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Antioxidant enzyme activities and glutathione levels in various areas of the rat brain after pentylenetetrazole-induced epilepsy: the effect of Centella asiatica (Gotu kola).

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Abstract

About 50 million individuals throughout the globe suffer from epilepsy, the most common neurological illness, which manifests itself in the form of convulsive episodes. Centella asiatica (CA) has a long history of medicinal usage in India's Ayurvedic culture. This research aims to identify the antiepileptic properties of the medicinal herb CA. Epileptic rats were induced with pentylenetetrazole (PTZ), and the fractionated extracts were shown to be effective against these animals. Control and test groups were compared with respect to their levels of enzymatic and non-enzymatic antioxidants such as reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT). The CA extracts considerably reduced the number of epileptic seizures caused in the rats by (PTZ).

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

Introduction

Epilepsy, which is characterized by recurring spontaneous seizures, is a common and heterogeneous neurological illness. It is generally accepted that epileptic seizures originate from an overexcited neuronal population1. It is widely recognized that decreased GABA ergic activity and/or excessive glutamatergic neurotransmission predominantly contribute to the different forms of epilepsies2, despite the fact that many molecular pathways have been hypothesized in producing and propagating epileptic discharges. Acute and chronic degenerative illnesses, such as epilepsy, have been suggested to include excitotoxicity, the death of neurons resulting from stimulation of glutamate receptors. Massive glutamate secretion during seizures has been hypothesized to activate many free radical producing mechanisms, leading to increased creation of oxygen free radicals3. Antiepileptic medications (AEDs) are developed to lessen the negative effects of epilepsy4 by correcting the complex neurochemical and neurophysiological disturbances that lead to seizures. When used to treat seizures, AED regimens have a mixed track record of efficacy and are plagued by issues including pharmacoresistance and neurotoxic consequences.5.

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The identification of active components from indigenous medicinal plants for many human maladies, including neurodegenerative illnesses like Alzheimer's disease, parkinsonism, etc., has made significant strides in recent years. In light of this, the current research seeks to learn more about the changes in antioxidant metabolism that occur during PTZ-induced epilepsy and antiepileptic therapy with CA.

The Stuff and How We Did It

Animals used in experiments

The rats were obtained from the Indian Institute of Science (IISc), Bangalore, and housed in polypropylene cages at a temperature of 2820C with a photoperiod of 12 hours of light and 12 hours of dark and 75% relative humidity at the department's animal house. Rats were provided with a regular pellet meal and water ad libitum (both from Hindustan Lever Ltd. in Mumbai). In accordance with the Animal Ethics Committee's 438/01/a/cpcsea/dt:17.07.2006 resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt.04.03.2006, the rats were kept in a humane environment.

Drug used to treat epilepsy

The convulsant medication pentylenetetrazole (PTZ) was used for this investigation. It was purchased from Sigma Chemical Company in the USA as a reputable commercial chemical.

Obtaining the Plant Parts

A member of the Department of Botany at S.V.University in Tirupati identified a plant believed to be Centella asiatica (CA) that had been harvested from the Tirumala hills. The S.V.University of Tirupati, Botany Department now has a voucher specimen (Voucher no. 1688) of this plant in its herbarium. The leaves were harvested from the plant, dried under cover, ground into a powder, and then the anticonvulsant principle or principles were extracted from the powder using several solvents.

Plant Extract Preparation

Since these solvents were largely employed by multiple scientists for extracting anticonvulsant principle(s) from diverse plants6, 7, they were used to extract the active principles of the leaves of the plant. Two days were spent at room temperature soaking powdered plant material in methanol before filtering out the solvent. About thrice or four times, we did this until the extract no longer changed color. A gum-like residue was obtained after the extract was distilled and concentrated under reduced pressure in a Buchi rotovapour R-114. This residue was then suspended in water and extracted using a series of organic solvents that increased in polarity from lipophilic n-Hexane to hydrophilic n-Butanol. Under low pressure, the Buchi rotavapour was used to distill and concentrate the solvent used in each extract. Finally, we employed freeze-dried extracts in our research.

Substance administration

One week before the injection of PTZ at the dosage of 60mg/kg body weight8, the animals were administered a saline solution of each fraction of CA extract (200mg/Kg body weight). A gavage tube was employed to give the drug via the oral route, which is the clinically predicted method of administration of CA7. One milliliter was all that was given to the animal. Animals were administered diazepam (2mg/kg body weight i.p.), an anticonvulsant medication, dissolved in normal saline for a period of one week (Reference control).

The Laboratory Setup

There were a total of 8 groups, each including 6 rats: Group 1-control rats given just saline (SC), Group 2-rats given PTZ (Epileptic group), Group 3-epileptic rats given both n-Hexane and chloroform extracts before PTZ treatent

(nHE+PTZ), and Group 4-epileptic rats given only PTZ treatment (CE+PTZ).

pretreatment Ethyl acetate extract (EAE+PTZ) rats, pretreatment n-Butanol extract (nBE+PTZ) rats, pretreated aqueous extract (AE+PTZ) rats, and pretreated diazepam (DP+PTZ) rats were compared.

Tissue Isolation

Brain areas such the cerebral cortex (CC), cerebellum (CB), pons (PM), and hippocampus (HC) were removed from the animals after the allotted time, and then frozen in liquid nitrogen and kept at -800C until examination.

Dissecting the biochemistry

The concentration of glutathione was calculated using the technique described by Theodorus et al.9 (1981). Methods based on a variation of Flohe and Gunzler10 (1984) were used to identify Se-Dependent Glutathione Peroxidase (Se-GSH-Px). The enzyme activity of glutathione reductase was measured using a modified version of Carlberg and Manervik11's (1985) technique. According to the technique of Habig et al12 (1974), glutathione-S-transferase activity was evaluated using 1-chloro-2, 4-dinitrobenzene (CDNB) as the standard substrate at a wavelength of 340 nm. Misra and Fridovich13's (1972) approach was used to calculate superoxide dismutase activity. An modified version of Aebi14 (1984) was used to determine catalase activity.

Mathematical dissection

Six duplicates from each group were tested in every experiment. SPSS statistical software (11.5 version) was used to calculate the mean, standard error (SE), and Analysis of Variance (ANOVA) for various parameters. Difference between control and experimental tests was deemed as significant at $P<0.05$.

What We Learned and Why

The GSH content of the brains of PTZ-induced mice was considerably lower than that of saline control animals across all areas, with the reduction being most pronounced in the cerebral cortex (CC). Extracts from CA as a pretreatment

For example, Table 1 shows that the GSH content in the brain was considerably raised after exposure to n-HE, CE, EAE, n-BE, AE, and diazepam (Reference control).

A comparison of control and experimental groups' brain tissue glutathione peroxidase (GPx), glutathione reductase (GR), glutathione s-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activity after receiving CA extracts.

Tables 2–6 demonstrate the levels of enzymatic antioxidant activity in various parts of the brain tissue of both normal and experimental rats. Each of the GPx, GR, GST, and SOD The hippocampus (HC) showed the greatest reduction in and CAT of all areas of the brain during PTZ-induced seizures (Group II). When compared to PTZ-bearing mice (Group II), the activities of GPx, GR, GST, SOD, and CAT were considerably elevated in animals treated with CA extracts (Groups III–VII) and diazepam (DP) (Group VIII).

The production of reactive oxygen species (ROS) is an inevitable byproduct of aerobic metabolism, and the antioxidant defense incorporates both enzymatic and non-enzymatic mechanisms15. Cancer, arthritis, and neurodegenerative diseases16 are just some of the many pathogenic outcomes of chronic oxidative stress. The metabolism of glutathione and related compounds is an important defense mechanism for cells against oxidative stress-inducing substances. Glutathione may scavenge free radicals, decrease peroxides, or be conjugated with electrophilic chemicals, all of which contribute to detoxification on various levels. Glutathione protects the cell in many

ways, both directly and indirectly, against ROS and the harmful byproducts they create.

Reduced glutathione (GSH) has long been known to prevent chemically induced injury17, 18. During PTZ-induced seizures, Glutathione S-Transferase (GST) activity and Glutathione S-Transferase (GSx) concentration were reduced in several brain areas. Earlier results from other epilepsy models19 corroborate the decreased glutathione metabolism. De Freitas et al20 have shown comparable drop in GSH levels in rat hippocampus after pilocarpine-induced seizures. Mullar et al.21 found reduced glutathione peroxidase in the plasma and brain of PTZ-induced epileptic rats, reporting a systemic disruption of the glutathione system. Similar decrease in Glutathione peroxidase activity was also documented in rats treated with PTZ. In addition, in vitro studies of glutamate toxicity in neuronal cell lines22 have shown that this toxin depletes Glutathione levels and causes oxidative stress. Consistent with these findings, it has been reported that PTZ-induced epilepsy is accompanied by a decrease in microsomal glutathione levels23, antioxidant enzyme activities24, a generalized diminution of antioxidant activity in PTZ-induced epilepsy25, and a significant decrease in GSH, Glutathione disulfide (GSSG) in the cerebral cortex of mouse after PTZ-induced seizure.

Lower glutathione levels are associated with worse cellular defense against toxic shock from PTZ, which in turn leads to increased superoxide and reactive oxygen species production.

radical H2O2. This research suggests that the decreased SOD activity may have restricted the potential for converting oxidized glutathione to reduced GSH. Pretreatment with several CA extracts reversed the altered glutathione metabolism seen in PTZ-induced seizures, and similar to diazepam, these extracts exhibited their antiepileptic activity via regulating antioxidant metabolism in various brain areas. Supplemental CA has also been shown to greatly protect against arsenic-induced oxidative stress by reestablishing blood GSH levels26. As shown by a reduced seizure score and restored oxidative stress indicators, Gupta et al.27 found that the injection of CA extracts significantly improved the learning deficit generated by PTZ kindling. The primary component of CA, asiatic acid, has been shown to have considerable neuroprotective benefits on cultured cortical cells by enhancing the cellular oxidative defense system, as described by Lee et al.28. Another component of CA, asiaticoside, increased the induction of antioxidant levels like SOD, CAT, and GPx during the early stages of wound healing, suggesting that these elements may be significant contributors to the substance's curative effects. These results, when added to those of previous studies, imply that CA extracts have a neuroprotective impact against PTZ-induced seizures and enhance the antioxidant capacity of the body.

Failure of dismutation of superoxide anions produced by xanthine oxidase activity may underlie the reduced SOD activity seen in many brain areas in PTZ-induced seizures. PTZ-induced epilepsy may result in oxidative damage due to the considerable reduction in SOD activity, as well as the increase in lipid peroxidation and purine catabolism shown in the current research. Inhibition of SOD has also been shown in many seizure models. Wilhelm et al.29 found that the pilocarpine model of seizures in rats inhibited Na+, K+ - ATP ases and SOD activity in the brain. During PTZ-induced seizures, Obay et al.24 found that lipid peroxidation and antioxidant enzyme activity both decreased. It has been hypothesized that the increase in ROS generation and suppression of SOD activity30 was caused by the widespread development of glutamate receptors (both NMDA and non-NMDA).

Superoxide dismutase (SOD) activity was increased in all brain areas of rats treated with various extracts of CA, suggesting a probable role of SOD in quenching superoxide anion radical. According to the findings of Shukla et al.31, Asiaticoside, an anticonvulsant produced 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, haveconcluded that supplementation of *Centella asiatica*significantly protected arsenic-induced oxidative stress. The findings of the present study coupled with theabove reports, it can be speculated that the bioactive factors present in different extracts of CA maymodulate 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 regionsof rat brain in PTZ-induced epileptic rats after pre-treatment with different extracts of CA. Similar induction of different antioxidant enzymes includingcatalase 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 itincreased enzymatic (SOD, CAT, GPx) and non-enzymatic (Vitamin-E and Ascorbic) antioxidant levels[33]. Significant increase in catalase activity hasalso been reported after oral treatment with the crudemethanol extracts of CA in lymphoma-bearing mice[32].Decreased lipid peroxidation and increased catalaseactivity 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 treatedrats after pre-treatment with chloroform, methanolicextract of CA[35].

From the present findings coupled with the earlier reports, it is obvious that the bioactive factors presentin *Centella asiatica* stimulated the antioxidant enzymessuch 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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