastrocnemius muscle of wistar male albino rat during PTZ induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

GN	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	7.033	5.704*	7.200	8.720*	7.942*	7.869*	7.374
TC							
	± 0.031	± 0.018	± 0.016	± 0.014	± 0.021	± 0.010	± 0.024
		(-18.89)	(2.38)	(23.99)	(12.93)	(11.88)	(4.85)
	0.885	1.210*	0.879	0.698*	0.752*	0.751*	0.881
GLY							
	± 0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(36.74)	(-0.64)	(-21.16)	(-15.04)	(-15.14)	(-0.433)
	0.694	1.019*	0.688	0.507*	0.561*	0.560*	0.690
GLU							
	± 0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(46.85)	(-0.816)	(-26.99)	(-19.18)	(-19.3)	(-0.552)
	2.62	3.018*	2.615	2.236*	2.393	2.459	2.570
LAC							
	± 0.011	± 0.068	± 0.007	± 0.105	± 0.295	± 0.019	± 0.019
		(15.2)	(-0.203)	(-14.66)	(-8.66)	(-6.14)	(-1.908)
	75.303	49.941*	75.501	79.659	101.26*	98.09*	78.812
PYR							
	± 2.455	± 4.448	± 2.093	± 6.505	± 3.138	± 4.599	± 5.38
		(-33.68)	(0.263)	(5.785)	(34.47)	(30.26)	(4.66)
	1.447	1.122*	1.453	1.634*	1.580*	1.581*	1.451
LDH							
	±0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(-22.47)	(0.39)	(12.94)	(9.2)	(9.26)	(0.264)

Cont…

GN	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	1.011	0.874*	1.013	1.054	1.240*	1.217*	1.028
ICDH							
	±0.043	± 0.024	± 0.051	± 0.015	± 0.038	± 0.031	± 0.026
		(-13.51)	(0.197)	(4.22)	(22.68)	(20.4)	(1.648)
	1.188	0.863*	1.194	1.375*	1.321*	1.322*	1.192
SDH							
	±0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(-27.37)	(0.476)	(15.76)	(11.2)	(11.27)	(0.32)
	1.839	1.578*	1.886	2.306*	2.255*	2.152*	2.032*
MDH							
	±0.028	± 0.023	± 0.017	± 0.027	± 0.031	± 0.017	± 0.017
		(-14.18)	(2.55)	(25.37)	(22.64)	(17.01)	(10.52)

All the values are mean, ± SE of six individual observations.

Values in '( )'parentheses are % change over saline control.

*Values are significant at $P < 0.05$ in Scheffe test.

Carbohydrates play not only a structural role in the cell but may serve as a reservoir of chemical energy. Carbohydrates are the major sources of energy fuels for metabolic process readily assimilable, though fats yield more energy[25]. Muscle utilizes carbohydrates as the major source of energy for mechanical activity and kinesiological efficiency of the animal. The immediate source of energy for muscular contraction is ATP and these biological currencies are replenished ultimately by carbohydrates or fats or proteins. Selected parameters of glycolytic and oxidative pathways of carbohydrate metabolism were studied in different muscles of rat during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*. The decrease in total carbohydrate levels in the white vastus, red vastus, soleus and gastrocnemius muscles of PTZ treated rats indicates utilization of carbohydrates to meet energy demands during PTZ induced epileptic conditions. On treatment with CA extracts the total carbohydrate levels were increased which might be due to the synthesis of carbohydrates through glycogenesis and gluconeogenesis replenishing the loss of carbohydrates that occur during epileptic seizures. The glycogen levels were increased in different muscles of PTZ treated animals, which indicate possible mobilization of stored reserves and mobilization of glycogen from liver to the skeletal muscle in order to meet the energy demands during epileptic condition. On par 8 with the glycogen, glucose levels were also increased in all the muscles during PTZ-induced epilepsy, which might be implicated to the increased conversion of glycogen to glucose for the onward glycolytic pathway. On contrary to this, glycogen and glucose levels were non-significantly decreased and/or recovered to normalcy during pre-treatment with CA extracts suggesting lesser utilization of these components through anaerobic glycolysis. Lactate is the end product of glycolysis under anaerobic conditions and the rate of lactate production is considered as an index of physiological stress in the biological systems[27-28]. The lactic acid production and accumulation suggest the tissue capacity to withstand anaerobiosis. The levels of lactic acid also indicate the prevalence of anaerobiosis in the tissues and the tissue specific resistance or susceptibility to anaerobic conditions. In the present study, lactate levels were increased during PTZ induced epileptic condition. Although, the extent of changes in lactate and pyruvate were not uniform in all tissues, lactate levels were decreased and pyruvate levels were increased during pre-treatment with CA extracts. Increased lactate content during PTZ treatment suggests induction of lacticacedemia in different muscles and CA extracts reduce such metabolic acidosis and protect muscles

from any architectural damage caused due to PTZ- induced epileptic seizures. The formation of pyruvate, an important end product of glycolysis was found to be low during PTZ-induced epilepsy indicating greater mobilization of pyruvate to lactate through reverse pathway of NADH2 dependent lactate dehydrogenase. The decreased levels of pyruvate and elevated levels of lactate during induced epilepsy indicate prevalence oxygen deficiency in the intracellular milieu with advancement of treatment. NAD-Lactate dehydrogenase (LDH) is a key enzyme of glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by LDH interlinks anaerobic and aerobic oxidation of glucose. The activity of LDH 9 was significantly decreased in all the muscles during PTZ- induced epileptic condition when compared to their respective controls indicating down regulation of oxidative metabolism due to lesser feeding of pyruvate into the TCA cycle. Pre-treatment with different CA extracts significantly increased the NAD-LDH activity in all the muscles which implies that the bioactive factors of CA extracts favor greater conversion of lactate to pyruvate and subsequent feeding of pyruvate into Kreb's cycle for further oxidation. The reduced levels of oxidative enzymes of TCA cycle i.e. ICDH, SDH and MDH during induced epilepsy indicate depressed oxidative metabolism in mitochondria and reduced turnover of carbohydrates and energy output[29]. The decreased activities of mitochondrial enzymes could also be attributed to the low feeding and /or availability of substrates, loss of structural integrity of mitochondria and prevalence of hypoxic condition ultimately leading to energy crisis during epilepsy. However, pre-treatment with different CA extracts to the epileptic animals caused marked elevation in the activities of all the oxidative enzymes thus promoting flux of reduced equivalents into oxidative phosphorylation and compensates the energy crisis that might have occurred during epilepsy. The present findings demonstrate that the CA extracts and the bioactive factors present in the CA extracts offer protection against induced epilepsy by restoring oxidative metabolism and reduce the risk of metabolic dysfunction that occurred during epilepsy.

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