Anticonvulsant and Antioxidant Effects of Musa sapientum Stem Extract on Acute and Chronic Experimental Models of Epilepsy

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ABSTRACT

Background: Musa sapientum (banana) plant extract has been shown to possess antioxidant activity in previous studies. Neuronal injury resulting from oxidative stress is an important factor involved in pathogenesis of epilepsy. Objective: The present study aimed to evaluate the anticonvulsant activity of M. sapientum stem extract (MSSE) in acute and chronic experimental models in mice and its effects on various markers of oxidative stress in the brain of pentylenetetrazole (PTZ)-kindled animals. Material and Methods: Maximal electroshock seizures (MES) and PTZ-induced convulsion models were used for acute studies. For the chronic study, the effect of MSSE on the development of kindling was studied. For the evaluation of the effects of MSSE on oxidative stress in brain, malondialdehyde (MDA) and reduced glutathione (GSH) levels were estimated in the brains of the kindled animals. Results: MSSE significantly increased the latency to onset of myoclonic jerks and the duration of clonic convulsions following PTZ administration. The MSSE pretreated group showed significantly reduced mean seizure score on PTZ-induced kindling. There was a significant increase in the brain MDA levels and decrease in GSH levels in response to PTZ-induced kindling. On MSSE pretreatment, there was a significant decrease in the MDA levels in the brains, though the increase in the GSH levels was not significant. Conclusion: The results from this study suggest the presence of significant anticonvulsant activity in MSSE, in both acute and chronic PTZ-induced seizure models, which could be due to its antioxidant activity, as it is reflected by the change in oxidative stress markers in brain.

Key words: Epilepsy, Musa sapientum, oxidative stress, seizure

SUMMARY

- Evaluation of the anticonvulsant activity of Musa sapientum and its effects on various markers of oxidative stress in the brain has not been done previously to the best of our knowledge
- M. sapientum stem extract (MSSE) significantly increased the latency to onset of myoclonic jerks and the duration of clonic convulsions in the experimental models
- The MSSE pretreated group showed significantly reduced mean seizure score on pentylenetetrazole (PTZ)-induced kindling
- There was significant increase in the brain malondialdehyde (MDA) levels and decrease in glutathione (GSH) levels in response to PTZ-induced kindling
- On MSSE pretreatment, there was a significant decrease in the MDA levels in the brain, though the increase in the GSH levels was not significant.

INTRODUCTION

Epilepsy is a common neurological disorder characterized by unpredictable recurrent seizures due to abnormal electrical activity arising from the cerebral neurons. More than 70 million people suffer from this disorder worldwide and most (about 90%) of these are from the developing parts of the world, with a median prevalence of 1.5% for studies conducted in the rural areas.[4,5] The current pharmacotherapy for epilepsy is limited to the control of the symptomatic seizures without any complete cure and often follows a chronic course for most of the patients. Maintaining a seizure-free status in the patients is challenging, as it generally involves the use of more than one drug after the initial months of therapy. The prolonged use of antiepileptic drugs is also associated with various types of mild-to-severe, established, and unavoidable adverse effects in the patients.[10] Apart from the burden of cost and the associated adverse effects with chronic therapy, the seizures remain poorly controlled in about 30%–40% patients, despite the advent of many newer drugs.[4,5]

Epileptogenesis has been linked to neuronal injury resulting from oxidative stress. Mitochondrial dysfunction due to oxidative injury has been implicated as one of the factors involved in the etiopathogenesis of many newer drugs.[4,5] This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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of epilepsy. On the other hand, seizures play an important role in neuronal cell death by causing mitochondrial dysfunction with increased levels of reactive oxygen species and apoptotic neuronal cell death, resulting in oxidative stress, leading to the cycle of subsequent seizures. Musa sapientum, commonly known as banana, is an herbaceous plant of Musaceae family. Different parts of the banana plant contain carotenoids, phenolic compounds, and biogenic amines such as dopamine, serotonin, noradrenaline, tryptophan, and tyrosine, which are relevant to the pathophysiology of various neurological disorders. The plant extract has also been shown to possess antioxidant activity in the previous studies. The evidence of antioxidant activity in M. sapientum in earlier research prompted us to assess it for the anticonvulsant activity, which to the best of our knowledge has not been done yet in any other study. The present study was conducted to evaluate antiepileptic potential of M. sapientum stem extract (MSSE) in acute and chronic experimental models of epilepsy and its effects on various markers of oxidative stress in the brain of pentylenetetrazole (PTZ)-kindled animals.

**MATERIALS AND METHODS**

**Animals**

Swiss albino mice of either sex weighing between 25 and 30 g were used for the study. The mice were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India, and were housed in the central animal house under standard laboratory conditions at an ambient temperature of 22°C ± 2°C and on natural light-dark cycle, in groups of six in polypropylene cages. Pellet diet was given to the animals and water was available ad libitum. The animals were acclimatized to laboratory conditions before experimentation and were given only water on the night before the day of experiments to avoid influence of food on drug absorption. Proper care of animals was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal facilities and approval from the Institutional Animal Ethics Committee (Regn No. 1173/ac/po/08/CPCSEA) was obtained.

**Plant material and preparation of aqueous extract**

Fresh banana stems were collected in the autumn season and the stem sample was authenticated by the National Institute of Science Communication and Information Resources (Ref. No. NISCAIR/RHMD/Consult/2014/2414-194). The upright concentric layers of leaf sheaths forming the pseudostem were peeled off to reveal the central pale-white stem. This central pale-white stem was cut into small pieces. One hundred gram stem was crushed with 20 ml distilled water in a mixer followed by filtration through a sterile muslin cloth to get aqueous extract. The whole process was carried out at room temperature. Extract was lyophilized and stored in refrigerator at 4°C, to be used for the study.

**Drugs and chemicals**

1. Lyophilized MSSE
2. Physiological saline
3. PTZ
4. Phenytoin as a standard anticonvulsant for convulsions produced by electric shock
5. Sodium valproate as a standard anticonvulsant for PTZ-induced convulsions
6. Other laboratory chemicals as required for the estimation of biochemical parameters.

**Experimental models for seizure**

**Acute studies (single dose)**

**Maximal electroshock seizures method**

The vehicle and the three test groups (n = 6 in each group) were orally administered distilled water (10 ml/kg) and incremental doses of 25, 50, and 100 mg/kg of MSSE, respectively, while the standard drug group was administered phenytoin, 25 mg/kg, intraperitoneal (i.p.), 1 h before the maximal electroshock seizures (MES) experiment. Electro-convulsions were produced by alternating current (50 mA, 0.2 s duration) delivered through ear-clip electrodes after applying a drop of 0.9 saline solution to the ear. This method produces convulsions characterized by tonic hindlimb extension (THLE), the duration of which was measured in seconds. A compound is said to possess anti-seizure potential, if it prevents THLE after the electrical stimulation.

**Pentylenetetrazole-induced convulsions**

PTZ, a powerful proconvulsant which acts by inhibiting the GABAergic activity in brain, was injected in a dose of 60 mg/kg (i.p.) to induce convulsions in the animals. The vehicle and the three test groups (n = 6, in each group) were orally administered distilled water (10 ml/kg) and incremental doses of 25, 50, and 100 mg/kg of MSSE, respectively, while the standard drug group was administered sodium valproate (400 mg/kg, i.p.), 1 h before the PTZ injection. The animals were observed after the PTZ injection, for the latency to myoclonic jerks and duration of clonic seizures within the next 30 min. No clonus within 30 min was inferred as the anti-seizure effect of the intervention. They were also monitored over the next 24 h for any mortality.

**Chronic Study**

**Pentylenetetrazole-induced kindling**

To study the effect of MSSE on the development of kindling, one group was administered only PTZ and the other group was administered MSSE (100 mg/kg, p.o.) followed 1 h later by PTZ. PTZ was given in an initial subconvulsive dose of 30 mg/kg, s.c., on alternate days at the same time (thrice a week) and the animals were observed for the appearance of seizure activity for 30 min. The evaluation of seizure activity was done from Stage 0 to Stage 5 as follows: Stage 0, no change; Stage 1, hyperactivity, restlessness, vibrissae twitching; Stage 2, head nodding, head clonus, myoclonic jerks; Stage 3, unilateral or bilateral limb clonus; Stage 4, forelimb clonic seizures; and Stage 5, generalized clonic seizures with loss of righting reflex. An animal was considered to have been kindled when it had a seizure score of 4–5 on three consecutive administrations and its treatment was discontinued.

**Biochemical tests for oxidative stress**

At the end of the above experiments, the PTZ-induced kindled mice in each group were sacrificed under deep anesthesia. The animal brain was exposed after making a midline incision on the skin and the dorsal neck to cut open the skull. Whole brain was gently removed from the cavity with the help of spatula and was immediately washed in ice-cold sodium phosphate buffer. After blotting the brain, its dry weight was taken and the whole brain tissue was homogenized with ten times (w/v) sodium phosphate buffer (ice-cold mixture of NaH2PO4 and NaHPO4, 7.4 pH). The homogenate was then centrifuged at 3000 rpm for 15 min.

**Estimation of malondialdehyde**

Malondialdehyde (MDA) (a lipid peroxidation marker) estimation was done based on the reaction between MDA and thiobarbituric acid-reacting substances, as described by Ohkawa et al. Acetic acid (20%, pH 3.5) 1.5 ml, thiobarbituric acid (0.8%) 1.5 ml, and sodium lauryl sulfate (8.1%) 0.2 ml were mixed with 0.5 ml of the supernatant from the brain tissue and heated at 100°C for 1
Effect of Musa sapientum stem extract on the duration of clonic convulsions following administration of pentylentetrazole (60 mg/kg, intraperitoneal) in mice

The results are presented in Table 3. MSSE produced decrease in the duration of clonic convulsions in a dose-dependent manner. The duration of clonic convulsions in the control group was 10.17 ± 1.08 (in seconds, mean ± SEM) which decreased to 8.67 ± 0.34 (on 25 mg/kg MSSE), 6.17 ± 0.31 (50 mg/kg MSSE), and 4.83 ± 0.48 (100 mg/kg MSSE) in treated groups. The difference in the duration of clonic convulsions was statistically significant with middle and higher dose, i.e., 50 and 100 mg/kg of MSSE when compared to the control group. Sodium valproate (400 mg/kg) showed decrease in the duration of clonic convulsions to 0.00 ± 0.00 which was highly significant (P < 0.01) when compared to the control. Sodium valproate produced more decrease in the duration of clonic convulsions as compared to the higher dose of MSSE (100 mg/kg), and the difference was statistically significant (P < 0.01).

Table 1: Effect of Musa sapientum stem extract on duration of tonic hindlimb extension in maximal electroshock seizures method in mice

| Group                  | Treatment     | Duration of THLE (s), mean±SEM |
|------------------------|---------------|---------------------------------|
| Control (distilled water) | 10 mL/kg, p.o. | 14.5±0.99                        |
| MSSE                   | 25 mg/kg, p.o. | 13.83±0.75                       |
|                        | 50 mg/kg, p.o. | 12.67±0.84                       |
|                        | 100 mg/kg, p.o.| 11.83±0.48                       |
| Phenytoin              | 25 mg/kg, i.p. | 0.00±0.00**                      |

**P<0.001 as compared to control group. THLE: Tonic hindlimb extension; MSSE: Musa sapientum stem extract; SEM: Standard error of mean

Table 2: Effect of Musa sapientum stem extract on latency to onset of myoclonic jerks following pentylentetrazole (60 mg/kg, i.p.) administration in mice

| Group                  | Treatment     | Latency to onset of myoclonic jerks (s), mean±SEM |
|------------------------|---------------|---------------------------------------------------|
| Control (distilled water) | 10 mL/kg, p.o. | 40.17±6.48                                        |
| MSSE                   | 25 mg/kg, p.o. | 52.17±2.29                                        |
|                        | 50 mg/kg, p.o. | 63.17±4.54                                        |
|                        | 100 mg/kg, p.o.| 127.17±7.54**                                    |
| Sodium valproate       | 400 mg/kg, i.p.| 142.50±5.65**                                    |

**P<0.001 as compared to control group. MSSE: Musa sapientum stem extract; SEM: Standard error of mean

Table 3: Effect of Musa sapientum stem extract on duration of clonic convulsions following pentylentetrazole (60 mg/kg, i.p.) administration in mice

| Group                  | Treatment     | Duration of clonic convulsions (s), mean±SEM |
|------------------------|---------------|----------------------------------------------|
| Control (distilled water) | 10 mL/kg, p.o. | 10.17±1.08                                    |
| MSSE                   | 25 mg/kg, p.o. | 8.67±0.34                                    |
|                        | 50 mg/kg, p.o. | 6.17±0.31**                                   |
|                        | 100 mg/kg, p.o.| 4.83±0.48**                                   |
| Sodium valproate       | 400 mg/kg, i.p.| 0.00±0.00**                                   |

**P<0.001 as compared to control group. MSSE: Musa sapientum stem extract; SEM: Standard error of mean

h in a boiling water bath. After cooling with tap water, 5 ml of butanol:pyridine mixture (15:1 v/v) and 1 ml of distilled water were added. Then, the mixture was vortexed vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was taken and its absorbance was measured at 532 nm using a spectrophotometer. Various samples of tetraethoxypropane (1–10 nmol) were used as standard to derive the linear standard curve and determine the concentration of MDA expressed as nmol/g of brain tissue.

Estimation of reduced glutathione

Glutathione (GSH) is important for cellular defense against free radicals, peroxides, and other toxic compounds. GSH was estimated by the method described by Ellman.[19] The supernatant (0.5 ml) obtained from the brain tissue was mixed with 1 ml of 5% trichloroacetic acid and the mixture was then centrifuged for 10 min at 2000 rpm to remove the proteins. The homogenate (0.1 ml) was added with 0.5 ml of the Ellman’s reagent (5,5’-dithiobis-2-nitrobenzoic acid prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution), 4 ml of phosphate buffer, and 0.4 ml of double-distilled water. This mixture was then vortexed and the absorbance of the solutions was measured at 412 nm against blank and compared with a standard curve generated from known GSH. The concentration of nonprotein thiols was determined by linear standard graph and expressed as µg/g of brain tissue.

Statistical analysis

Data were presented as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance followed by post hoc Tukey’s test. Differences with P < 0.05 were considered statistically significant in all the experiments.

RESULTS

Acute studies in seizure models

Effect of Musa sapientum stem extract on duration of tonic hindlimb extension in maximal electroshock seizures method

The results are presented in Table 1. MSSE produced decrease in the duration of THLE in a dose-dependent manner. The THLE in control group was 14.5 ± 0.99 (in seconds, mean ± SEM), which decreased to 13.83 ± 0.75 (on 25 mg/kg MSSE), 12.67 ± 0.84 (on 50 mg/kg MSSE), and 11.83 ± 0.48 (on 100 mg/kg MSSE) in treated groups. The difference in the duration of THLE was not statistically significant with 50 mg/kg MSSE when compared to the control group. Sodium valproate (400 mg/kg) showed decrease in the duration of clonic convulsions to 0.00 ± 0.00 which was highly significant (P < 0.01) when compared to the control. Sodium valproate produced more decrease in the duration of clonic convulsions as compared to the higher dose of MSSE (100 mg/kg), and the difference was statistically significant (P < 0.01).

Effect of Musa sapientum stem extract on latency to onset of myoclonic jerks following pentylentetrazole (60 mg/kg intraperitoneal) administration in mice

The results are presented in Table 2. MSSE in a dose-dependent manner produced increase in the latency to onset of myoclonic jerk following PTZ (60 mg/kg i.p.). The latency to onset of myoclonic jerk in control group was 40.17 ± 6.48 (in seconds, mean ± SEM), which increased to 52.17 ± 2.29 (on 25 mg/kg MSSE), 63.17 ± 4.54 (on 50 mg/kg MSSE), and 127.17 ± 7.54 (on 100 mg/kg MSSE) in treated groups. The difference in the latency to onset of myoclonic jerk between control and test group was statistically significant with 50 mg/kg (P < 0.05) and 100 mg/kg (P < 0.01) when compared to the control group. Sodium valproate (400 mg/kg) increased the latency to 142.50 ± 5.65 s which was highly significant (P < 0.01) when compared to control. Although sodium valproate (400 mg/kg) caused more increase in the latency to onset of myoclonic jerk as compared to the higher dose of MSSE (100 mg/kg), the difference was not statistically significant.

Effect of Musa sapientum stem extract on the duration of clonic convulsions following administration of pentylentetrazole (60 mg/kg, intraperitoneal) in mice

The results are presented in Table 3. MSSE produced decrease in the duration of clonic convulsions in a dose-dependent manner. The duration of clonic convulsions in the control group was 10.17 ± 1.08 (in seconds, mean ± SEM) which decreased to 8.67 ± 0.34 (on 25 mg/kg MSSE), 6.17 ± 0.31 (50 mg/kg MSSE), and 4.83 ± 0.48 (100 mg/kg MSSE) in treated groups. The difference in the duration of clonic convulsions was statistically significant with middle and higher dose, i.e., 50 and 100 mg/kg of MSSE when compared to the control group. Sodium valproate (400 mg/kg) showed decrease in the duration of clonic convulsions to 0.00 ± 0.00 which was highly significant (P < 0.01) when compared to the control. Sodium valproate produced more decrease in the duration of clonic convulsions as compared to the higher dose of MSSE (100 mg/kg), and the difference was statistically significant (P < 0.01).
Chronic study

**Effect of Musa sapientum stem extract (100 mg/kg) on kindling (mean seizure score) following administration of pentylenetetrazole (30 mg/kg) in mice**

The results are presented in Table 4 and Figure 1. The group on MSSE (100 mg/kg, p.o.) before PTZ (30 mg/kg, i.p.) showed significantly (P < 0.01) reduced mean seizure score as compared to the only PTZ (30 mg/kg, i.p.)-treated group.

**Biochemical tests for oxidative stress**

**Effect of Musa sapientum stem extract on amount of malondialdehyde in nmol/g of brain tissue in pentylenetetrazole-kindled mice**

The results are presented in Table 5. There was a significant increase in the brain MDA levels in PTZ-induced kindled group as compared to control group. On treatment with MSSE, there was a statistically significant (P < 0.01) decrease in the brain MDA levels.

**Table 4: Effect of Musa sapientum stem extract on kindling (mean seizure score) following administration of pentylenetetrazole (30 mg/kg, s.c.) in mice**

| Days | Mean±SEM | PTZ (30 mg/kg)-treated group | MSSE (100 mg/kg) + PTZ (30 mg/kg)-treated group |
|------|----------|------------------------------|-----------------------------------------------|
| 1    | 0.75±0.17 | 0.25±0.11*                   |                                               |
| 3    | 1.00±0.00 | 0.25±0.11**                  |                                               |
| 5    | 1.00±0.00 | 0.25±0.11**                  |                                               |
| 7    | 1.25±0.11 | 0.50±0.00**                  |                                               |
| 9    | 1.25±0.11 | 0.50±0.00**                  |                                               |
| 11   | 1.42±0.08 | 0.75±0.17**                  |                                               |
| 13   | 1.42±0.08 | 1.00±0.00**                  |                                               |
| 15   | 1.50±0.00 | 1.17±0.10*                   |                                               |
| 17   | 1.75±0.11 | 1.50±0.00                   |                                               |
| 19   | 2.00±0.00 | 1.42±0.08**                  |                                               |
| 21   | 2.00±0.00 | 1.50±0.00                   |                                               |
| 23   | 2.08±0.08 | 1.17±0.10**                  |                                               |
| 25   | 2.17±0.17 | 1.17±0.10**                  |                                               |
| 27   | 2.17±0.17 | 1.17±0.10**                  |                                               |
| 29   | 2.33±0.17 | 1.42±0.08**                  |                                               |
| 31   | 3.75±0.11 | 1.75±0.11**                  |                                               |
| 33   | 4.00±0.00 | 1.75±0.11**                  |                                               |
| 35   | 4.00±0.00 | 1.75±0.11**                  |                                               |
| 37   | 4.50±0.13 | 1.75±0.11**                  |                                               |

*P<0.05 as compared to PTZ (30 mg/kg)-treated group; **P<0.01 as compared to PTZ (30 mg/kg)-treated group. PTZ: Pentylenetetrazole; SEM: Standard error of mean; MSSE: Musa sapientum stem extract

**Table 5: Effect of Musa sapientum stem extract on amount of malondialdehyde in nmol/g brain tissue in pentylenetetrazole-kindled mice**

| Group | Treatment | Amount of GSH in µg/g brain tissue (mean±SEM) |
|-------|-----------|-----------------------------------------------|
| Control | 10 mL/kg, p.o.(distilled water) | 640.0±25.30 |
| PTZ | 30 mg/kg, s.c. | 500.0±36.88* |
| MSSE + PTZ treated | 100 mg/kg, p.o. + 30 mg/kg, s.c., respectively | 680±40.00 |

*P<0.05 as compared to control group. GSH: Glutathione; PTZ: Pentylenetetrazole; SEM: Standard error of mean; MSSE: Musa sapientum stem extract

**Table 6: Effect of Musa sapientum stem extract on amount of glutathione in µg/g brain tissue in pentylenetetrazole-kindled mice**

| Group | Treatment | Amount of MDA in nmol/g brain tissue (mean±SEM) |
|-------|-----------|-----------------------------------------------|
| Control | 10 mL/kg, p.o. (distilled water) | 6.30±0.19 |
| PTZ | 30 mg/kg, s.c. | 8.12±0.49* |
| MSSE + PTZ treated | 100 mg/kg, p.o. + 30 mg/kg, s.c., respectively | 4.91±0.13** |

*P<0.05 as compared to control group; **P<0.001 as compared to PTZ group. PTZ: Pentylenetetrazole; SEM: Standard error of mean; MSSE: Musa sapientum stem extract; MDA: Malondialdehyde

**Effect of Musa sapientum stem extract on amount of glutathione in µg/g of brain tissue in pentylenetetrazole-kindled mice**

The results are presented in Table 6. There was a significant decrease in the brain GSH levels in PTZ-induced kindled group as compared to the control group. Even though there was an increase in the brain GSH levels in MSSE-treated group, the increase was not statistically significant.

**DISCUSSION**

The current pharmacotherapy of epilepsy has the limitations of a chronic course, involving unavoidable adverse effects, economical burden, and falls short of the therapeutic goal of a seizure-free status in nearly one-third of the patients. Use of plant-based products for therapy of convulsions has been a part of long-standing tradition in Asia, Africa, and South America. Many plant extracts have shown the presence of anticonvulsant activity in animal seizure models, which has been attributed to the action of flavonoids, furanocoumarins, phenylpropanoids, and terpenoids on gamma-amino butyric acid (GABA) receptors and voltage-gated ion channels. These phytochemicals facilitate the maintenance of normal physiological function of the major inhibitory neurotransmitters. With the emphasis on evidence-based medicine and the advent of modern laboratory technologies, renewed interest in research on herbal products, to identify an effective and safe antiepileptic compound has been generated.

M. sapientum is a widely available plant in Southeast Asia which has been evaluated for the presence of antioxidant activity in a few earlier
studies. The plant extract has been shown to decrease serum lipid peroxidation and prevent rise in MDA. It was also found to increase the levels of GSH and superoxide dismutase level in treated group. Oxidative potentials such as hepatoprotection and antiinflammatory activity have also been attributed to the antioxidant activity in the plant. Oxidative injuries leading to mitochondrial respiration deficits have been implicated in epileptogenesis. Oxidative stress has also been linked to altered physiological functions of calcium, resulting in mitochondrial dysfunction and neuronal damage. Such neuronal damage is understood to be one of the most important factors responsible for etiopathogenesis of seizure. Based on the previous reports of antioxidant activity of \textit{M. sapientum}, in this study, we evaluated its antiepileptic potential and its effect on neuronal oxidative stress in experimental models of seizure, the assessment of which has not been done yet in any previous study.

The results of the present study showed that in MES-induced convulsions, the MSSE did not provide significant protection as there was no significant difference in the duration of THLE in the control and MSSE-treated group of animals. However, in PTZ-induced convulsions, the MSSE showed a significant increase in the latency to the onset of myoclonic jerks and also decreased the duration of clonic convulsions significantly when compared to the control group. PTZ is supposed to induce seizure by interfering with the function of inhibitory neurotransmitter GABA. Decreased GABAergic activity is one of the factors for triggering seizure and drugs facilitating GABA action in brain, such as valproate, benzodiazepines, and barbiturates, are well known for their role in the treatment of epilepsy. Increased glutamate activity has also been suggested to play a role in seizure induction by PTZ as glutamate receptor antagonists have been shown to decrease the PTZ-induced activity. The compound showing a protective effect in PTZ-induced convulsions has been reported to be effective clinically in absence seizures. The results of the present study indicate that MSSE may be effective in absence seizures but not in generalized tonic-clonic seizures as it failed to modulate seizure activity in MES model, an animal model for evaluation of drugs in generalized tonic-clonic seizures. Although the mechanism of these effects of MSSE are not clear at this stage of experiments, a possibility of GABA facilitatory or glutamate inhibitory effect cannot be ruled out.

The results also suggest that MSSE 100 mg/kg has a protective effect against PTZ-induced kindling in mice as it significantly reduced the seizure score in treated group. PTZ-induced kindling is a well-established chronic animal model of epilepsy commonly used to explore epileptogenesis and novel antiepileptic compounds. It has been generally used as a laboratory model of human partial complex epilepsy. In this method, a subconvulsive dose of PTZ is applied repetitively and intermittently for a number of days leading finally to the development of seizures. Seizure score is calculated after each PTZ injection. Results from the previous studies have suggested that PTZ-induced kindling is associated with increased glutamate activity and subsequent generation of reactive oxygen species and oxidative stress in the neurons. Oxidative stress plays an important role in brain tissue damage during seizure in the PTZ kindling model of epilepsy. Increase in lipid peroxidation and a decrease in antioxidant enzymes has been observed following PTZ-induced kindling in earlier experimental studies. The results suggest that the protective effect against development of kindling may be due to antioxidant activity in MSSE.

Free radical injury has been shown to be one of the important factors involved in the development of seizures and seizure-induced neuronal damage. The damage at the polyunsaturated sites in the neuronal membranes leads to increased lipid peroxidation reflected as the rise in the levels of MDA. There is also a decrease in central nervous system GSH concentration in animals exhibiting generalized seizures. GSH is an endogenous antioxidant, the reduced form of which reacts with free radicals and prevents the oxidative toxic injury. In this study also, the repeated PTZ administration increased the oxidative stress as indicated by a significant increase in the MDA level and a decrease in the GSH level. Pretreatment with MSSE at 100 mg/kg decreased the MDA levels significantly and appeared to restore the reduced GSH level in the brain tissues of PTZ-kindled mice. This suggests that MSSE has the potential to prevent the neuronal oxidative injury.

**CONCLUSION**

The results from this study suggest the presence of significant anticonvulsant activity in MSSE against PTZ-induced convulsions. MSSE also showed protective effect against kindling induced by PTZ. These effects could be due to its antioxidant activity, as reflected by a decrease in the MDA levels and an increase in the GSH levels in the brains of the PTZ-kindled animals pretreated with MSSE. The study suggests that MSSE could be a potential natural compound for use in epilepsy; however, further studies are required to identify the active constituents of the extract and ascertain its pharmacodynamic profile.

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**Conflicts of interest**

There are no conflicts of interest.

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Pharmacognosy Research, Volume 10, Issue 1, January-March, 2018
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