IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

# ANTI EPILEPTIC PROPERTIES OF *CENTELLA ASIATICA* AGAINST PENTYLENETETRAZOLE INDUCED EPILEPSY IN RATS WITH REFERENCE TO LIPID METABOLISM

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***Abstract:*** The aim of this study is to investigate the anticonvulsant effect of aqueous extract of *Centella asiatica* (CA) in functionally different muscles with reference to lipid metabolism during pentylenetetrazole (PTZ) induced epilepsy and also during pre-treatment with aqueous extract of CA. Epilepsy is a convulsive episode and is the most frequent neurodegenerative disorder affecting about 50 million people world-wide. Lipid profiles are important components of the neurochemical environment in the muscles of rat. They 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 specifity and tissue immunity. The rats were divided into 4 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 aqueous extract (AE) and (4) Epileptic group pretreated with diazepam 2mg/kg body weight. PTZ-induced epilepsy increased the Lipid peeroxidation and decreased the Phospholipids, Total Cholesterol and Triglycerides in all the muscles (White vastus, Red vastus, Soleus and Gastrocnemius). Pre-treatment with aqueous extract (AE) showed a conspicuous recovery. From the results, it is acknowledged that the bioactive factors present in aqueous extract of CA offered protection against PTZ-induced alterations occurred in different muscles.

**Key words**: *Centella asiatica*, Epilepsy, Pentylenetetrazole, Lipids, Rat muscles.

## I. INTRODUCTION

Lipid profiles 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 (Lehninger, 1995). Lipid peroxidation is a well-defined mechanism of cellular damage in animals and plants. Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), natural bi-products of lipid peroxidation. Oxidative modification of lipids can be induced *in vitro* by a wide array of pro-oxidant agents and occurs *in vivo* during aging and in certain disease conditions. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. These aldehydic secondary products of lipid peroxidation are generally accepted markers of oxidative stress.

Free radical reactions are critical for the normal operation of a wide spectrum of biological processes. A free radical is defined as 'any chemical species (any atom, group of atoms or molecules) in a particular state with one or more unpaired electrons in its outer orbit' (Halliwell and Gutteridge 1989) thereby making it more reactive than a non-radical. Generally, all aerobic organisms utilize oxygen as an electron acceptor to oxidize various metabolic substrates, so that stored energy is released for biological activities. During this process, many oxygen molecules are reduced to water, but a fraction of oxygen (2-5%) is univalently reduced to various intermediates representing the electron reductant of oxygen: superoxide ($O_2$); hydrogen peroxide ($H_2O_2$); and hydroxyl radical ($^{\cdot}OH$) (Halliwell and Gutteridge 1989).

Free radicals are formed during the normal metabolic processes (Yoshika *et al*. 1990), in addition to being generated by exposure to toxic agents (Halliwell and Gutteridge 1985) and several other disease states (Lambert and Bondy 1989). The survival of an organism may depend upon its ability to overcome the toxic effects of reactive oxygen species (ROS) because oxidation of thiol groups during oxidative stress can inactivate enzymes and hydroxyl radicals are known to attack cellular proteins, lipids and nucleic acids (Slater 1984).

Free radicals can peroxidise polyenoid acids and lead to the formation of malondialdehyde (MDA) and also participates in the oxidation of proteins, which causes enzymatic inactivation (Dean *et al*. 1991). Free radicals inactivate enzymes, break DNA and initiate chain reactions that peroxidise the lipids. Hence, the present investigation is taken up to ascertain the alterations in lipid profiles in different rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica*.

## II. Materials and Methods

### 2.1. 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.

### 2.2.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.

### 2.3. 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 this studies.

### 2.4. Induction of Epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) in saline (Rizwan *et al.*, 2003).

### 2.5. Administration of Test substance

Each aqueous 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).

### 2.6. 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). 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/physiological analyses.

### 2.7. 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, red vastus, soleus and gastrocnemius 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.

### Biochemical assays

### 2.8. Phospholipids

Phospholipids were estimated by the method of Zilversmidth and Davis (1950).

### 2.9. Total cholesterol (TC)

The total cholesterol content was estimated using Liebermann Burchard reaction as described by Natelson (1971).

### 2.91. Triglycerides (TG)

Triglycerides were estimated by the method of Natelson (1971).

### 2.92. Lipid peroxidation (LP)

This assay was used to determine MDA levels as described by Ohkawa *et al.*, (1979).

### 2.93. 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 $P<0.05$.

## III. RESULTS AND DISCUSSION

The changes in the Phospholipids content in different rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica* were represented in Table.1

The percent change of depletion in the Phospholipids content during PTZ-induced epilepsy can be represented as: TC: GN (-80.152)>SOL (-76.697)>RV (-63.539)>WV (-48.022)

The phospholipid content was increased in all the muscles of rat after pre-treatment with the aqueous extract of CA and diazepam.

**Table 1: Changes in the Phospholipids content in different rat muscle during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica***

| MUSCLE | SC | PTZ+AE | PTZ | DP+PTZ |
|---|---|---|---|---|
| **WHITE VASTUS (WV)** | 37.124 | 48.122* | 19.296* | 60.485* |
| | ±0.122 | ±0.304 | ±1.018 | ±0.664 |
| | | (29.625) | (-48.022) | (62.927) |
| **RED VASTUS (RV)** | 18.845 | 29.066* | 6.871* | 31.684* |
| | ±0.856 | ±0.159 | ±0.248 | ±0.154 |
| | | (54.237) | (-63.539) | (68.129) |
| **SOLEUS (SOL)** | 7.065 | 15.756* | 1.647* | 15.945* |
| | ±0.136 | ±0.920 | ±0.621 | ±1.345 |
| | | (123.01) | (-76.687) | (125.69) |
| Gastrocnemius **(GN)** | 10.354 | 18.334* | 2.055* | 19.627* |
| | ±0.154 | ±0.654 | ±0.145 | ±0.648 |
| | | (77.072) | (-80.152) | (89.56) |

All the values are mean, ±SD 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)

The changes in the Total cholesterol content in different rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica* were represented Table.2

The percent change of depletion in the Phospholipid content during PTZ-induced epilepsy can be represented as: TC: SOL (-58.424) > RV (-54.711) GN > (-40.564) >WV (-35.296)

The total cholesterol levels were significantly increased in all the rat muscles during treatment with aqueous extract of CA and also with diazepam treatment (Reference control).

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

| MUSCLE | SC | PTZ+AE | PTZ | DP+PTZ |
|---|---|---|---|---|
| **WHITE VASTUS (WV)** | 31.658 | 41.226 | 20.484* | 52.369* |
| | ±0.235 | ±0.456 | ±0.257 | ±0.354 |
| | | (30.223) | (-35.296) | (65.421) |
| **RED VASTUS (RV)** | 69.254 | 88.684* | 31.364* | 102.154* |
| | ±0.124 | ±0.254 | ±0.349 | ±2.613 |
| | | (28.056) | (-54.711) | (47.506) |
| **SOLEUS (SOL)** | 48.654 | 68.789* | 20.228* | 82.364* |
| | ±0.125 | ±3.642 | ±1.647 | ±2.657 |
| | | (41.384) | (-58.424) | (69.285) |
| Gastrocnemius **(GN)** | 82.264 | 105.085 | 48.894* | 135.621* |
| | ±3.364 | ±5.865 | ±3.64 | ±7.964 |
| | | (27.741) | (-40.564) | (64.861) |

All the values are mean, ±SD 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 total cholesterol / g wet wt of the tissue)

The changes in the triglycerides content in rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica* were represented in table Table.3
The percent change of depletion in the triglycerides content during PTZ-induced epilepsy can be represented as: TG: SOL (-36.648) > GN (-31.902) > RV (-25.745) >WV (-6.331)

The triglyceride content was elevated in rat muscles during pre-treatment with aqueous extract of CA. Similar elevation in triglycerol content was also observed in diazepam treated reference control.

**Table .3: Changes in the Triglycerides content in different rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica***

| MUSCLE | SC | PTZ+AE | PTZ | DP+PTZ |
|---|---|---|---|---|
| **WHITE VASTUS (WV)** | 3.159 | 4.954* | 2.959* | 3.544* |
| | ±0.112 | ±0.147 | ±0.251 | ±0.142 |
| | | (56.822) | (-6.331) | (12.187) |
| **RED VASTUS (RV)** | 4.564 | 6.614* | 3.389* | 6.744* |
| | ±0.116 | ±0.247 | ±0.242 | ±0.017 |
| | | (44.917) | (-25.745) | (47.765) |
| **SOLEUS (SOL)** | 5.621 | 7.198* | 3.561* | 7.434* |
| | ±0.145 | ±0.178 | ±0.346 | ±0.321 |
| | | (28.056) | (-36.648) | (32.254) |
| **Gastrocnemius (GN)** | 6.454 | 8.954 | 4.395* | 8.654* |
| | ±0.245 | ±0.125 | ±0.621 | ±0.324 |
| | | (38.736) | (-31.902) | (34.087) |

All the values are mean, ±SD 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)

The changes in the Lipid peroxidation content in different rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica* were represented in Table. 4.

The percent change of elevation in the Lipid peroxidation content during PTZ-induced epilepsy can be represented as: LP: WV (97.579) > GN (42.976) SOL (38.341) > > RV (37.534)

The lipid peroxidation was significantly decreased in rat muscles after pre-treatment with the aqueous extract of CA and diazepam.

**Table 4: Changes in the Lipid peroxidation content in different rat muscles during PTZ-induced epilepsy and pre-treatment with aqueous extract of *Centella asiatica***

| MUSCLE | SC | PTZ+AE | PTZ | DP+PTZ |
|---|---|---|---|---|
| **WHITE VASTUS (WV)** | 18.545 | 9.654* | 36.641* | 9.995* |
| | ±1.654 | ±1.984 | ±1.645 | ±1.642 |
| | | (-47.942) | (97.579) | (-46.104) |
| **RED VASTUS (RV)** | 49.654 | 32.621* | 68.291* | 35.366* |
| | ±2.641 | ±1.612 | ±2.164 | ±1.994 |
| | | (-34.303) | (37.534) | (-28.775) |
| **SOLEUS (SOL)** | 52.164 | 35.364* | 72.164* | 36.425* |
| | ±2.195 | ±1.941 | ±1.668 | ±1.919 |
| | | (-32.206) | (38.341) | (-30.172) |
| **Gastrocnemius (GN)** | 35.785 | 26.585* | 51.164* | 28.954* |
| | ±2.995 | ±1.357 | ±1.195 | ±1.654 |
| | | (-25.709) | (42.976) | (-19.089) |

All the values are mean, ±SD 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)

Membrane phospholipids have a dual role as structural building blocks of cell membranes and as precursor molecules involved in signal transduction such as the lipid second messengers diacyl glycerol, phosphatidic acid, lysophosphatidic acid and arachidonic acid (Hodgkin *et al.,* 1998). The phospholipid content was decreased in the present study in all the rat muscles during PTZ-induced epilepsy which might be implicated to the enzymatic hydrolysis of membrane phospholipids by phospholipases leading to loss of membrane integrity. Excessive $Ca^{+2}$ secretion through glutamate receptors activates large array of potential neurotoxic mechanisms, including activation of phospholipases, calpaines, proteases, proteins kinases, endonucleases as well as Reactive oxygen species (Sattler and Tymianski, 2000). Further more, Farooqui *et al.*, (1997) have reported phospholipid hydrolysis by glutamate receptor over activation leads to the degradation of membrane phospholipids and excitotoxic neuronal death. From the observation of present study coupled with the above reports, it can be speculated that both the inhibition of phospholipid synthesis and activation of phospholipases have been involved in the reduced levels of phospholipids in different rat muscles during glutamate induced excitotoxicity that prevails in PTZ-induced epileptogenesis.

The phospholipid content was increased in epileptic animals pre-treated with aqueous extract of CA which suggest that the bioactive factors present in CA might possibly inhibit the over activation of glutamate receptors and subsequent inhibition of $Ca^{+2}$ overload in the epileptic cells, thus causing a overall reduction in the phospholipase activity.

In the present study the PL content was decreased during PTZ-induced epilepsy (Munjal *et al.*, 2007). The decreased PL content may serve as a metabolic alarm to the animal in the sense that the membrane integrity is lost. Treatment with aqueous extract of  showed a significant recovery in the PL content. Hence, it is evident that the different bioactive factors of CA offered protection against PTZ-induced epilepsy.

From the results it is clear that PTZ-induced epilepsy reduced cholesterol levels in different muscle of rat, which might be implicated to the either reduced synthesis / augmented degradation by lipoprotein lipase activity. Since epilepsy causes glutamate excitotoxicity and subsequent oxidant stress, it is possible that the PTZ-might alter the membrane lipid metabolism resulting in cellular architectural damage causing synaptic dysfunction and neuronal degeneration.

The treatment with extracts of *Centella asiatica* and diazepam restored the levels of cholesterol in different muscles of  epileptic rats. Similar increase in cholesterol levels were reported in rats fed both CA extract and powder during $H_2O_2$ induced oxidative stress (Hussin *et al.*, 2009). 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. However, in depth studies are needed to understand the altered lipid metabolism in muscle during epilepsy and also during antiepileptic treatment with aqueous extract of CA.

The decreased levels of triglycerides in different rat muscles 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 neurodegenerative diseases such as Parkinson's disease, Alzheimer's and Huntington's disease (Lipton and Rosenberg, 1994). There is general agreement that glutamate excitotoxicity leads to $Ca^{+2}$ over loading exceeding the capacity of $Ca^{+2}$ regulating mechanisms which activate several enzymes including calcium dependent nucleases, lipases, proteases and neuronal nitric oxide synthase thus increasing the oxidative stress.

Pre-treatment with extract of CA caused an elevation in triglyceride content in different rat muscles which suggest that the bioactive factors present in CA might have counteracted the glutamate excitotoxicity resulting a reduction in $Ca^{+2}$–over–influx into neurons and thus protected the cells from neurodegenerative processes evoked by induced epilepsy.

Pre-treatment with extract of CA caused significant diminution in the MDA content in all the functionally different rat muscle. Although not related to the anti-convulsant activity, it has been reported that CA offers significant protection against oxidative stress.  Decreased MDA content and an increase in glutathione and catalase activities have been reported in rats treated with aqueous extract of CA in intra cerebro ventricular 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 (Shukla *et al.*, 1999). 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.

**Summary and Conclusion**

The depleted lipid peroxidation during treatment with CA extracts suggest that bioactive factors present in CA extracts imparted significant recovery towards normalcy and lessen the free radical mediated toxicity that occurred during PTZ-induced epilepsy.

The decrease in the levels of cholesterol, triglycerides and phospholipids contents during PTZ-induced epilepsy may suggest disruption of membrane architecture of different rat muscles. The decreased phospholipid content served as a metabolic alarm to the animal in the sense that the membrane integrity is lost. However, treatment with CA extracts caused significant elevation in muscle lipid profiles which suggest that the CA extracts protect the membrane architectural damage that was occurred during epilepsy. It is suggesting that the CA extracts or the bioactive compounds present in the extracts may be beneficial in the antiepileptic treatment. Hence, the information gained from the present study can be used for proposing a better pharmacological utensil to treat the epilepsy and related disorders. The present study also helps in the discovery of new neuroprotective components from the medicinal plants.

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