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Evaluation of anti-epileptic activity of fresh fruit juice of *Moringa oleifera* against maximal electroshock (M.E.S) and Picrotoxin (PTX) induced convulsions in mice

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Abstract

Moringa oleifera(Moringaecea)are used in Indian traditional medicine and in folklore for many diseases and extensively used as CNS depressant traditionally. The interesting things of this plant are each part of *Moringa oleifera* is used as medicines. The present work has been carried out to evaluate the anti-epileptic activity of fresh fruit juice of *Moringa oleifera* against maximal electroshock (M.E.S) and Picrotoxin (PTX) induced convulsions in mice at different dose level (100ml/kg. p.o & 50ml/kg. p.o). Diazepam (5mg/kg. p.o) and Phenytoin(25mg/kg. i.p) were used as reference standard drugs.The data obtained indicates that the fresh fruit juice of *Moringa oleifera* at the dose of (50ml/kg p.o & 100ml/kg p.o) shows anticonvulsant activity against Maximal electroshock and Picrotoxin induced convulsion in mice.
Keywords: Fresh fruit juice of Moringa oleifera, Antiepileptic activity, Maximal electroshock, Picrotoxin.

1. Introduction

Epilepsy is the name given to the condition where a person has recurrent seizures – abnormal discharges of electrical activity in the brain cells which may lead to abnormal behaviors such as- Involuntary muscle movements, unusual perceptions, Disturbed level of consciousness.[1]

Epilepsy is one of the most common neurological disorders. India is home to about 10 million people with epilepsy (prevalence of about 1%)[1] this being higher in the rural (1.9%) as compared with the urban counterpart (0.6%).[2-4]. It affects people of all ages, currently available antiepileptic drugs has some adverse effects therefore Phytomedicines provides idea for producing new antiepileptic drugs.

Moringa oleifera is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia.[5] It is known as thedrumstick tree based on the appearance of its immature seed pods, the horseradish treebased on the taste of ground root preparations, and the bean oil tree from seed-derivedoils. *Moringa oleifera* is called a miracle tree because of its medicinal values. In the traditional medicinal system almost all the parts of this plant has been used in the treatment of various ailments. So present study was carried out to evaluate anticonvulsant activity of fresh fruit juice of *Moringa oleifera* against Maximal electroshock and picrotoxin induced convulsions in mice at the dose of 50ml/kg p.o and 100ml/kg p.o.

2. Materials and Method

2.1 Collection of Plant materials

Fresh Fruit*s of Moringa oleifera* was collected from local area of Buldhana district (M.S.) India. The fruits was authenticated by Department of Botany Shivaji Senior College Chikhali, Dist-Buldhana.

2.2 Chemical

All the drugs and chemicals used in this experiment were of analytical grade. The Drugs and other chemicals used are as follow, Picrotoxin (Ozone), Diazepam (Neon Laboratories Ltd).

Phenytoin (Abott Pharmaceuticals), Thibarbituric acid (Dolphin Chemicals), Tricholoro acetic acid (Merck), n-butanol (Dolphin chemicals Mumbai), Malonaldehyde standard (Cayman Chemicals),

2.3 Animals

Swiss Albino mice of body weight 20-30 gm were procured from Animal house of Anuradha College of Pharmacy, Chikhli & were used in this study With the exception of the short time that the animals were removed from their cages for testing; all animals were maintained on an adequate normal laboratory pellet diet and allowed free access to food and water.

2.4 Instruments

Electro convulsometer (Dolphin), Electronic Juicer (Jyoti).

2.5 Methods

A) Maximal electroshock (M.E.S) induced convulsions in mice.

All animals were divided into 4 groups (n=6) as mentioned below. Phenytoin was administered orally 30 min prior to electroshock. 50 mA current was applied to the animal through corneal electrodes for 0.2 sec. To enhance electroconductivity two drops of 0.9 % NaCl were applied on each eye before applying current. The incidence and duration of extensor tonus were noted.[6-7]

B) Picrotoxin (PTX) induced convulsions in mice.

The animals were grouped containing six in each labeled I-IV. Convulsion was induced by administration of picrotoxin (3mg/kg i.p). Time for onset of action (Clonic and tonic Seizures) and death was recorded.[8-9]

C) Estimation of Thibarbituric acid reactive substances:

(MDA)

The blood sample was withdrawn from the Tip of tail. To 0.5 ml serum, 2.5ml of20mg/dl trichloroacetic acid was added and the tube was left to stand for 10 min atroom temp. After centrifugation at 3500rpm for 10 min. The supernatant was decantedand precipitate was washed one with 0.05 sulphuric acids. Then 2.5ml of 0.05mol.Sulphuric acid and 3.0ml of 0.2mg /dl Thiobarbituric acid in 2Mol. Sodium sulphateare added to this precipitate and the coupling of lipid peroxide with TBA carried outby heating in boiling water bath for 30 min.[10] after cooling in cold water. The resultingchromogen was extracted with 4.0ml of *n*-butyl alcohol by vigorous shaking [9]

Separation of organic phase was faciliatated by centrifuigaton at 300 rpm for 10 minand its absorbence was determined of wavelength 530nm.

2.6 Statistical analysis

Statistical analysis Data is presented as mean ± SD. Data of 'latency'&'mortality' was analyzed by one way ANOVA followed by Dunnett's t- test.

3. Results

3.1 Effect of fresh fruit juice of *Moringa oleifera* on Maximal electroshock (MES) induced convulsions in mice.

Maximal electroshock caused convulsions as well as lethality in mice. Mice (Group III) were pretreated with fresh fruit juice of *Moringa oleifera* (50ml/kg. p.o) for 07 days showed significant (P<0.05) decrease in duration of convulsion and significantlyreduced mortality. However fresh fruit juice of *Moringa oleifera* (100ml/kg. p.o.) for 7 days and Phenytoin (25mg/kg) showed most significant decrease in duration of convulsions and reduced mortality.

Table 1: Effect of fresh fruit juice of *Moringa oleifera* and Phenytoin on convulsions induced by MES in mice

Groups	Treatment (Mg/kg, i.p.)	Duration of convulsions in sec	Mortality dead/used (%)
I (Negative Control)	MES	27.26± 1.70	6/6(100)
II (std)	Phenytoin (25mg/kg i.p) + MES	---	0/6(0)
III (Test 1)	Fresh fruit juice of *Moringa oleifera* (50ml/kg p.o) + MES	14.64± 1.48	2/6 (32.33%)
IV (Test 2)	Fresh fruit juice of *Moringa oleifera* (100ml/kg p.o) + MES	8.10± 1.10	1/6 (16.66)

Values are mean ± SD (n=6). P<0.05 was considered to be statistically significant

3.2 Effect of fresh fruit juice of *Moringa oleifera* on lipid peroxidation level in MES-induced oxidative stress in mice.

TBARS level, the indicator of lipid peroxidation, was significantly increased in serum of MES- induced epileptic mice when compared with controls. Phenytoin (25mg/kg i.p.) and fresh fruit juice of *Moringa oleifera* at the dose of (50ml/kg p.o & 100ml/kg p.o) significantly decreased elevated level of lipid peroxidation in serum when compared with toxicant control group

Table 2: Effect of fresh fruit juice of *Moringa oleifera* on lipid peroxidation level in MES- induced oxidative stress in mice

Group	Treatment	Concentration of MDA nMol/ml
I	Saline.	3.41± 0.48
II	MES	5.8 ± 0.56
III	Phenytoin (25mg/kg i.p)	3.81± 0.39
IV	Fruit juice of MO (50ml/kg p.o) + MES	4.1±0.48
V	Fruit juice of MO (100ml/kg p.o) + MES	2.35±0.24

Values are mean ± SD (n=6). P<0.05 was considered to be statistically significant

3.3 Effect of fresh fruit juice of *Moringa oleifera* on Picrotoxin induced convulsions in mice.

Single dose, intraperitonial administration of Picrotoxin (3mg/kg i.p) caused clonic convulsions as well as lethality in mice. Mice were pretreated with fresh fruit juice of *Moringa oleifera* (50ml/kg p.o) for 7 days showed significant ($P<0.05$) and increase in latency to clonic convulsion & decrease in duration of convulsions & also significantly reduced mortality (Table 4). However fresh fruit juice of *Moringa oleifera* (100ml/kg. p.o) for 7 days and Diazepam (5mg/kg p.o) showed most significant decrease in duration of convulsions and reduced mortality.

Table 3: Effect of fresh fruit juice of *Moringa oleifera* on Picrotoxin induced convulsions in mice.

Groups	Treatment (mg/kg)	Onset of action in sec	Duration of Convulsions in sec	Mortality dead / used (%)
I (Negative Control)	Picrotoxin (3mg/kg,i.p.)	120± 13.10	510± 51.12	6/6(100)
II (Std)	Diazepam (5mg/kg, p.o)+PTX	---	---	0/6(0)
III (Test 1)	fruit juice of M.O(50ml/kg p.o)+PTX	255± 25.6	326± 31.2	2/6(16.66 %)
IV (Test 2)	fruit juice of M.O(100ml/kg p.o)+PTX	463± 47.10	240± 18.16	1/6(32.33)

Values are mean ± SD (n=6). $P<0.05$ was considered to be statistically significant

3.4 Effect of fresh fruit juice of Moringa oleifera on lipid peroxidation level in Picrotoxin induced oxidative stress in mice:-

TBARS level, the indicator of lipid peroxidation, was significantly increased in serum of Picrotoxin-induced epileptic mice when compared with controls. Diazepam (5mg/kg p.o) and fresh fruit juice of *Moringa oleifera* at the dose of 50ml/kg p.o & 100ml/kg p.o. Significantly decreased elevated level of lipid peroxidation in serum when compared with toxicant control group (Table 4).

Table 4: Effect of fresh fruit juice of *Moringa oleifera* on lipid peroxidation level in Picrotoxin-induced oxidative stress in mice

Group	Treatment	Concentration of MDA nMol/ml
I	Saline.	3.41± 0.48
II	Picrotoxin	6.25 ± 0.62
III	Diazepam (5mg/kg p.o)+Picrotoxin	3.92 ± 0.38
IV	Fruit juice of MO (50ml/kg p.o) + Picrotoxin	4.3±0.40
V	Fruit juice of MO (100ml/kg p.o) + Picrotoxin.	2.7 ± 0.28

Values are mean ± SD (n=6). $P<0.05$ was considered to be statistically significant

4. Discussion

The result of present study indicates that the fresh fruit juice of *Moringa oleifera* (50ml/kg p.o & 100ml/kg p.o) posses anticonvulsant activity in mice. GABA is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitory neurotransmitter in the brain [11]. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factor in epilepsy. Our study shows that the fresh fruit juice of *Moringa oleifera* for 7 days treatment protected some of the animals against seizure induced by MES and Picrotoxin. Diazepam and Phenytoin were standard antiepileptic drugs and posse's anticonvulsant effect on Maximal electroshock and Picrotoxin induced seizures respectively [12].

Picrotoxin also produced seizure in all the mice used. Picrotoxin is known to elicit seizures, by antagonizing the effect of GABA via blocking of the chloride channels linked to GABA-A receptor. In this study, Diazepam was shown to antagonize the effect of Picrotoxin while the fresh fruit juice of *Moringa oleifera* was also shown to delay the latency of Picrotoxin-induced seizures, suggesting that the fresh fruit juice of *Moringa oleifera* may be affecting GABAergic mechanism, probably by opening the chloride channels associated with GABA receptors.

The maximal electroshock test is the most widely used animal's model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high. This method identifies the drug with activity against generalized tonic-clonic seizures and partial seizures using clinically established antiepileptic drugs. The study also suggests that the Juice of *Moringa oleifera* effective against generalized tonic-clonic and partial seizures.

Thus the fruit juice of *Moringa oleifera* (50ml/kg p.o & 100ml/kg p.o) possess anticonvulsant activity against MES and picrotoxin induced seizures which could be by interfering with GABA, Glutaminergic mechanism, Na+ channels and Cl clannels.

5. Conclusion

In conclusion this study has demonstrated that the Fresh fruit juice of *Moringa oleifera* at the dose of (50ml/kg p.o & 100ml/kg p.o) shows anticonvulsant activity against Maximal electroshock and Picrotoxin induced convulsion in mice. Picrotoxin administration and MES produced an increased lipid peroxidation in serum of the mice and therefore, demonstrated and confirmed the possible involvement of free radical oxygen in the picrotoxin induced seizures. Treatment with fresh fruit juice of *Moringa oleifera* decreased serum MDA activity increased by administration of picrotoxin & maximal electroshock, thereby suggesting that these drug acts positively on lipid peroxidation. From above we can conclude that the fresh fruit juice of *Moringa oleifera* (50ml/kg p.o & 100ml/kg p.o) may be promising for the treatment of Picrotoxin and Maximal electroshock induced epilepsy could be by interfering with GABA glutaminergic mechanism and Na^+, Cl^- channels. Further studies of experimental epilepsy should determine whether the beneficial effects of fresh fruit juice of *Moringa oleifera* persist over longer periods.

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