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Research Article

Effect of Ethanol Extract of *Brophyllum pinnatum* on Pentylenetetrazole-Induced Kindling, Cognition and Oxidative Stress in Rats

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Abstract

Epilepsy is a debilitating non communicable neurological disorder caused by a variety of factors. There are limited drugs for the management of epilepsy. Thus this study evaluates the effect of ethanol extract of *Brophyllum pinnatum* (EEBP) on epileptogenesis in a pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Thirty male Wistar rats weighing 150-200 g were randomly divided into five groups (n = 5), all the groups except group one were administered 30 mg/kg pentylenetetrazole (PTZ) on alternate days, till at least 25 PTZ administrations. Groups one and two were administered distilled water, and groups 3 – 5 were given 75, 150 and 300 mg/kg EEBP. The rats were subsequently scored for kindling, while cognitive effect was assessed with Y-maze test, and markers of oxidative stress and glutamic acid decarboxylase (GAD) were also estimated in the whole brain after sacrifice. Kindling scoring results showed that EEBP decreased the progression of epileptogenesis. Pentylenetetrazole-induced seizure intensity and duration were reduced by EEBP. The result also revealed that the extract ameliorates the impaired working memory and the negative symptoms of epilepsy. These beneficial effects of the extract might be due to its modulation of oxidative stress markers. This study provides valuable and reliable evidence that *Bryophyllum pinnatum* possess antiepileptogenic and antioxidant properties.

Key Words: *Epileptogenesis, Seizure scores, Glutamic acid decarboxylase, Kindling, Brophyllum pinnatum*

INTRODUCTION

Epilepsy is reported to be among the most common non communicable neurological diseases globally with over fifty million people suffering from the disease (WHO, 2022). It is more prevalent in less developed regions of the world, especially Africa and Asia due to poor prognosis and religious bias for the cause of the disease. A great number of people with epilepsy in these regions of world rely almost exclusively on herbal remedy since conventional therapy is only symptomatic and has low efficacy, controlling seizures in only about 25% of patients. There is also neither effective prophylaxis nor cure for the disease (Park *et al*., 2017). Epilepsy is characterized by periodic, unpredictable seizures caused by disordered, synchronous and repetitive firing from a population of brain neurons (Pale *et al*., 2022). The trigger for seizures and eventually epilepsy include psychosocial stress, supernatural forces or infection due to contagion, head injury at birth and congenital origin (Komolafe *et al*., 2011). Despite the various causes, the pathophysiological mechanisms are still largely similar.

In the past years, poor prognosis of epilepsy has necessitated the surge in neuroscience research resulting in development and deployment of vast number of new antiepileptic drugs. A major limitation to most AEDs is development of serious adverse effects and tendencies for drug-drug interactions associated with their use (Sarangi, *et al*., 2020). However, newer AEDs exhibit lesser drug-drug interaction in comparison to the earlier AEDs (Sarangi, *et al*., 2020). The ideal AED will subdue all types of seizures with minimal unwanted effects. Unfortunately, drugs currently used in clinical setting for management of epileptic seizure are associated with many side effects such as lethargy, dizziness, euphoria, and headache.

The mechanism of epileptogenesis is often studied with an experimentally induced phenomenon called kindling (Aksoz *et al*., 2020). It involves sub-acute administration of sub-lethal dose of convulsants, particularly pentylenetetrazol and monitoring for development of signs of seizures. The involvement of several other neuropsychiatric problems such as cognitive dysfunction with epilepsy has made it a more complex and complicated disorder. Many neurological diseases including epilepsy are considered to involve

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oxidative stress in their pathogenesis (Folbergrová *et al*., 2016). This disease is associated with an increased amount of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxide (Schweikl *et al*., 2017). Neuronal changes responsible for these behaviors observed in neurological diseases have been attributed to these free radicals (Feinstein *et al*., 2016). Furthermore, the resultant effects of the oxidative damages on the brain tissues are psychiatric and cognitive problems such as depression, anxiety and memory loss may also be responsible for decreasing of the life span in the epileptic individuals (Mei *et al*., 2016). Anxiety which is defined as an unpleasant, uncontrollable and unavoidable emotional state is a common event in epileptics.

Medicinal plants with the potential of preventing oxidative stress are lately being investigated for their biochemical effects in modulation of epilepsy and associated conditions such as anxiety and memory deficit (Diniz *et al*., 2015). The extract of *Bryophyllum pinnatum* leaves is used in traditional medicine for the treatment of various neurological conditions including convulsion (Salahdeen and Yemitan, 2006). However, the therapeutic importance of *B. pinnatum*, particularly in animal models of neurological diseases to justify its traditional and ethnopharmacological use is yet to be adequately established scientifically. This study investigates the effect of *Bryophyllum pinnatum* on the central nervous system and convulsion in-vivo.

MATERIALS AND METHODS

Plant material and preparation of extract: Fresh leaves of *B. pinnatum* were collected in April 2017 from the wild in a suburb of Ibadan; it was identified and authenticated at the Department of Botany, University of Ibadan, Nigeria. Fresh leaves of *B. pinnatum* was washed clean with water and air dried. One hundred and eighty six grams (186 g) of pulverized dry leaf was macerated in 70% ethanol for 48 hours, followed by filtration using muslin sieve and double filtration on absorbent cotton and on Whatman 3 mm paper. The filtrate was then concentrated with a rotary evaporator at 40 ºC before allowing to dry forming a dark green colored paste extract. The dried extract was kept in a desiccator till use.

Animals: Adult male rats weighing between 150 - 200 g were obtained from Central Animal House (CAH), College of Medicine, University of Ibadan. The rats were allowed to acclimatize for about 7 days with adequate access to food (standard rodent pellet) and water. The study procedures strictly complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Drugs and chemicals: Glutamate, pentylenetetrazole, Tris-Potassium chloride, Adrenaline, 5′, 5′-Dithiobis- (2-nitrobenzoate) (Sigma-Aldrich, USA), Trichloroacetic acid (Burgoyne Burbidges and Co., India), Thiobarbituric acid (Guanghua Chemical Factory Co. Ltd., China), Sodium Carbonate (Fidsons, England), Sodium bicarbonate ($NaHCO_3$), Sodium chloride (NaOH) (J.T Baker Chemicals Co., USA). Hydrogen peroxide (H_2O_2) (BDH Chemicals, England).

Experimental Design

LD_{50} Determination: The LD_{50} of ethanol extract of *B. pinnatum* (EEBP) was determined as described by Lorke (1983). Initial dose finding procedure involved administering 10, 100 and 1000 mg/kg of extract orally to three groups of three mice each. General behavior of the animals and mortality was monitored for 24 hours in the treated animals. Four doses (800, 1600, 3200 and 6400 mg/kg) were then chosen based on the result of the dose finding procedure above, and administered to four groups of one animal per group (n = 1) to ascertain the safety of the extract at doses above 2000 and 5000 mg/kg. The LD_{50} of EEBP was calculated as

$$LD_{50} = \sqrt{A \times B}$$

Where A = Lowest dose showing death

B = Highest dose showing death

Drug Preparations and Treatments: Ethanol extract of *B. pinnatum* (EEBP) and other drugs used were regularly weighed out daily and dissolved in distilled water (vehicle). Ethanol extract of *B. pinnatum* was administered orally at chosen doses between 75 and 300 mg/kg. Pentylenetetrazole (PTZ) was administered at a subconvulsive dose of 30 mg/kg (i.p.). The animals were randomly divided into five groups (n = 6) and treated as follows; group 1 and 2 (10 mL/kg, distilled water p.o), group 3-5 (75, 150, and 300 mg/kg EEBP). All the groups except group 1 which served as the control, received 30 mg/kg PTZ every other day till the 25th administration or when group 2 showed a seizure score of 5. Twenty four hours after the last administration of PTZ, animal behavior was scored in activity cage for kindling induction and effect on cognition was evaluated using Y–maze test. Immediately after the behavior tests, the whole brain of the animal was removed for estimation of oxidative stress biomarkers (malondialdehyde, superoxide dismutase, catalase, nitrite, hydrogen peroxide and glutathione) and glutamic acid decarboxylase (GAD).

Kindling induction: Kindling is an important model for studying epilepsy and investigating long term structural and neurochemical changes in the brain (de Souza *et al*., 2019). Pentylenetetrazole is one of the most common chemical used to induce brain excitability. It has been used since invention by Goddard in 1967 as a chronic animal model for temporal lobe and complex partial epilepsy (Morimoto *et al*., 2004). It involves intraperitoneal injection of subconvulsive dose of convulsants on alternate or every other day till the animal shows sign of adequate kindling score of 5 on three consecutive administrations. If kindling score of 5 is not achieved on the 43rd day (i.e. 22nd injection) the PTZ administration is stopped. The animals were observed for 30 min after every PTZ administration. The kindling score or resultant seizure are as follows: no response (0); restlessness, hyperactivity and vibrissae twitching (1); head clonus and myoclonic jerks (2); unilateral or bilateral limb clonus (3); forelimb clonic seizures (4); generalized clonic seizure (Malhotra and Gupta, 1997).

Learning and memory: The effect of EEBP on cognitive dysfunction in epilepsy was investigated using the Y-maze. In this, following the end of the 43 days treatment, on the 44th day, each rat was gently placed individually in the Y-maze apparatus and allowed to explore all the three arms (labeled A, B, C) freely for 5 minutes, taking the following parameters:

the number of arm visits, and sequence (alternation) of arm visits (Luszczki *et al.*, 2005). The procedure was repeated for each animal after properly cleaning the apparatus to avoid odour from the previous animal (Brocco *et al.*, 2002). Alternation is entry into all three arms consecutively; consecutive entries is ABC, BCA, and CAB.
Percentage alternations was calculated as

$$\left[\frac{Actual\ alternation}{Maximum\ alternation}\right] \times 100$$

Where maximum alternation was calculated as
$[Total\ number\ of\ arms\ entered] - 2$

Biochemical assay: Rats were sacrificed through anaesthetized immediately after the behavioral analysis, and the brain was dissected using the dissecting kits. The whole brain was removed, weighed and kept in the refrigerator. Afterward, the brain was homogenized in 5 mL phosphate buffer (10% w/v, 0.1 M, pH 7.4) centrifuged (10,000 rpm for 10 min at 4 ºC) and supernatant separated immediately before discarding the pellet. The supernatant was aliquotted into portions for different biochemical assays.

Determination of Superoxide dismutase (SOD) activity
According to Misra and Fridovich, (1972), this method was based on the ability of superoxide dismutase to inhibit the adrenaline (epinephrine) autoxidation at pH 10.2 because, superoxide ($O_2^{\bullet-}$) radical generated by the xanthine oxidase reaction causes the oxidation of epinephrine to adrenochrome. A 1 in 10 dilution of brain supernatant (0.5 mL) in distilled water (4.5 mL) was prepared, and 0.2 mL of the diluted sample was added to 2.5 ml carbonate buffer (0.05M, pH 10.2) for equilibration. Freshly prepared adrenaline (0.3 mL, 0.3 mM) was then added (and quickly mixed by inversion) to start the reaction in the spectrophotometer. The blank or reference cuvette contained 2.5 mL carbonate buffer, 0.3 mL adrenaline (substrate) and 0.3 mL distilled water. The increase in absorbance was monitored for 60, 120 and 180 seconds at 480 nm. Superoxide dismutase (SOD) activity was measured as 1 unit necessary for 50% inhibition of the oxidation of adrenaline.

Determination of Catalase (CAT) activity: Catalase activity was determined according to the method described by Sinha (1971), which is based on the enzymes ability to decompose hydrogen peroxide (H_2O_2). A 1: 20 dilution of the supernatant was made by mixing supernatant (0.5 mL) with distilled water (9.5 mL). Phosphate buffer (2.5 mL; pH 7.0) and hydrogen peroxide solution (2.0 mL) in a 5 mL flat bottom conical flask to make the assay mixture. The diluted sample (0.5 mL) was rapidly mixed the assay mixture by gentle swirling at room temperature to make the reaction mixture. Reaction mixture (1 mL) was dispensed into 2 mL dichromate/acetic acid (5% solution of $K_2Cr_2O_7$ with glacial acetic acid) at 60 seconds intervals. Absorbance of the mixture was measured at 570 nm at 60 and 180 seconds intervals respectively. The catalase activity was expressed as µmoles of H_2O_2 decomposed per minute per mg protein.

Determination of Reduced Glutathione (GSH) activity: Reduced glutathione activity was measured according to method described by Jollow *et al*., (1974) which is based on reduced glutathione's ability to react with 5`, 5`-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a stable chromophoric product. The chromophoric product (2-nitro-5-thiobenzoic acid) formed by the reaction of DTNB with sulfhydryl compounds possess a characteristic absorbance at 412 nm which is proportional to the amount of reduced glutathione in the sample. The supernatant (0.4 mL) was added to equal volume of 20% trichloroacetic acid (TCA) and mixed by gentle swirling motion. The mixture was centrifuged (10000 rpm for 20 min at 4 ºC) and the supernatant was removed. The supernatant (0.25 mL) was added to 2 mL DNTB (0.6 mM) and the resulting solution was made up to 3 mL with phosphate buffer (0.75 mL; 0.2 M. pH 8.0). The resulting solution absorbance was read against blank (1 mL phosphate buffer + 2 mL DNTB 0.6 mM) at 412 nm using a spectrophotometer. Reduced GSH concentration was expressed as micromoles per gram tissue (µmol/g tissue).

Determination of Lipid Peroxidation: Lipid peroxidation was measured as malondialdehyde content according to the Okhawa *et al*., (1979) which is based on the fact that the unstable polyunsaturated lipids (fatty acid peroxides) generates a complex series of reactive carbonyl compounds (malondialdehydes) on decomposition. This carbonyl compounds (MDA) forms adduct (1:2) with thiobarbituric acid giving rise to pink coloured product when heated in acidic pH. The product is read at an absorbance of 532 nm. Briefly, the reacting mixture (containing brain supernatant 0.4 mL + tris-potassium chloride buffer 1.6 mL + 30% trichloroacetic acid 0.5 mL + 0.75% thiobarbituric acid 0.5 mL) was placed in water bath at 80 ºC for 45 min. The mixture was cooled in ice before centrifuging (3000 rpm, 15 min) to obtain a clear supernatant whose absorbance was against a blank (distilled water) at 532 nm. MDA concentration was calculated using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ CM^{-1}$ and expressed as µmole of MDA per gram tissue.

Determination of GAD activity: The brain supernatant (1 mL) was adjusted to pH 7 and transferred in the cuvette and incubated at 37°C for 5 min. The reaction was then started by addition of 100 µL glutamate solution (10 mM) and decarboxylation of glutamate at 340 nm was measured against a blank containing all components except glutamate using spectrophotometer (Cozzani, 1970).

Statistical Analysis: The data were expressed as mean ± S.E.M. (standard error of mean). The data was analyzed using Kruskal–Wallis test (non-parametric) and one–way analysis of variance (ANOVA) followed by post–hoc test (Dunnet's test) for multiple comparisons where appropriate using Graph Pad Prism software version 5. A level of $p < 0.05$ was considered as statistically significant for all tests.

RESULTS

Effect of ethanol extract of *Bryophyllum pinnatum* leaves (EEBP) on seizure score in PTZ-kindled rats: Treatment with EEBP (75 – 300 mg/kg) decreased the mean seizure score in PTZ-kindled rats in comparison with 30 mg/kg PTZ only group (Figure 1).

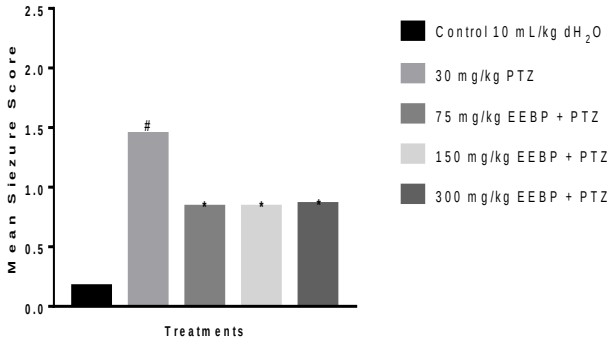

Figure 1:
Ethanol extract of *Bryophyllum pinnatum* leaf pretreatment decreased the seizure score in PTZ-kindled rats.
Seizure scores are expressed as 0 = no response; 1 = facial movements; 2 = myoclonic jerks (limbs and body); 3 = myoclonic jerks (upright position); 4 = clonic seizures; 5 = generalized clonic seizure. Each bar represent the mean score for six animals per group. All the values were expressed as Mean ± SEM, Data were analysed using one way ANOVA for multiple comparison followed by Kruskal-Wallis test as a *post-hoc* test. *significant difference from control (dH_2O, $p < 0.05$), #significance in comparison with PTZ only treated group. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole, dH_2O: distilled water.

Effect of ethanol extract of *Bryophyllum pinnatum* leaves (EEBP) on learning and memory in Y-maze test in PTZ-kindled rats: Treatment with EEBP leaves (75 – 300 mg/kg) increased the percentage alternation in PTZ-kindled rats. EEBP (75 - 300 mg/kg) significantly [F (4, 20) = 909.6; p < 0.05] increased the percentage alternation in comparison with the PTZ-only treated group. The percentage alternation in PTZ treated group was significantly [F (4, 20) = 909.6; p <0.05] lower than dH2O-treated group (Figure 2).

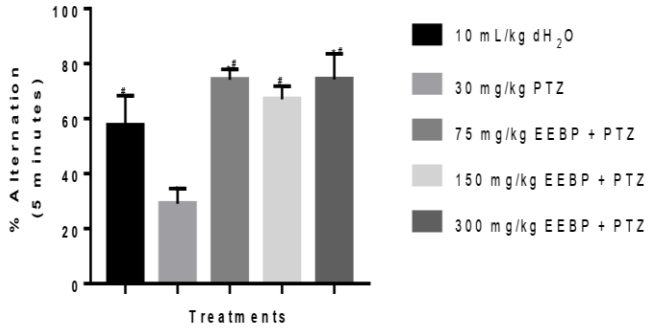

Figure 2:
Ethanol extract of *Bryophyllum pinnatum* leaves (EEBP) increased the percentage alternation in PTZ-kindled rats
All the values were expressed as Mean ± SEM, Analysis of data were done using one way ANOVA and Dunnet's multiple comparison test.*significant difference from control (dH_2O, $p < 0.05$), #significance in comparison with PTZ only treated group. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole, dH_2O: distilled water

Effect of ethanol extract of *Bryophyllum pinnatum* leaves (EEBP) on glutamic acid decarboxylase (GAD) in PTZ-kindled rats: The treatment with EEBP leaves (75 - 300 mg/kg) increased the level of GAD in PTZ-kindled rats. EEBP (150 - 300 mg/kg) significantly [F (4, 20) = 909.6; p < 0.05] increased the level of GAD in comparison with PTZ-only treated group. The GAD level in the PTZ-treated group was significantly [F (4, 20) = 909.6; p <0.05] lower than dH2O-treated group (Figure 3).

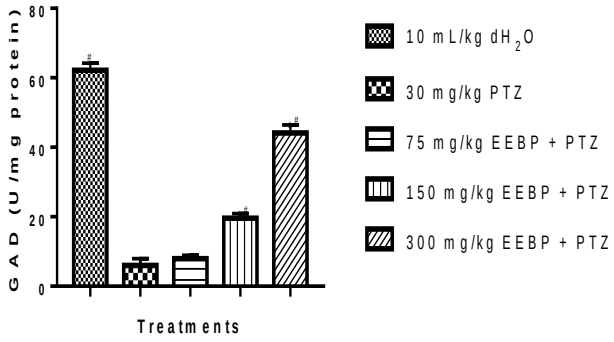

Figure 3:
Ethanol extract of *Bryophyllum pinnatum* leaf increased the level of glutamic acid decarboxylase GAD in PTZ-kindled rats
All the values were expressed as Mean ± SEM. Analysis of data were done using one way ANOVA and Dunnet's multiple comparison test. .*Significant difference from control (dH_2O, $p < 0.05$),#Significance at $p < 0.05$ in comparison with 30 mg/kg PTZ only. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole and dH_2O: distilled water.

Effect of ethanol extract of *Bryophyllum pinnatum* (EEBP) leaves on glutathione levels in PTZ-kindled rats: The treatment with EEBP leaves (75 - 300 mg/kg) increased the level of reduced glutathione in PTZ-kindled rats. EEBP (150 - 300 mg/kg) significantly [F (4, 20) = 909.6, p < 0.05] increased the level of reduced GSH in comparison with PTZ-treated groups. The reduced glutathione level in the PTZ-treated group was significantly [F (4, 20) = 909.6, p < 0.05] lower than dH2O-treated group (Figure 4).

Effect of ethanol extract of *Bryophyllum pinnatum* leaves on malonaldehyde (MDA) level in PTZ-kindled rats: The treatment with EEBP leaves (75 – 300 mg/kg) significantly [F (4, 20) = 2979; P<0.05] decreased the level of malonaldehyde (MDA) in PTZ-kindled rats in comparison with the PTZ alone group. Treatment with PTZ alone significantly [F (4, 20) = 2979; p <0.05] increased MDA level when compared the group that received dH2O only (56.95±1.217 vs. 0.1924±0.009) (Figure 5).

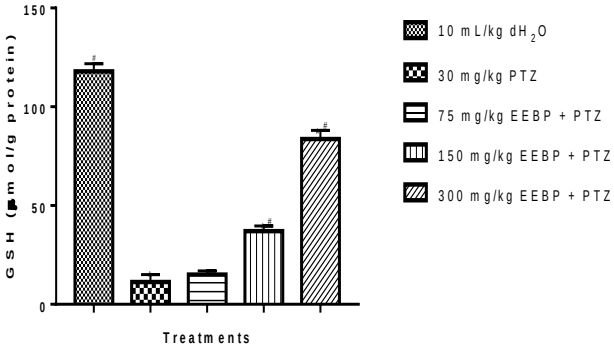

Figure 4: Ethanol extract of *Bryophyllum pinnatum* leaves (EEBP) increased level of reduced GSH in PTZ-kindled rats.
All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's multiple comparison test. *Significant difference from control (dH_2O, $p < 0.05$), #significance in comparison with PTZ only treated group. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole and dH_2O: distilled water.

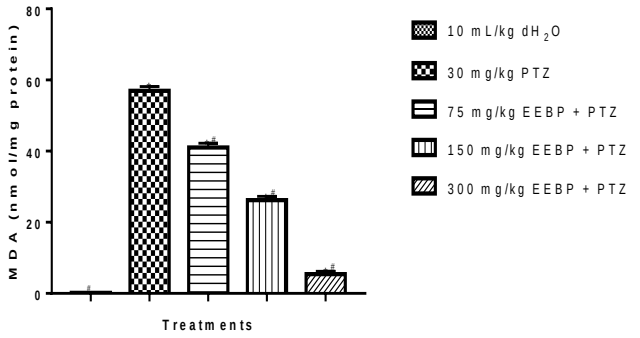

Figure 5:
Effect of ethanol extract of *Bryophyllum pinnatum* leaf on malonaldehyde levels in PTZ-kindled rats
All the values were expressed as Mean ± SEM, Analysis of data were done using one way ANOVA and Dunnet's multiple comparison test. *Significant difference from control (dH_2O, $p < 0.05$), #significance at $p < 0.05$ in comparison with 30 mg/kg PTZ only. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole and dH_2O: distilled water

Effect of ethanol extract of *Bryophyllum pinnatum* leaves on superoxide dismutase (SOD) level in PTZ-kindled rats: The SOD level in the PTZ-kindled rats (PTZ 30 mg/kg) was significantly [F (4, 20) = 909.6; p <0.05] lower than the group treated with dH2O or PTZ-kindled rats treated with EEBP (75-300 mg/kg). Treatment with EEBP (75-300 mg/kg) significantly [F (4, 20) = 909.6; p <0.05] increased the level of SOD in PTZ-kindled rats in comparison with group treated with PTZ alone (Figure 6).

Effect of ethanol extract of BP on catalase level in PTZ-kindled rats: There was a significantly [F (4, 20) = 909.6; p <0.05] lower level of catalase in the PTZ only (30 mg/kg PTZ) treated group in comparison with the dH2O only-treated control group. The administration of ethanol extract of *Bryophyllum pinnatum* leaf (150 - 300 mg/kg) significantly [F (4, 20) = 909.6; p <0.05] increased catalase level in comparison with the PTZ only-treated group (Figure 7).

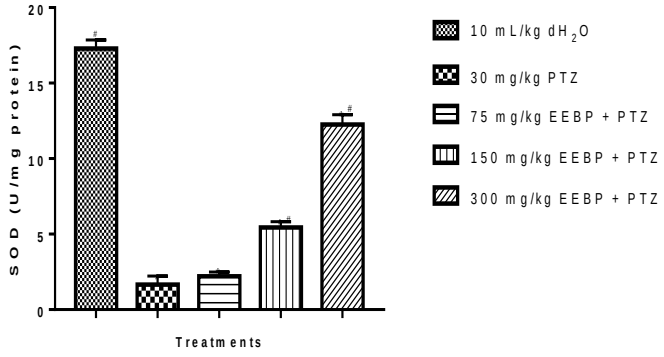

Figure 6:
Effect of ethanol extract of *Bryophyllum pinnatum* leaf on superoxide dismutase levels in PTZ-kindled rats
All the values were expressed as Mean ± SEM, Analysis of data were done using one way ANOVA and Dunnet's multiple comparison test. *Significant difference from control (dH_2O, $p < 0.05$)#Significance at $p < 0.05$ in comparison with PTZ only. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole and dH_2O: distilled water.

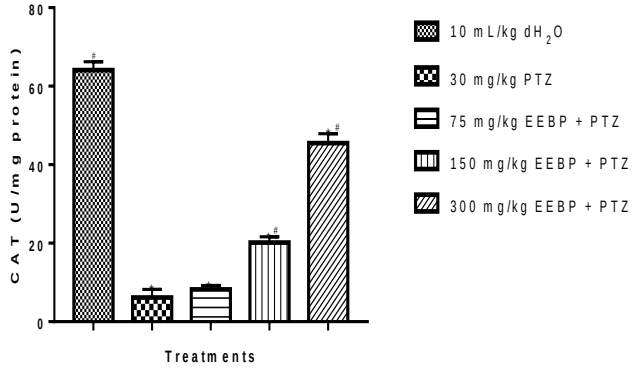

Figure 7:
Effect of ethanol extract of *Bryophyllum pinnatum* leaf on catalase levels in PTZ-kindled rats
All the values were expressed as Mean ± SEM, Analysis of data were done using one way ANOVA and Dunnet's multiple comparison test.*Significant difference from control (dH_2O, $p < 0.05$), # = significance at $p < 0.05$ in comparison with PTZ only. EEBP: ethanol extract of *Bryophyllum pinnatum* leaves, PTZ: pentylenetetrazole and dH_2O: distilled water

DISCUSSION

The Pentylenetetrazole-induced kindled test represents a valid and reliable model for human generalized and absence seizure (Chen *et al*., 2017). Pentylenetetrazole has been used severally in experiments to study seizure phenomenon and to identify potential drugs that may control seizure susceptibility (Patsoukis *et al*., 2004). Although direct mechanism of PTZ is not known in detail, literature records reveal that it causes alterations in GABAergic systems, glutamergic systems and antioxidant defense systems (Viswanatha *et al*., 2020). As shown in the study administration of EEBP to rats decreased the progression of epileptogenesis induced by PTZ kindling. On analysis of the result, lower doses of EEBP significantly reduced the period that rats remain in phase 5 seizure. The reduction in intensity and duration of seizure observed showed that EEBP could have effect on epileptogenesis.

Antiepileptic agents such as diazepam and phenobarbitone inhibit PTZ-induced seizure by binding to GABAA receptors, thus enhancing the opening of chloride channels mediated by GABA (Gilani *et al*., 2014). GABAA receptors on the postsynaptic membranes are multi-unit complexes containing binding sites for endogenous ligand GABA, benzodiazepine, barbiturate and similar ligands with a central chloride ion channel. Although the mechanism of induction of epilepsy by PTZ is unclear, single or repeated administration of convulsive or subconvulsive doses cause seizures in rodents via effects mediated through the GABAergic system (Erkeç and Arihan, 2015). Shimada and Yamagata 2018 reported that PTZ has the

ability to block flow of GABA receptor. Conversely, GABA dependent flow and uptake of chloride ion is increased by flumazenil in cultured cortical neurons. Flumazenil an antagonist of benzodiazepine which binds to GABAA receptor at the benzodiazepine binding site has shown in PTZ kindled mice that PTZ effect is mediated via benzodiazepine receptor (Ahmadiani, 2003). Thus, the reversal of PTZ-induced seizure by ethanol extract of *Bryophyllum pinnatum* leaves suggest that the effect might be due to its effect on GABAergic neurotransmission; although another possibility could be that glutamate-mediated excitations are depressed (Rubio-Casillas and Fernández-Guasti, 2016). Glutamic acid decarboxylase (GAD) is responsible for basal production of GABA from glutamate, and it also control neurogenesis (Tajabadi *et al*., 2015). Inhibition of GAD by ethylketopentenoate has been shown to induce treatment resistant epileptic seizure (Zhang *et al*., 2015). Thus, overexpression of GAD could be beneficial in an epileptic brain due to its ability to convert more glutamate to GABA, thereby reducing the excitatory action of glutamate and enhancing GABAergic transmission. The ability of EEBP in restoring the PTZ-induced reduction of GAD portends a possible mechanism for its action in epilepsy. PTZ-induced kindling is associated with cognitive deficit, changes in emotional behavior and neuronal cell loss in hippocampus, dentate gyrus and hilus (Alhaj *et al*., 2015). Similarly, psychiatric comorbidities especially depression, cognitive dysfunction and anxiety-like problems are often found in patients with epilepsy (Leo *et al*., 2019). Also, Tavakoli reported an associated decrease in the learning ability in epileptic patients in his clinical studies on memory disorders in such patients (Tavakoli *et al*., 2011). These problems have obviously significant impact on the quality of life of the patients. In the present study, PTZ-induced kindling greatly impaired cognitive performance in the elevated performance task which is often used for assessment of spatial long term memory and anxiety. However, a significant decline in the number of arm entries was also observed indicating that locomotor activity (and not cognitive decline) was responsible for effects on percentage alternation. Consistent with these observations, Y-Maze evaluation in this study also revealed that PTZ impaired cognitive function in the negative control groups treated with PTZ alone. Therefore, the finding that PTZ has potent ability to induce cognitive dysfunction in rats, a typical symptom associated with epilepsy, further supports how relevant it is as an animal model predictive of antiepileptogenic activity (Singh *et al*., 2021). In this study, EEBP was able to reverse the decrease in percentage of cognition induced by PTZ, producing a cognition-enhancing effect. This suggests that this extract has the ability to ameliorate the impaired working memory, set shifting, and other cognitive functions that are related to epilepsy (Zhao *et al*., 2014).

Free radicals are generated during generalized epilepsy, and they are responsible for progression of convulsion due to the deleterious effect of some of these reactive oxygen species and superoxide in the brain (Vishnoi *et al*., 2016). The detrimental effects of free radicals is destruction of cell membrane and cell dysfunction. The body in normal state is able to mop-up the free radicals through actions of various endogenous enzymes (glutathione reductase GR, glutathione peroxidase GP, superoxide dismutase SOD) and exogenous antioxidants (vitamins A, C and E) (Ali *et al*., 2014). There is significant involvement of free radicals in the pathogenesis of a wide range of neurological diseases including epilepsy (Suleymanova, 2021). Using rats with amygdale convulsion, Zhang *et al*., (2015) demonstrated that oxygen radicals were produced particularly in the hippocampus following seizure, and also posited that these radicals play a part progression of the epilepsy. Pentylenetetrazole-induced seizure and seizure-induced stress have been reported to be significantly reduced by antioxidants (Moezi *et al*., 2015). Furthermore, patients with epilepsy have been observed to have low serum level of antioxidants and increased malondialdehyde (Keskin Guller *et al*., 2016). PTZ initiates various processes such as membrane phosphorylation, proteolysis, and consequently release of free fatty acids, diacylglycerols, eicosanoids, lipid peroxides and free radicals (Doctor *et al*., 1982). In the present study, EEBP decreased MDA level in PTZ treated rats, and this effect might be due to its ability to reduce the spate of tissue injury and hence antioxidant effect. Consequently, the observed antiepileptic potential of *Bryophyllum pinnatum* extract might be attributable to its antioxidant properties.

Superoxide dismutase (SOD) and catalase (CAT) are endogenous antioxidants enzymes whose level are increased by treatment with EEBP in PTZ-induced oxidative stress. The level of glutathione GSH, an endogenous antioxidant with critical role in the cellular detoxification of reactive oxygen species is increased by antioxidants (Naha *et al*., 2015). Its defensive mechanism is by getting itself oxidized to prevent formation of toxic hydroxyl radical. The level of GSH is significantly depleted in the brain of epileptic patients and significant depletion in GSH level has been observed in chronic models of epilepsy as well as in the brains of patients with chronic epilepsy (Du *et al*., 2013). In this study, treatment with EEBP restores the depleted level of GSH caused by PTZ-induced oxidative stress. The antioxidant property of EEBP in PTZ-induced oxidative stress might be mediated via restoration of depleted GSH level and increase the level of antioxidant enzymes.

In conclusion, this study provides valuable evidence that *Bryophyllum pinnatum* possess antiepileptogenic and antioxidant properties. The plant might be involved in the physiological regulation of glutamatergic system as well as the modulation of reduction-oxidation (redox) system. Thus, justifying its ethnomedicinal claims in the management of epilepsy.

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