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Research Article

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

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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 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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