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EFFECT OF *SAPINDUS EMERGINATUS* VAHL FRUITS EXTRACT ON NEUROCHEMICAL CONCENTRATIONS IN RAT BRAIN

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ABSTRACT

Sapindus emerginatus belonging to family sapindaceae is used as traditional Indian medicine to treat epilepsy. The purpose of the present study is to investigate the effect of methanolic fruit extract of sapindus emerginatus (MESE) on neurochemical concentrations in rat brain after induction of seizures by Maximal electroshock. Our aim of study was relationship between seizure activities and altered monoamines such as Noradrenaline, Dopamine, Serotonin and Gamma amino butyric acid in forebrain of rats in Maximal electroshock seizure models. In Maximal electroshock model, extracts (200 & 400 mg/kg) significantly restored the decreased levels of brain monoamines such as Noradrenaline, Dopamine, Serotonin and Gamma amino butyric acid. Thus, this study suggests that methanol extract of sapindus emerginatus increased the monoamine levels on rat brain, which may be decreased due to Maximal electroshock induced seizure in rats.

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INTRODUCTION

Epilepsy is among the most prevalent of the serious neurological disorders, affecting from0.5 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 paroxymal 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-aminobutyric acid (GABA), noradrenaline, 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 soapnuts in treating migraine and epilepsy. Therefore, the present study was performed to examine the effect of *sapindus emerginatus* on neurochemical concentrations in rat brain after induction of seizure by MES model.

MATERIALS AND METHODS:

Plant material:

Fruits of *Sapindus emarginatus* were collected from RangaReddy district and authenticated from Dept. of Botany, Osmania University,with vocher 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 a soxhlet 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, triterpinoids, saponins, flavonoids, phytosterols, and volatile oils in the extract. So, the anticonsulvant activity of methanolic extract of plant in different dose levels (200mg/kg and 400mg/kg) were studied.

Acute Oral Toxicity Study[6]:

For the LD50 dose determination, hydro methanolic 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 methanolic 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-24-11-2000/282

Experimental design:

Maximal electroshock induced seizre model

The rats were divided into four groups (n=6) and group I animals served as control and receive 1 ml of 5% CMC p.o, group II serve as drug control receiving phenytoin 20 mg/kg, p.o and group III and IV animals are administered with the fruit extract of *Sapindus emarginatus* at two different doses (200mg/kg and 400mg/kg body weight, respectively) p.o for 15 days respectively. On the 15th day, seizures were induced to all the groups of animals using electro convulsio meter. A 60 Hz alternating current of 150 milliamps intensity elicited maximal electro shock (MES) seizures for 0.2 second. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of

fatalities[7]. The observed duration of various phases of epilepsy was tabulated.

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary.

Estimation of Serotonin , Nor -adrenaline and Dopamine :

Preparation of tissue extracts by method Schlumpf M et al1974[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 upto 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 weight and was homogenized in5ml 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 Noradrenaline 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 upto 100 ml withdistilled water) +27.33 ml of 0.3M sodium acetate (4.08 g of sodium acetae 100 ml with distilled water) and volume is made up to 100 ml with distilled water). $_P$H is adjusted with sodium hydroxide sol.
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 ($_P$H6. 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. HCl without OPT was added.Internal Standard: (500 µg/ml each of noradrenaline, 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 tartarate 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 Dunnet's test p values less than 0.05 were considered as significance.

RESULTS

Table-1 EFFECT OF MESE ON MES INDUCED SEIZURES:

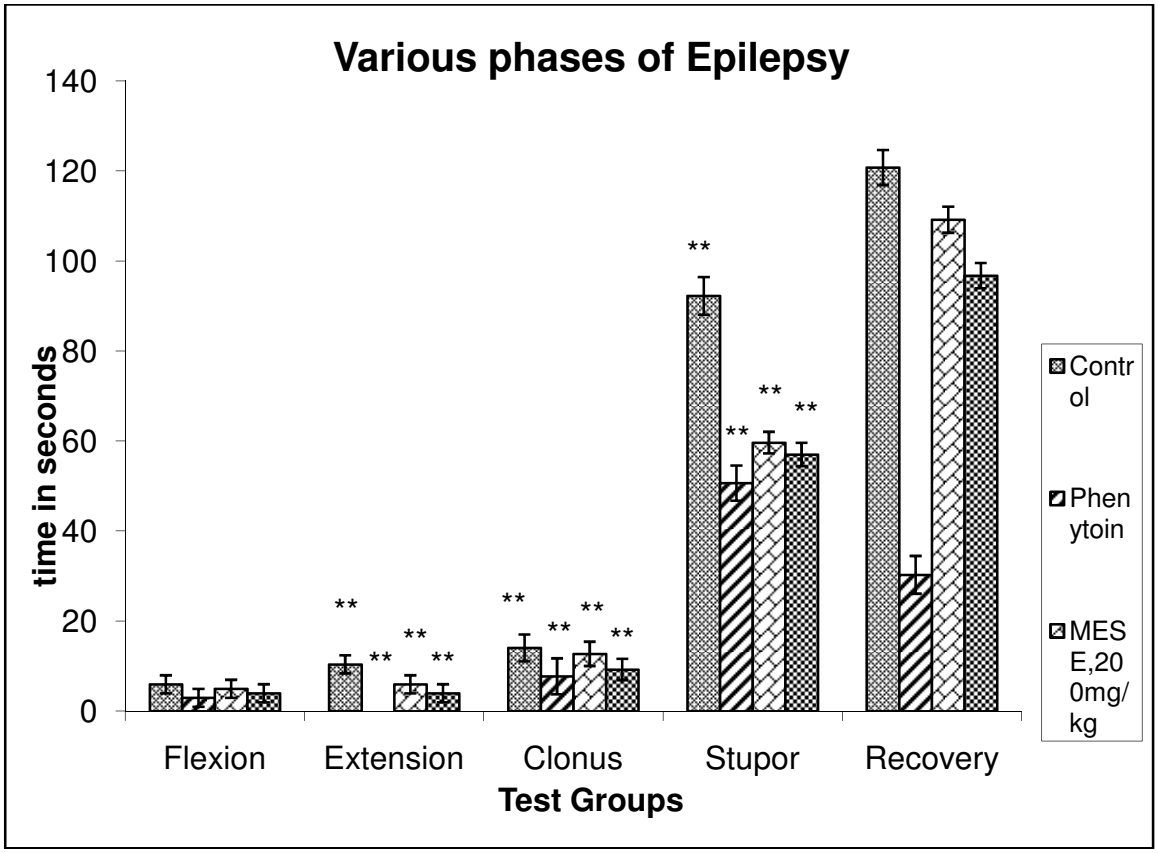
GROUP	DOSE mg/Kg	Flexion (sec)	Extension(HLTE) (sec)	Clonus (sec)	Stupor (sec)	Recovery (sec)
Control	1ml of 5% CMC p o	3.81±0.016	10.42±0.020	14.07±0.60	92.28±0.106	120.8±1.40
Phenytoin	(20 mg/kg, p.o)	1.323±0.218^{a}***	0.00 ^{a}**	7.77±0.169^{a}***	50.69±0.134^{a}***	30.32±0.811
MESE	(200mg/kg, p.o)	2.94±0.025^{b}**	4.11±0.043^{b}**	12.77±0.162^{b}***	59.69±0.859^{b}***	109.20±0.663
MESE	400mg/kg, p.o	1.62±0.030^{b}**	2.85±0.308^{b}**	9.29±0.014^{b}**	58.05±0.226^{b}***	96.78±0.410

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 Dunnet's "t" test.*P< 0.05, **P< 0. 01, ***P < 0. 001.

The MESE exhibited a significant (P<0.01 and P<0.001) reduction in various phases of epileptic seizure on comparison with the reference standard phenytoin 20 mg/kg, p.o. There was also a significant reduction in the time required for the righting reflex (recovery) in the MESE treated groups (Table 1). The data observed indicated that both extracts exhibited significant anti-seizure effect against MES induced seizures. Control group animals exhibited hind limb tonic extension (HLTE) of 10.42±0.020 sec. after the delivery of an electroshock. MESE at dose of 200 mg/kg and 400 mg/kg body weight reduced the duration of HLTE to 4.11±0.043 and 2.85±0.308 sec. respectively. Statistically significant results were observed with MESE at the dose of 200 and 400mg/kg with P<0.01.

EFFECT OF MESE ON MES INDUCED CONVULSIONS IN RATS



GRAPH-1

EFFECT OF MESE ON BIOGENIC AMINES ESTIMATION:

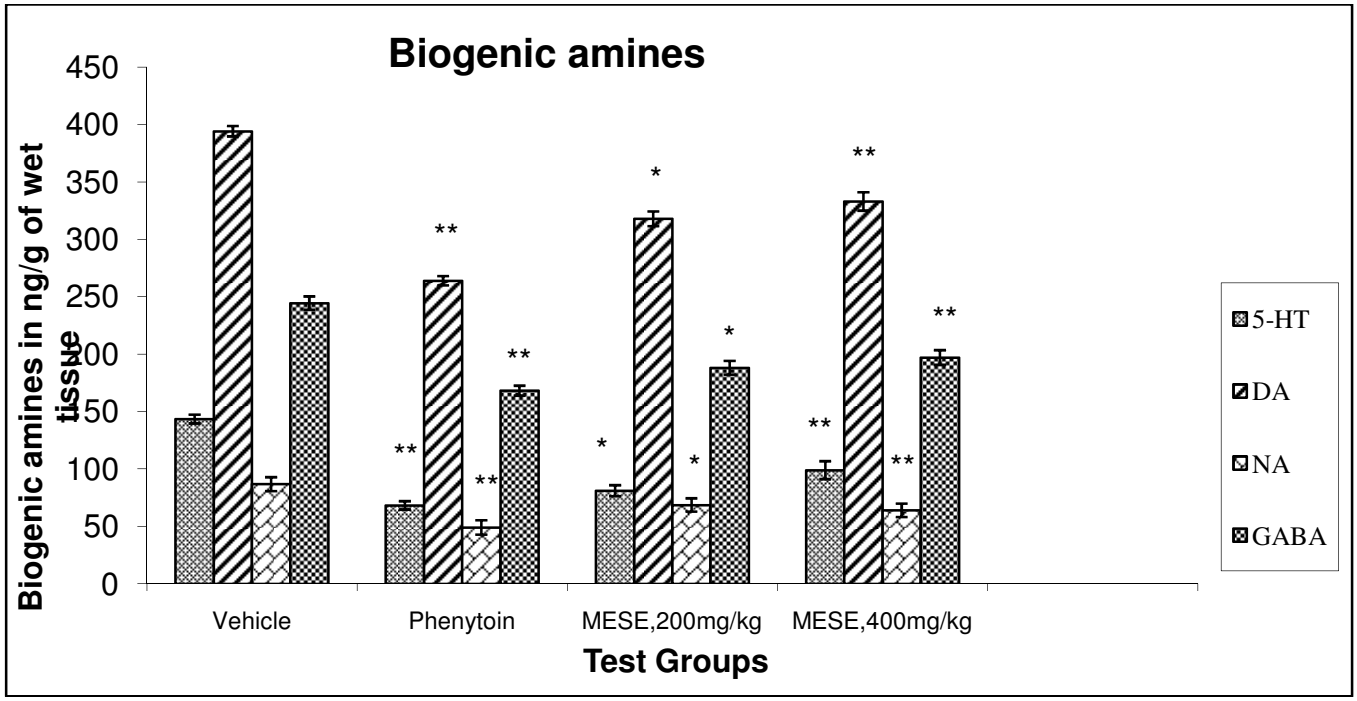
Table-2 Effect of MESE on levels of biogenic amines in forebrain of MES convulsion induced rat.

GROUP	**DOSE**	**Serotonin (ng/g of wet tissue)**	**Dopamine (ng/g of wet tissue)**	**Noradrenaline (ng/g of wet tissue)**	**GABA (ng/g of wet tissue)**
Control	1ml of 5% CMC p o	143.58±1.04	394.27±2.78	86.94±1.04	244.57±1.02
Phenytoin	20 mg/kg, p.o	68.43±0.55[a]**	264.11±3.70[a]**	49.23±1.03[a]**	168.33±0.65[a]**
MESE	200mg/kg, p.o	81.2±0.71[b]*	318.03±0.46[b]*	68.79±1.09[b]*	188.2±0.81[b]*
MESE	400mg/kg, p.o	99.08±0.32[b]**	333.14±1.13[b]**	64.17±2.50[b]**	197.18±0.42[b]**

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 Dunnet's "t" test. ** P<0.01,* P<0.05 significant when compared to control.

EFFECT OF MESE ON BIOGENIC AMINES ESTIMATION.



Noradrenaline

In MES model, Noradrenaline levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. MESE at the doses of 200&400mg/kg, standard drugs Phenytoin treated animals showed a significantly ($p<0.05$ and $p<0.01$) increased in Noradrenaline levels in forebrain of rats. Table-2

Dopamine

In MES 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 drugs phenytoin treated animals showed a significantly ($p<0.05$ and $p<0.01$) increased in Dopamine levels in forebrain of rats. Table-2

Serotonin

In MES 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 drugs Phenytoin treated animals showed a significantly ($p<0.05$ and $p<0.01$) increased in Serotonin levels in forebrain of rats**.** Table-2

Gamma amino butyric acid

In MES 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 drugs Phenytoin treated animals showed a significantly ($p<0.05$ and $p<0.01$) increased in GABA levels in forebrain of rats. Table-2

DISCUSSION

Effect of extract in maximal electroshock seizures :

The extract, MESE, was not able to abolish tonic hind limb extension at all the doses used in this study but significantly reduced the duration of the tonic hind limb extension. Tonic hind limb extension is the universal feature of maximal electroshock in mice, rats, rabbits, cats, monkeys and human[11]. Abolishing tonic hind limb extension in MEST predicts the ability of testing material to prevent the spread of seizure discharge from the epileptic focus and its effectiveness in MEST correlates well in suppressing generalized tonic-clonic seizures. Also, abolishing hind limb extension indicates the ability of testing material to inhibit or prevent seizure discharge within brainstem seizure substrate. All the currently available drugs that are clinically effective in the treatment of generalised tonic seizures (phenytoin, carbamazepine, phenobarbitone, valproate, lamotrigine, oxycarbamazepine, etc) are effective

in MEST[12]. MESE in this study was not able to abolish tonic hind limb extension but significantly reduced its duration. Phenytoin in this experiment caused significant reduction of the tonic hind limb extension phase and completely abolished this behaviour at 20 mg/kg. This validates the activity of the extract in this model.

Reduction in the duration of tonic hind limb extension but inability to completely abolish it by MESE indicated weak anticonvulsant activity in MEST but suggested strongly the presence of anticonvulsant compounds in the extract.

The role of neurochemicals in epileptogenesis and in recurrent seizure activity is well-documented. Spontaneous and experimentally induced deficiencies in gamma amino butyric acid (GABA), noradrenaline (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 Phenytoin restored the monoamine levels on brain. Similarly MESE significantly ($p<0.05$ and $p<0.01$) increased monoamines levels in forebrain of rats. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by MES[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. We therefore studied the effect of MESE on brain GABA content.MESE showed significant ($p<0.05$ and $p<0.01$) 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 Norepinephrine was inferred from studies that showed harmful effects of a damage of Norepinephrine system on seizures induced by electrical stimulation. In present study, MESE significantly ($p<0.05$ and $p<0.01$) 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 methanolic extract of Sapindus emerginatus (fruit) possess anticonvulsant effects in wister 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 principal and elucidate the exact mechanism of action.

The decreased neurotransmitter levels in the Maximal electroshock 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, 5-HT and GABA levels significantly restored on forebrain. Thus MESE increases the seizure threshold and decreased the susceptibility to MES induced seizure in rats. Hence this work suggest 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 ethnomedical 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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