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Research Article

ANTI EPILEPTIC ACTIVITY OF *SAPINDUS EMERGINATUS* VAHL FRUIT EXTRACT IN PENTYLENETETRAZOLE INDUCED SEIZURE MODEL

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ABSTRACT

Objective: *Sapindus emerginatus* (sapindaceae) is used as traditional Indian medicine to treat epilepsy. The purpose of the present study is to investigate the effect of methanol extract of *Sapindus emerginatus* (MESE) on neurochemical concentrations in rat brain after induction of seizure by Pentylenetetrazole (PTZ).

Method: Method includes relationship between seizure activities and altered monoamines such as nor adrenaline, Dopamine, Serotonin and Gamma amino butyric acid in forebrain of rats in Pentylenetetrazole model.

Result: In Pentylenetetrazole model, extracts (200 & 400 mg/kg) significantly restored the decreased levels of brain monoamines such as nor adrenaline, Dopamine, Serotonin and Gamma amino butyric acid.

Conclusion: Thus, this study suggests that methanol extract of *Sapindus emerginatus* had increased the monoamine levels on rat brain, which were decreased due to the susceptibility to Pentylenetetrazole induced seizure in rats.

Keywords: Antiepileptic activity, Pentylenetetrazole, *Sapindus emerginatus*, Nor adrenaline, Dopamine, Serotonin and Gamma amino butyric acid.

INTRODUCTION

Epilepsy is among the most prevalent of the serious neurological disorders, affecting from 0.5 to 1.0% of the world's population[1]. Interestingly, the prevalence of epilepsy in developing countries is generally higher than in developed countries[2]. Epilepsy is a common neurological disorder characterized by paroxysmal dysrhythmia, seizure, with or without body convulsion and sensory or psychiatric phenomena[3]. There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include, 1) Diminition of inhibitory mechanism (especially synaptic inhibition due to GABA) 2) Enhancement of the excitatory synaptic mechanism (especially those mediated by NMDA). 3) Enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents). Different forms of human epilepsy may be caused by any one or combination of the above mechanisms**.** The agitated neuronal activity that occurs during a seizure is caused by a sudden imbalance between the inhibitory and excitatory signals in the brain with δ-amino butyric acid (GABA), nor adrenaline, serotonin, and dopamine respectively; being the most important neurotransmitters involved[4]. Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening can be an invaluable source for search of new antiepileptic compounds.

Sapindus emerginatus (soap nuts, soap berry, reetha) have historically been used in folk remedies as a mucolytic agents, emetic, contraceptive and for treatment of excessive salivation, epilepsy, and to treat chlorosis. Modern scientific medical research has investigated the use of soap nuts in treating migraine and epilepsy. Therefore, the present study was performed to examine the Anti Epileptic activity of *Sapindus emerginatus* vahl fruit extract in Pentylenetetrazole induced seizure model

MATERIALS AND METHODS

Plant material

Fruits of *Sapindus emarginatus* were collected from RangaReddy district and authenticated from Dept. of Botany, Osmania University, with voucher no.0167. Then they are washed under running tap water, air dried and then grinded coarsely and stored in air tight containers.

Preparation of the extract

Coarsely powdered fruits 500g were packed in sox let apparatus and extracted using 2000 ml methanol as solvent. After extraction, solvent was filtered and concentrated under reduced temperature and pressure. The resulted extract yield was 7.45% and the appearance of the extract was dried gum resin in nature.

Preliminary phytochemical studies[5]

Preliminary photochemical studies indicate the presence of Carbohydrates, Proteins, Flavonoids, Phytosterols, Triterpinoids, and Volatile oils in the extract. So, the anticonvulsant activity of methanol extract of plant in different dose levels (200mg/kg and 400mg/kg) was studied.

Acute Oral Toxicity Study[6]

For the LD50 dose determination, methanol extract of *Sapindus emerginatus* fruits were administered up to dose 2000 mg/kg body weight and extract did not produce any mortality, thus 1/5th, 1/10th, of maximum dose tested were selected for the present study. LD50 of methanol extract of *Sapindus emerginatus fruits* were found to be 2000 mg/kg.

Experimental Animals

Male albino wistar rats weighing between 180-220 gm were procured from the Departmental Animal House, Nishka laboratories, Uppal, Hyderabad, India. Animals were housed in polycarbonate cages at a room with a 12 h day-night cycle, temperature of 22 ± 2°C and humidity of 45-64%. During the whole experimental period, animals were fed with a balanced commercial diet and water ad *libitum.* The experimental study was conducted according to CPCSEA norms, after obtaining Animal Ethical Committee approval from the Institutional Animal Ethical Committee, Ref. No-22-11-2000/282

Experimental design

Pentylenetetrazole (PTZ)-induced seizures model

The method used was adapted from that described by Swinyard *et al.,* (1985) [7]. Wistar rats were divided in to four groups of six animals each. Group I served as control (vehicle treated i.e. Tween 80, 2%), Group II served as standard received Diazepam 4mg/kg body weight (i.p.), Group III and Group IV were treated with methanol extract as 200 and 400mg/kg body weight (p. o) respectively 30 min after i.p.

injection of Diazepam and 60 min after oral administration of extracts, 60mg/kg PTZ was injected intraperitoneally. Onset to forelimb cloni, as well as hind limb extension (tonic convulsion) was recorded. The onset and number of death after showing tonic hind limb extension were also recorded. Rats that did not convulse 30 min after pentylenetetrazole administration will be considered protected.

Estimation of Serotonin, Nor - adrenaline and Dopamine

Preparation of tissue extracts by method Schlumpf M et al 1974[8]

Reagents

1. HCl – Butanol sol.: (0.85 ml of 37% hydrochloric acid in one-litre *n*-butanol)

2. Heptane

3. 0.1 M HCl: (0.85 ml conc. HCl up to 100 ml H_20)

Procedure

At the end of experiment rats were sacrificed, whole brain was dissected out and the sub cortical region (including the striatum) was separated. Tissue was weighed and was homogenized in 5ml HCl–butanol for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 ml) was removed and added to centrifuge tube containing 2.5 ml heptane and 0.31ml HCl of 0.1 M. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2 ml) was then taken either for 5-HT or NA and DA assay. All steps were carried out at 0^0C. (N.B: It taken in between 50-75 mg of tissue for homogenate with 5 ml of HCl-Butanol in correlation of same tissue concentration 1.5-5 mg/0.1 ml of HCl-butanol used in Schlumpf M et al, 1974. This is done to get adequate amount of supernatant liquid for analysis)

Estimation of nor adrenaline and dopamine [Dilip Kumar, 2009] [9]

Reagents

1. 0.4M HCl: 3.4 ml conc. HCl up to 100 ml H_20

2. Sodium acetate buffer (pH 6.9): 2.88 ml of 1M acetic acid (5.7 ml of Glacial acetic acid up to 100 ml with distilled water) +27.33 ml of 0.3M sodium acetate (4.08 g of sodium acetate 100 ml with distilled water) and volume is made up to 100 ml with distilled water). pH is adjusted with sodium hydroxide solution.

3. 5M NaOH: 20 g of sodium hydroxide pellets dissolved in distilled water and volume is made up to 100 ml with distilled water)

4. 0.1 M Iodine solution (in Ethanol): 4 g of pot. Iodide +2.6 g of iodine dissolved in ethanol volume is made up to 100 ml)

5. Na_2SO_3 sol. ((0.5 g Na_2SO_3 in 2 ml H_2O + 18 ml 5 M NaOH)

6. 10M Acetic acid: 57 ml of glacial acetic acid dissolved in distilled water up to 100 ml.

Procedure

To the 0.2 ml of aqueous phase, 0.05 ml 0.4 M HCl and 0.1 ml of EDTA / Sodium acetate buffer (pH 6. 9)were added, followed by 0.1 ml iodine solution(0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.1 ml Na_2SO_3 solution. 0.1 ml Acetic acid is added after 1.5 min. The solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375 nm for dopamine and 395-485 nm for nor-adrenaline.

Estimation of Serotonin

The serotonin content was estimated by the method of Schlumpf et al 1974.

Reagents

O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 0.2 ml aqueous extract 0.25 ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Tissue blanks for Dopamine and nor-adrenaline were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). For serotonin tissue blank, 0.25 ml cont. HCI without OPT was added. Internal Standard: (500 µg/ml each of nor adrenaline, dopamine and serotonin are prepared in distilled water: HCl-butanol in 1:2 ratio.

Estimation of brain GABA content[10]

The brain amino butyric acid (GABA content was estimated according to the method of Lowe et al., (1958). Animals were sacrificed by decapitation and brains were rapidly removed, and separated forebrain region. It was blotted, weighed and placed in 5ml of ice-cold trichloroaceticacid (10% w/v), then homogenized and centrifuged at 10,000rpm for 10min at 0°C. A sample (0.1ml) of tissue extract was placed in 0.2ml of 0.14 M ninhydrin solution in 0.5M corbonate-bicorbonate 1 buffer (pH9.95), kept in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartar ate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10min fluorescence at 377/455nm in a spectofluorimeter was recorded.

Statistical Analysis

The Data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dun net's test p values less than 0.05 were considered as significance.

RESULTS

Effect of MESE on Pentylenetetrazole induced seizures (Graph 1)

Values represent mean of six observations.

Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III and Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dun net's "t" test.

** P<0.01,* P<0.05 significant when compared to control.

In PTZ induced seizures, MESE at dose 200 and 400 mg/kg body weight exhibited delayed onset of clonus 88.10±0.220 and 92.66±0.545 sec. respectively in comparison to control 75.12±0.540 sec. For the extensor phase MESE at dose 400mg/kg showed 320.80±0.505sec as significant anticonvulsant activity in comparison to control extensor (275.11±0.220sec) with P<0.01.

Effect of MESE on Biogenic amines estimation

Values represent mean of six observations.

Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III and Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dun net's "t" test. ** P<0.01,* P<0.05 significant when compared to control.

Serotonin

In pentelenetetrazole model, Serotonin levels significantly (p<0.01) decreased in forebrain of epileptic control animals were observed. MESE at the doses of 200&400mg/kg, standard drug Diazepam treated animals showed a significantly (p<0.05) increased in Serotonin levels in forebrain of rats.

Dopamine

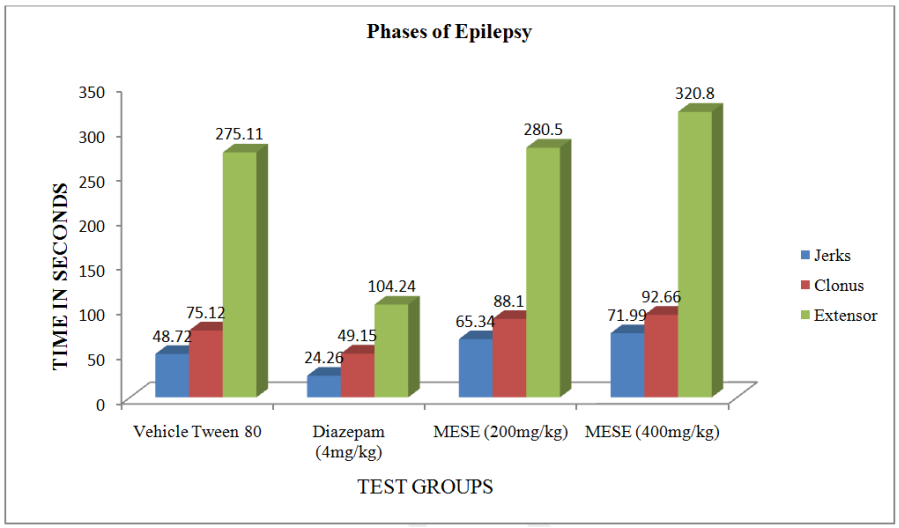
In pentelenetetrazole model, Dopamine levels significantly (p<0.01) decreased in forebrain of epileptic control animals were observed. MESE at the doses of 200&400mg/kg, standard drug Diazepam treated animals showed a significantly (p<0.05) increased in Dopamine levels in forebrain of rats.

Nor adrenaline

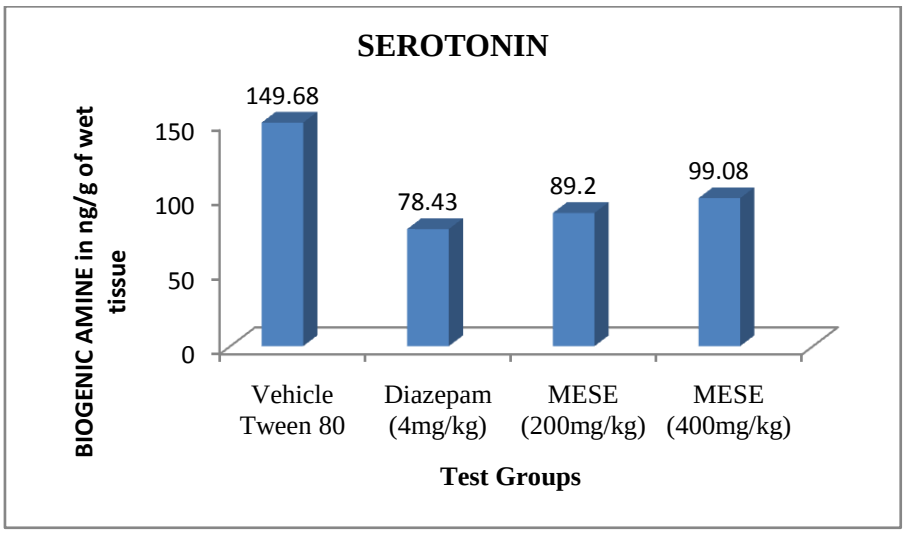
In pentelenetetrazole model, nor adrenaline levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. MESE at the doses of 200&400mg/kg, standard drugs Diazepam treated animals showed a significantly ($p<0.05$) increased in nor adrenaline levels in forebrain of rats.

Gamma amino butyric acid

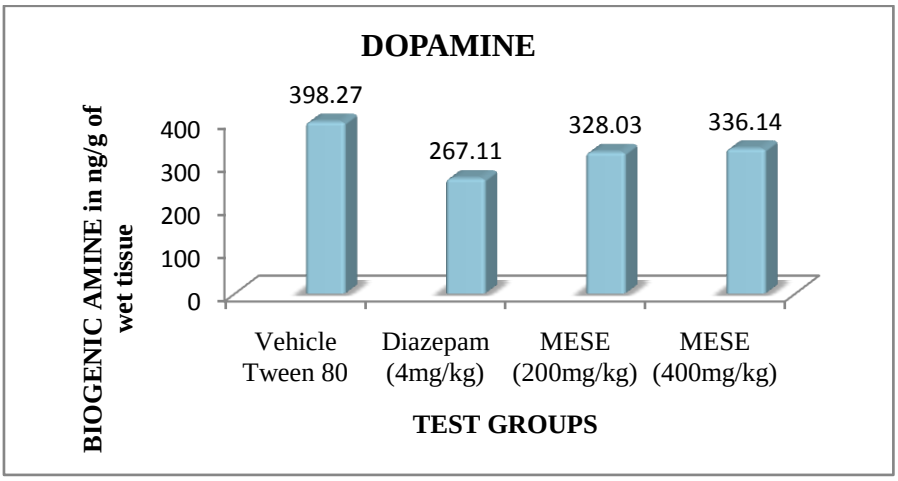
In pentelenetetrazole model, GABA levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. MESE at the doses of 200&400mg/kg, standard drug Diazepam treated animals showed a significantly ($p<0.05$) increased in GABA levels in forebrain of rats.



Graph 1



Graph 2



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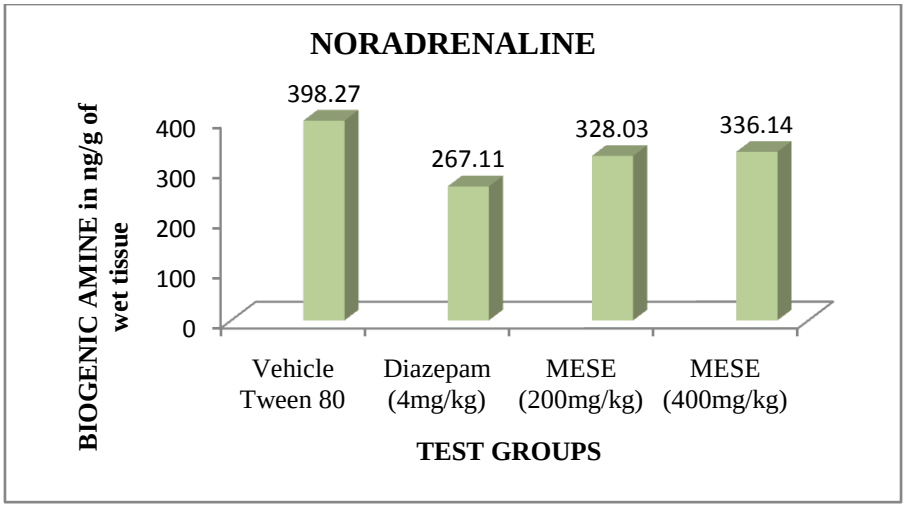
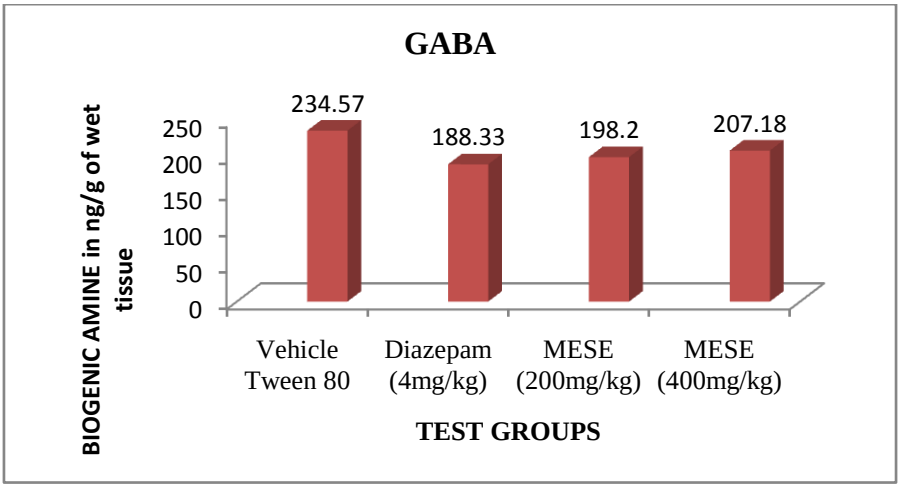


Group 4



Graph 5

DISCUSSION

Effect of extract on Pentylenetetrazole-induced seizures

The ability of an agent to prevent or delay the onset of tonic and tonic-clonic convulsion induced by PTZ in animals is an indication of anticonvulsant activity[11]. In this study, the extract MESE, caused significant anticonvulsant effect against PTZ-induced seizures by delaying the onset of myoclonic jerks and clonic convulsions in mice[12]. It also caused profound decrease in the duration of the tonic convulsions. Anticonvulsant activity in PTZ-induced seizures identifies compounds that can raise seizure threshold in brain. AEDs effective in the therapy of generalized seizures of petit mal type (absence of myoclonic) i.e. phenobarbitone, valproate, ethosuximide and benzodiazepines suppress PTZ-induced seizures in a significant manner. Diazepam which was used in this study as a reference anticonvulsant agent showed significant activity by delaying the onset of myoclonic jerks and clonic convulsions and decreasing the frequency and duration of tonic convulsions.

PTZ may be exerting its convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors. GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA attenuates and enhances convulsion. Standard antiepileptic drugs such as diazepam and phenobarbitone are thought to produce their effects by enhancing GABA-mediated inhibition in the brain and in this study with diazepam showed anticonvulsant activity against PTZ seizures.Seizures induced by PTZ are also blocked by drugs such as ethosuximide, by reducing T-type Ca^{2+} currents. Activation of N-methyl-D-aspartate (NMDA)receptor system is also involved in the initiation and propagation of PTZ-induced seizures. In this regard, drugs such as Felbamate that block glutamatergic excitation mediated by NMDA receptor have demonstrated anticonvulsant activity against PTZ-induced seizures. Since the extract delayed the occurrence and decreased the duration of convulsions induced by PTZ, it is possible that the anticonvulsant effects might be due to enhancement of GABA-mediated inhibition and/or inhibition of Ca^{2+} currents or blockade of glutamatergic neurotransmission mediated by NMDA receptor; which is not tested in this study.

The role of neurochemical in epileptogenesis and in recurrent seizure activity is well-documented. Spontaneous and experimentally induced deficiencies in Gamma amino butyric acid (GABA), nor adrenaline (NA), Dopamine (DA) and/or Serotonin (5-hydroxy- tryptamine or 5-HT). It has been implicated in the onset and perpetuation of many seizure disorders many experimental procedures designed to increase monoaminergic activity have proven antiepileptic properties[13].

In present study, the established antiepileptic drugs such as Diazepam restored the monoamine levels on brain. Similarly, MESE significantly ($p<0.05$) increased monoamines levels in forebrain of rats. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by PTZ[14]. MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect[15]. In addition to the GABA binding site, the $GABA_A$ receptor complex appears to have distinct allosteric binding

sites for benzodiazepines, barbiturates, ethanol etc. Therefore the effect of MESE on brain GABA content was studied. MESE showed significant ($p<0.05$) increased GABA content in brain dose dependently. This suggests that the anticonvulsant activity of MESE is probably through elevation of brain GABA content.

In Norepinephrine-lesioned rats showed a greater susceptibility to seizures induced by the electroconvulsive shock[16]. The antiepileptic role of endogenous nor epinephrine was inferred from studies that showed harmful effects of a damage of nor epinephrine system on seizures induced by electrical stimulation. In present study, MESE significantly ($p<0.05$) increased the NA in forebrain of rats and proves the antiepileptic activity of MESE.

Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines. Subsequent the present studies confirmed and extended these results. It became clear that MESE significantly increased the serotonin (5-HT) and DA and NA. It produces significantly decreased the susceptibility to various epileptic stimuli.

CONCLUSION

Results of the present study revealed that methanol extract of *Sapindus emerginatus* (fruit) possess anticonvulsant effects in Wistar rats with reduced mortality and the extract may be due to enhancing GABA receptor and block multi neuronal pathways in the spinal cord to treat the disease. Thus *Sapindus emerginatus* (fruit) may be considered as a valuable plant in both ayurvedic and modern drug development areas of its versatile medicinal uses. The present work did not include the identification of the active principal and its mechanism of action. Therefore, further research should be carried out to identify the active principle and elucidate the exact mechanism of action.

The decreased neurotransmitter levels in the pentelenetetrazole in control rat models were observed and the results showed the decreased neurotransmitter levels in rat's brain after induction of seizures. In MESE treated rats, monoamines such as NA, DA, and 5-HT and GABA levels significantly restored on forebrain. Thus MESE increases the seizure threshold and decreased the susceptibility to pentelenetetrazole induced seizure in rats. Hence this work suggests that methanol extract of fruits of *Sapindus emerginatus* possess antiepileptic properties that may be due to restoring the neurochemicals in rat brain. These results support the ethno medical uses of the plant in the treatment of epilepsy. However more experimentation and experimental analysis are required for a definitive conclusion.

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