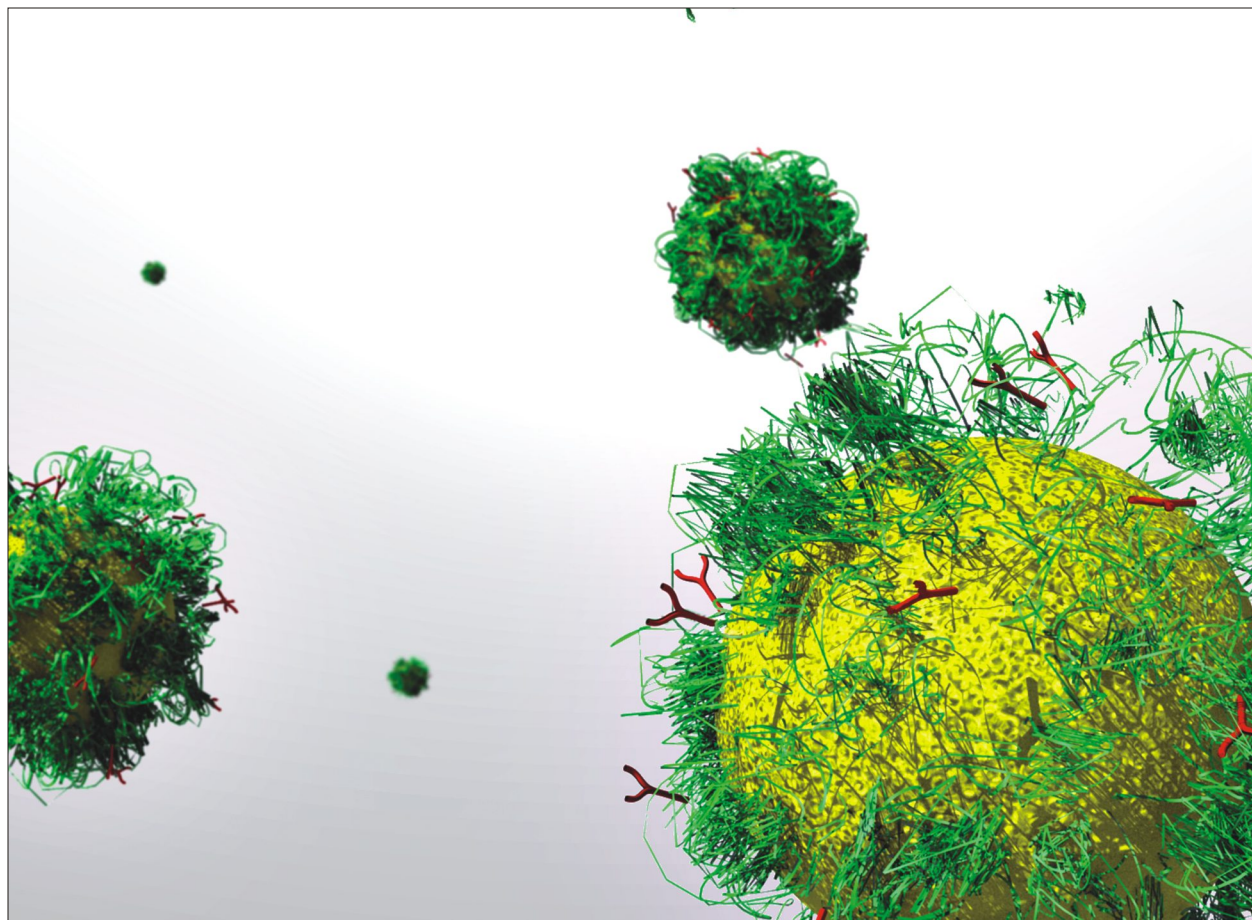




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Effect of *Momordica dioica* Roxb. Fruits on Pentylentetrazole Induced Convulsions and Oxidative Stress in Mice

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ABSTRACT

The main objective of the study is to evaluate anticonvulsant and antioxidative effect of Ethanolic extract of *Momordica dioica* fruits in mice. The effect of alcoholic extract of *Momordica dioica* Roxb. fruits on convulsions induced using Pentylentetrazole, changes in Acetyl cholinesterase (AChE) activity, Levels of Glutamate and GABA were studied using standard procedures. Markers of oxidative stress such as Malondialdehyde, Glutathione reductase, Superoxide dismutase and catalase were evaluated in mice using standard procedure. The ethanolic extract of *Momordica dioica* Roxb. increased duration of onset of convulsions ($p<0.001$), decreased the levels of AChE, glutamate ($p<0.001$) and increased the levels GABA ($p<0.001$) in brain as compared with disease control group. A marked effect on oxidative parameters such that lower MDA levels ($p<0.01$), increased levels of GSH, SOD, Catalase ($p<0.001$) were estimated comparing to disease group. From the study it has been proved that the plant extract showed anticonvulsant and antioxidative effect.

Key words: Epilepsy, pentylentetrazole (PTZ), diazepam, ethanolic extract of *Momordica dioica* (EEMD), seizures

INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons (Goodman and Gilman's, 2001). It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients (Rasilingam *et al.*, 2009; Mattson, 1992). The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valporate carry with them several serious side effects notably neurotoxicity (Gupta and Malhotra, 1997). As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. The aim of treating an epileptic is not only to abolish the occurrence of seizures but also to lead a self sustained life (Goodman and Gilman's, 2001).

In the present study, we have selected a plant namely *Momordica dioica* (Roxb.) belonging to family Cucurbitaceae. Fruit of plant are green and generally used as vegetable. It possesses many medicinal properties. Fruit are diuretic, alexiteric stomachic laxative, hepatoprotective and have antivenum property (Kirtikar and Basu, 1999). Phytoconstituents of *Momordica dioica* are traces of alkaloids, steroids, triterpenoids (Luo and Li, 1997), flavonoids, glycosides, saponins (Sadyojatha and Vaidya, 1995), triterpenes of urisolic acid dark brown semidrying oil and saturated fatty acids, ascorbic acids, vitamin A, thiamine, ribnofflavins, niacin, protein carbohydrates, lectins (Ghosh *et al.*, 1981), ascorbic acids, carotenes, bitter principles, oleanoic acid, stearic acid, gypsogenin, alpha-spiranosterol hederagenin, momordicaursenol (Ali and Srivastava, 1998). The alkaloid present in seed called momordicin and present in root called momordicafoetida.

Drugs and chemicals: Pentylentetrazole (sigma, USA), Diazepam, Glutamate, GABA, Thiobarbituric acid.

MATERIALS AND METHODS

Plant material and preparation of the extract: Fruits were procured from the local commercial market and authenticated for their correct botanical identity by chief botanist. The fruits are dried and coarsely powered with a grinder. For the preparation of alcoholic extract, the coarse powder of fruits was extracted with 5 parts of alcohol by maceration for 7 days and was filtered through a 400 mesh cloth to collect the extract. The extract was concentrated and finally dried to obtain the dried extract. The percentage w/w yield of aqueous extract was 12.

Animals: Study was carried out using Swiss albino mice weighing about 20-25 g. They were obtained from sainath laboratories, Hyderabad. The mice were group housed in polyacrylic cages with not more than four animals per cage and were maintained under standard laboratory conditions with natural light and dark cycles (approximately 14 h light-10 h dark cycle) and room temperature of $25\pm 1^{\circ}\text{C}$. They were allowed free access to standard dry diet and tap water ad libitum. All procedures described were reviewed and approved by Institutional Animal Ethics Committee (IAEC) (1047/ac/07/CPCSEA, Dated 24-04-2007).

Experimental design: Animals were randomly divided into five groups of each six animals. The first group received distilled water and normal saline through i.p. route, second group.

Assessment of anticonvulsant activity: Mice of either sex with a body weight between 18-22 g were used. Only vehicles are given through i.p. and oral route in group-1. Group-2 serving as disease control administered with PTZ (80 mg kg^{-1}) i.p. while in group-3 i.p. administration of PTZ (80 mg kg^{-1}) is done after 15 min of s.c. administration of diazepam (5 mg kg^{-1} , standard drug). The test compound was given to groups of 6 mice in group-4 (100 mg kg^{-1}) and group-5 (300 mg kg^{-1}) orally; 60 min after 80 mg kg^{-1} PTZ was injected intaperitoneally. Each animal was placed into an individual plastic cage for observation lasting for 30 min. Seizures and onset of tonic-clonic convulsions were recorded (Kulkarni, 2005).

Biochemical tests: After recordings of seizures and tonic-clonic convulsions, the animals were decapitated under ether anesthesia and the brains were quickly removed, cleaned with ice-cold saline and stored at 8°C .

Tissue preparation: Brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4).

Estimation of AChE activity: Acetyl cholinesterase (AChE) enzyme activity was estimated by Elman method (Ellman *et al.*, 1961), 0.4 mL aliquot of the homogenate is added to a cuvette containing 2.6 mL phosphate buffer (0.1 M, pH 8) and 100 μ L of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a spectrophotometer. When absorbance reaches a stable value it is recorded as the basal reading. 20 μ L of substrate i.e., acetylthiocholine is added and change in absorbance is recorded. Change in the absorbance per minute is thus determined.

Estimation of glutamate and GABA: The level of Glutamate was estimated by multiple development paper chromatography as described by Raju *et al.* (2004). The 1.0 mL of the supernatant from brain homogenate was evaporated to dryness at 70°C in an oven and the residue is reconstituted in 100 mL of distilled water. Standard solutions of glutamate and GABA at a concentration of 2 mM along with the sample are spotted on Whatman No. 1 chromatography paper using a micropipette. It was placed on a chamber containing butanol: Acetic acid: Water (12: 3: 5 v/v) as solvent. When the solvent front reached the top of the paper it was removed and dried. A second run is performed similarly, after which the papers are dried sprayed with ninhydrin reagent and placed in an oven at 100°C for 4 min. The portions which carry glutamate corresponding with the standard are cut and eluted with 0.005% CuSO₄ in 75% ethanol. Their absorbance is read against blank at 515 nm in spectrophotometer.

Lipid peroxidation: MDA, a measure of lipid peroxidation, was measured as described by Ohkawa *et al.* (1979). The reagents acetic acid 1.5 mL (20%) pH 3.5, 1.5 mL Thiobarbituric acid (0.8%) and 0.2 mL sodium dodecyl sulphate (8.1%) were added to 0.1 mL of processed tissue samples and then heated at 100°C for 60 min. The mixture was cooled with tap water and 5 mL of nbutanol/pyridine (15:1), 1 mL of distilled water was added. The mixture was vortexed vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. The concentration of MDA is expressed as nmol g⁻¹ tissue.

Glutathione reductase: Glutathione was measured according to the method of Ellman *et al.* (1961). The equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 mL of this supernatant, 2 mL of phosphate buffer (pH 8.4), 0.5 mL of 505-dithiobis (2-nitrobenzoic acid) and 0.4 mL of double distilled water were added. The mixture was vortexed and the absorbance read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as μ mg g⁻¹ tissue.

Catalase: Catalase activity was measured by the method of Aebi, 1974. 0.1 mL of supernatant was added to cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H₂O₂. The rate of decomposition of H₂O₂ was measured spectrophotometrically from changes in absorbance at 240 nm.

Super oxide dismutase: The SOD activity in supernatant was measured by the method of Misra and Fridovich (1976). The supernatant (500 μ L) was added to 0.800 mL of carbonate buffer

(100 mM, pH 10.2) and 100 μ L of epinephrine (3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec.

Statistical analysis: The data represented as Mean $\pm$ S.D. statistical comparisons were performed by one-way ANOVA followed by Dunnett's test using Graph Pad Prism 5.0, USA. $p < 0.05$ was considered significant.

RESULTS

Effect of EEMD (100 and 300 mg kg⁻¹) on seizure activity in PTZ induced groups: All the animals showed complete convulsions in PTZ group. Pre-treatment with EEMD showed 33.3 and 66.67% protection against PTZ-induced convulsions at doses of 100 and 300 mg kg⁻¹, respectively (Table 1). Complete protection against seizures was observed with Diazepam (5 mg kg⁻¹). All the groups were compared significantly with disease group.

Biochemical parameters

Effect of EEMD (100 and 300 mg kg⁻¹) on cholinesterase activity in PTZ induced groups: A decrease in cholinesterase activity has been observed in group dosed at 300 mg kg⁻¹ of plant extract at *** $p < 0.001$ as compared with disease control * $p < 0.05$ (Fig. 1).

Table 1: Effect of Diazepam and doses of ethanolic extract of *Momordica dioica* (EEMD) on PTZ induced seizures in mice

Treatment groups	Dose (mg kg ⁻¹)	Onset of Jerks convulsions	Onset of tonic convulsions	Onset of clonic convulsions	Status of animals after 30 min (Dead/Alive)	Protection against mortality (%)
Control	Vehicle	-	-	-	0/6	-
PTZ	80	1.046 $\pm$ 0.024	1.508 $\pm$ 0.273	3.376 $\pm$ 0.414	6/0	-
Diazepam	5	23.756 $\pm$ 1.596***	32.632 $\pm$ 0.589***	39.386 $\pm$ 0.637**	0/6	100
EEMD-1	100	1.533 $\pm$ 0.253 ^{ns}	2.968 $\pm$ 0.371*	6.713 $\pm$ 0.493**	4/2	33.33
EEMD-2	300	3.166 $\pm$ 0.302***	4.345 $\pm$ 0.424**	6.166 $\pm$ 0.594***	2/4	63.67

Data are shown in Mean $\pm$ S.D. In each group n = 6 and EEMD indicates ethanolic extract of *M. dioica* *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$ and ns: non-significant vs PTZ control

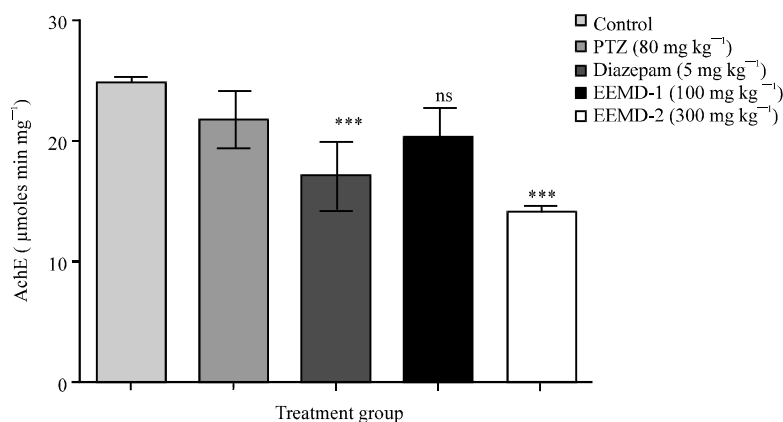


Fig. 1: Effect of EEMD on AChE enzyme levels in mice brain of PTZ induced convulsions. Data are shown in Mean $\pm$ S.D (No. of animals n = 6). *** $p < 0.001$ vs disease group, ns indicates non-significant

Effect of EEMD (100 and 300 mg kg⁻¹) on glutamate levels in PTZ induced groups: In PTZ+vehicle treated groups, administration of PTZ (80 mg kg⁻¹) resulted in convulsive nature. A decrease in the concentration of glutamate in animals given EEMD 100 and 300 mg kg⁻¹ (*p<0.05, ***p<0.001, respectively) as compared with the disease control group administered with vehicle and PTZ is observed (Table 2) (Fig. 2).

Effect of EEMD (100 and 300 mg kg⁻¹) on GABA levels in PTZ induced groups: Increased levels of GABA, **p<0.001 in groups of EEMD (100 and 300 mg kg⁻¹) were compared with animals treated with only PTZ. Increased levels of GABA showed lower seizure activity in the groups.

Effect of EEMD (100 and 300 mg kg⁻¹) on malondialdehyde (MDA) levels in PTZ induced groups: A difference was found in the brain MDA levels in between the groups ***p<0.001. The post Dunnett's analysis revealed the MDA level was increased in PTZ groups ***p<0.001 as compared to vehicle treated control groups. Reversal of the increased MDA levels was observed in EEMD pre treated groups in comparison to PTZ group (Fig. 3) (Table 2).

Effect of EEMD (100 and 300 mg kg⁻¹) on levels of glutathione reductase in PTZ induced groups: In PTZ-induced convulsions, decrease ***p<0.001 in GSH levels in PTZ treated groups is observed. EEMD pre-treated groups reversed the PTZ induced decrease in GSH levels at doses tested ***p<0.001, **p<0.001 in 100 and 300 mg kg⁻¹ dose (Table 2).

Table 2: Effect of diazepam and doses of ethanolic extract of *Momordica dioica* (EEMD) on MDA, GSH levels and catalase, SOD activity after PTZ induction in mice

Treatment groups	MDA (n moles g ⁻¹ tissue)	GSH (µg g ⁻¹ tissue)	SOD(U)	Catalase (n moles g ⁻¹ tissue)
Control	14.20±1.86	143.62±2.00	0.127±0.04	64.11±6.41
PTZ	87.49±4.00	46.68±1.74	0.098±.008	37.37±4.40
Diazepam	41.87±3.10***	120.77±2.37***	0.121±0.018***	53.04±2.91***
EEMD-1	66.34±3.81*	76.33±2.49**	0.133±0.011*	46.08±4.11**
EEMD-2	51.70±1.85***	95.94±1.83***	0.136±0.018**	50.10±3.58***

Data are shown in Mean±S.D. In each group n = 6 and EEMD indicates ethanolic extract of *M. dioica*. ***p<0.001, **p<0.01, *p<0.05 and ns as nonsignificant vs PTZ control

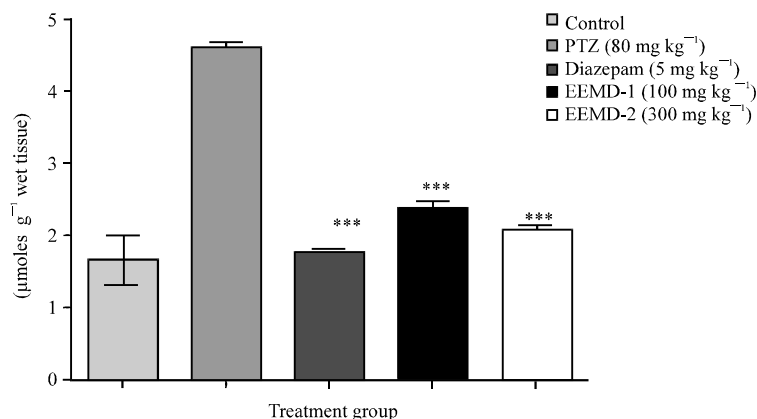


Fig. 2: Effect of EEMD on glutamate levels in mice brain of PTZ induced convulsion. Data are shown in Mean±S.D (No. of animals n = 6). ***p<0.001 vs disease group

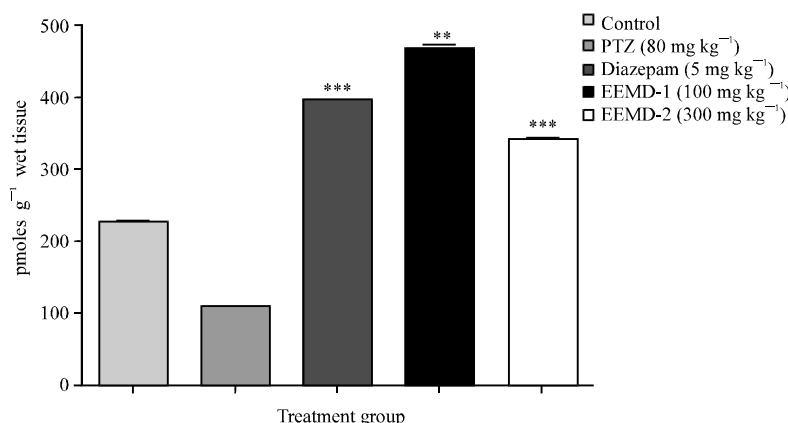


Fig. 3: Effect of EEMD on GABA levels in mice brain of PTZ induced convulsions. Data are shown in Mean±S.D (No. of animals n = 6). **p<0.01, ***p<0.001 vs disease group

Effect of EEMD (100 and 300 mg kg⁻¹) on levels of catalase in PTZ induced groups: High levels of catalase in PTZ+vehicle treated groups than the EEMD 100 **p<0.01 and 300 mg kg⁻¹ ***p<0.001 groups was observed (Table 2).

Effect of EEMD (100 and 300 mg kg⁻¹) on levels of SOD in PTZ induced groups: Low SOD activity in PTZ treated groups was observed ***p<0.001. The EEMD 100 (*p<0.05) and 300 mg kg⁻¹ (**p<0.01) treated groups showed increased SOD activity compared with PTZ groups (Table 2).

DISCUSSION

In the present study, convulsions were induced after PTZ (80 mg kg⁻¹) administration in all groups of mice.

EEMD (300 mg kg⁻¹) pre-treated groups showed increase in onset of seizure reflexes after PTZ administration and protection against seizure susceptibility. GABA_A receptor channels blocking play a crucial role in epileptic seizure development (Rebrov *et al.*, 2004). The lower dose of EEMD (100 mg kg⁻¹) also showed a lower seizure activity after PTZ administration.

The decrease in AChE enzyme levels brains, after PTZ induced seizures have also been reported (Appleyard *et al.*, 1986; Visweswari *et al.*, 2010). In agreement with earlier studies, our results also showed decrease in AChE following PTZ induced seizure. Glutamate and GABA levels in EEMD (100 and 300 mg kg⁻¹) pre-treated groups showed protective effect against seizure generation.

Seizure induces oxidative stress inhibition has also been observed which significantly inhibit PTZ induced seizures.

Lipid peroxidation was measure through estimation of MDA which inidactes the free radical generation in the brains of PTZ treated groups. PTZ is a inducer of various processes such as membrane phosphorylation, proteolysis and nuclease and consequently release of free lipid peroxides and free radicals. The lowered levels of MDA in the brains of 100 and 300 mg kg⁻¹ pre-treated groups were compared with PTZ treated groups indicating attenuation of lipid peroxidation.

A significant low level of reduced glutathione in PTZ treated mice was observed. Glutathione is an endogenous antioxidant present mainly in the reduced form within the cells. Free radical generation in the brain of mice after PTZ administration shows effect on depleting the reduced glutathione by combating oxidative stress (Schulz *et al.*, 2000).

The enzyme SOD itself is an antioxidant enzyme which catalyses the conversion of superoxide to hydrogen peroxide and in this way protects the cell against the oxidative stress. In the present study it was observed that EEMD at all doses could increase SOD level relative to PTZ mice. This can lead to the conclusion that probably EEMD with antioxidant effect is able to preserve the antioxidant enzyme SOD and consequently affect seizure intensity and duration.

In conclusion, the present study demonstrates that *Momordica dioica* significantly prevented the seizure susceptibility and attenuated the oxidative stress induced by PTZ kindling. Therefore it could offer a useful support to the basic antiepileptic therapy in preventing the development of cognitive impairment reported with several AEDs.

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