ORIGINAL ARTICLE

Antimigraine activity study of *Moringa oleifera* leaf juice

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Background: Migraine is characterized by a pulsating headache, usually restricted to one side, which comes in attacks lasting for 4-48 hours. Before the headache some of the patients also may experience visual disturbances called 'aura'. The attacks are also associated with nausea, vomiting and sensitivity to light and sound. **Aim**: The aim of the present investigation was to explore the antimigraine potential of alcoholic fraction of leaf juice of *Moringa oleifera Lamm,* which is traditionally used in the treatment of migraine. **Materials and Methods:** Three animal models, viz, Apomorphine induced climbing behavior; *l*-5-HTP induced syndrome and MK 801 induced hyperactivity were used to carry out the antimigraine activity studies. **Statistical Analysis:** The results were analyzed with ANOVA followed by Dunnett's multiple comparison tests. $P<0.05$ is considered significant. Prism 5 was used for the statistical analysis. **Results and Conclusion:** *Moringa oleifera* significantly reduced the Apomorphine induced climbing behavior, *l*-5-HTP induced syndrome and MK 801 induced hyperactivity in a dose dependent manner. These results indicated that *Moringa oleifera may* be acting via dopaminergic and serotonergic receptors. It could be concluded that *Moringa oleifera* may be effectively used in the treatment or management of migraine.

Key words: Apomorphine, *l*-5-HTP, migraine, MK 801, *Moringa oleifera*

INTRODUCTION

Migraine is a mysterious disorder characterized by a pulsating headache, usually restricted to one side, which comes in attacks lasting for 4-48 hours. Some of the patients experience a more specific disturbance prior to headache, called 'aura' which usually has visual disturbance. Migraine attacks are episodic and resolve with time. Associated symptoms such as nausea vomiting and increased sensitivity to light (photophobia) and sound (phonophobia) occur during the headache phase.[1]

The molecular pathophysiology of migraine is not fully understood and this has made the treatment and/or management of migraine difficult. Currently available drugs such as ergotamine and its derivatives, several synthetic drugs like NSAIDS, 5HT receptor agonists (Triptans), 5 HT_2 receptor antagonists and even antiemetics[2,3] are associated with various side effects and the treatment is based on trial and error method.

Due to several drawbacks associated with the conventional drug therapy in migraine treatment, there has been a wide research to find out natural products which could be effectively used for the treatment of migraine. Plants such as *Tanacetum parthenium*[4] *Petasites hybridus*,[5] and others have been studied for relief of migraine.

Moringa oleifera Lamm, family Moringaceae is a medium sized tree with tripinnate leaf[6] and is cultivated throughout India. The leaves of the plant are a rich source of amino acids, proteins and vitamin A and C. Apart from this they are also contain flavonoids, phenolics, glucosinolates and isothiocyanates.[7] The ethanolic fraction of the leaves contain thiocarbamates such as niazinin A and B, niazimicin, niazirin and isothiocyanates.[8] The leaves of *Moringa oleifera* are reported to show antioxidant,[9] antihypertensive,[10] hypocholesterolemic,[11] antifungal,[12] radio protective,[13] wound healing[14] and antinociceptive activities.[15] The roots and barks of the plant possess diuretic,[16] and antiepileptic properties.[17] The leaves are used in folklore medicine for the treatment of migraine. The present paper thus attempts to screen the usefulness of the alcoholic extract of the leaf juice in the treatment of migraine.

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MATERIALS AND METHODS

Drugs

Apomorphine, pargyline, *l*-5-HTP, MK 801 and methysergide were obtained from Sigma chemicals co. America and haloperidol was obtained in the form of injection (Serenace injection, 5 mg/ml; RPG Life sciences).

Plant Material

Fresh leaves of *Moringa oleifera Lamm* (Moringaceae) were collected and authenticated at Department of Botany herbarium, Nagpur University, Nagpur (voucher number 9116).

Animals

Male Swiss albino mice (20-25 g) obtained from the animal house of our institute was used. The animals received standard pellet diet (M/s Hindustan Lever Foods, Calcutta, India), water *ad libitum* and were maintained under standard environmental conditions (22°±5°C with 12- h of light/dark cycle). All experimental protocols were approved by the Institutional Animal Ethical Committee (648/02/c/CPCSEA).

Extraction of Plant Material

The fresh leaves (1 kg) were crushed with little amount of water to obtain the leaf juice. The leaf juice was filtered through a muslin cloth and later through Whatman filter paper to obtain a greenish brown juice. The juice was shade dried and a little amount of absolute alcohol was added to the juice to prevent the growth of microorganisms. The dried leaf juice was collected as a brown colored powder (about 30 g). It was refluxed at 50°C for 5-6 hours with absolute alcohol. The alcohol fraction was separated from the residue and dried to obtain the alcoholic fraction of *Moringa oleifera* leaf juice (MOA). The weight of MOA was about 6 g.

Preliminary Phytochemical Screening

The prepared extract (MOA) was tested for the presence of various chemical constituents with the help of different phytochemical tests.

In vivo Studies

Apomorphine induced climbing behavior

Each mouse was placed in a specially fabricated cylindrical wire mesh cage (height 13 cm, diameter 14 cm and mesh size 3 mm) for 1 hour prior to the experiment. Saline (10 ml/kg i.p.) or haloperidol (1 mg/kg i.p.) was administered 30 minutes prior to apomorphine (2.5 mg/kg. i.p.) and MOA (25, 50 and 100 mg/kg p.o.) was administered one hour prior to apomorphine (2.5 mg/kg. i.p.). Climbing behavior was assessed in 5 minute intervals up to 20 minutes, starting 10 minutes after apomorphine, using the following scoring system. 0-no paws on the cage, 1-two paws on the cage, 2-four paws on the cage.[18] The score recorded for each animal was based on the position of the animal at the moment it was first observed. The total time spent on the cage was also recorded for each animal. An observer unaware of the specific treatments was made to record the observations.

l-5-HTP Induced Serotonin Syndrome

The syndrome was measured after placing the mouse in a Perspex cage for a 30 minutes habituation period. The animal was then injected with pargyline (75 mg/kg i.p.) in order to prevent rapid degradation of *l*-5-HTP. Thirty minutes later saline (10 ml/kg i.p.) or methysergide (10 mg/kg i.p.) or MOA (25, 50 and 100 mg/kg p.o.) were administered. The methysergide and saline treated animals received *l*-5-HTP (50 mg/kg i.p.) after 30 minutes while the MOA treated animals received *l*-5-HTP (50 mg/kg i.p.) after 1 hour and were returned to the test cage. After 20 minutes, the serotonin syndrome was assessed every 10 minutes through 50 minutes. Five behavioral parameters, such as tremors, hind limb extension, forepaw treading, head weaving and head twitch were monitored using the following scoring system: 0- absent, 1- moderate, 2- marked.[18] These observations were done by an observer unaware of the specific treatments given.

MK 801 Induced Hyperactivity

Mouse locomotor activity was measured using Actophotometer (Medicraft, Inco Model 600-6D). The animals were placed in the Actophotometer for a thirty minute period. After this time, they were administered Saline (10 ml/kg i.p.), haloperidol (0.1 mg/kg, i.p.) or MOA (25, 50 and 100 mg/kg p.o.) and were then returned to test boxes. The recording was begun immediately in the case of haloperidol treated animals and saline treated animals whereas after 30 minutes in case of MOA treated animals. After a 30 minutes period, the animals were injected with MK-801 (0.3 mg/kg, i.p.) and the recording was continued immediately and the activity was measured for a further 90 minutes. The photocell counts that were measured every minute were grouped into 10 minute time intervals.[18]

Statistical Analysis

The results are reported as mean±S.E.M and analyzed with ANOVA followed by Dunnetts multiple comparison test. $P<0.05$ is considered significant. Prism 5 was used for the statistical analysis.

RESULTS

Preliminary Photochemical Screening

The various phytochemical tests performed revealed the presence of proteins, amino acids, glycosides, flavonoids and triterpenoids.

Apomorphine Induced Climbing Behavior

MOA (25, 50 and100 mg/kg p.o.) and haloperidol (1 mg/kg i.p.) inhibited apomorphine induced climbing behavior in mice significantly (P<0.01). The inhibition by MOA followed a dose dependent pattern with a maximum inhibition by MOA 100 mg/kg [Figures 1 and 2].

l-5-HTP Induced Serotonin Syndrome

MOA (25, 50 and 100 mg/kg p.o.) inhibited all the behavioral syndromes induced by *l*-5-HTP significantly (P<0.05) except for the hind limb extension [Figure 3].

MK 801 Induced Hyperactivity

MOA (25, 50 and 100 mg/kg p.o.) and haloperidol (1 mg/kg i.p.) significantly inhibited MK 801 induced hyperactivity (P<0.01). The inhibition by MOA followed a dose dependent pattern with a maximum inhibition by MOA 100 mg/kg [Figure 4].

DISCUSSION

Apomorphine induced climbing behavior was inhibited by MOA in a dose dependent manner. This behavior is reported to be due to activation of both D_1 and D_2 receptors[19] and hence D_1 and D_2 antagonists are effective in this model.[20] Thus it can be concluded that MOA shows a similar action as the D_1 and D_2 antagonists.

The *l*-5-HTP induced behavioral syndromes except for hind limb extension were very significantly reduced by all three doses of MOA. This action correlates with the prophylactic antimigraine drug methysergide, a serotonin antagonist.[21]

The noncompetitive NMDA channel blocker MK801 is reported to stimulate locomotor activity by increasing the dopamine and serotonin release in brain and hence

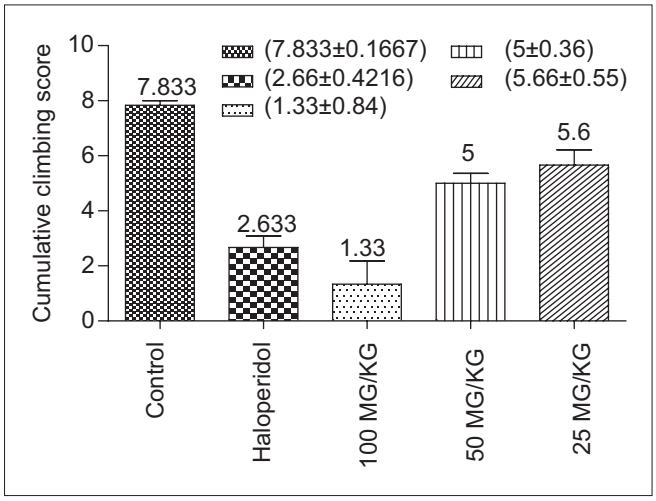

Figure 1: The effect of *Moringa oleifera* on Apomorphine induced climbing behavior. Each column represents±S.E.M of total climbing score for a group of six mice. A maximum score of 10 is possible. *P<0.01

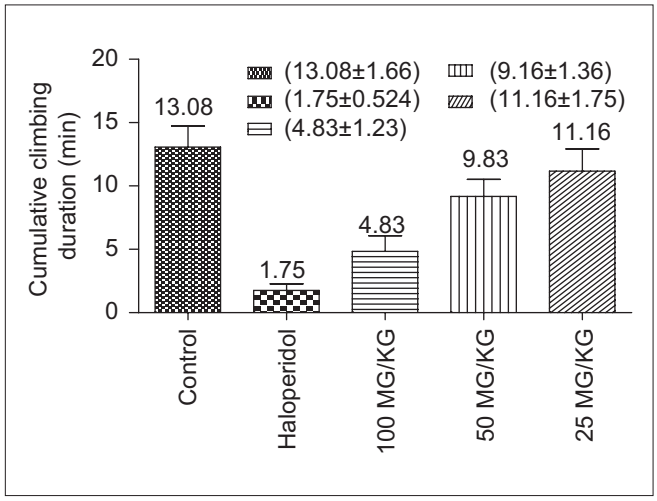

Figure 2: The effect of *Moringa oleifera* on Apomorphine induced climbing behavior. Each column represents±S.E.M of total time spent by the mice on the cage for a group of six mice. *P<0.01

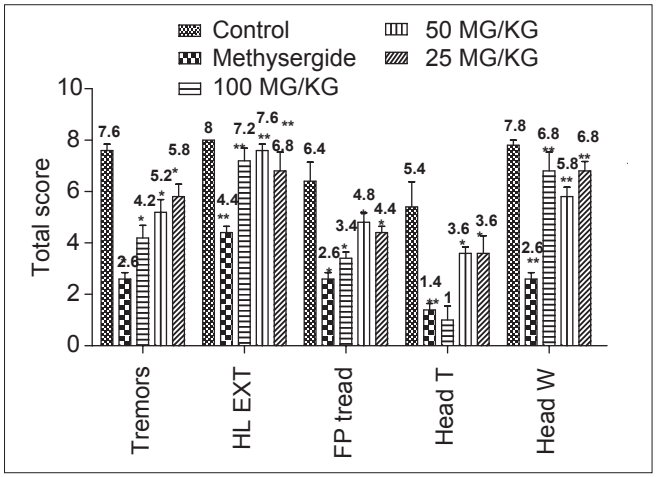

Figure 3: The effect of *Moringa oleifera* and methysergide on serotonin syndrome induced by l-5-HTP (50 mg/kg, i.p.) Each column represents±S.E.M of total score for a group of six mice. A maximum score of 8 is possible. *P<0.0001, **P<0.05

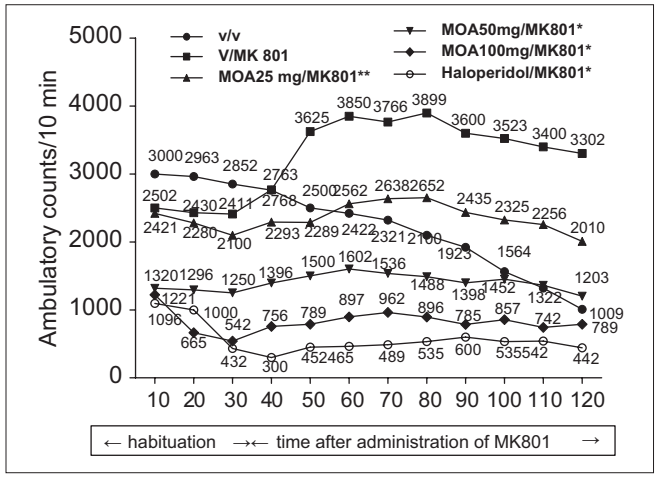

Figure 4: The effect of *Moringa oleifera* and haloperidol on MK 801 (0.3 mg/kg, i.p.) induced hyperactivity Each point represents±S.E.M of ambulatory counts for a group of six mice. *P<0.01, **P<0.05

dopamine and serotonin antagonists prove to be efficacious in antagonizing this MK801 induced hyperactivity.[22] MOA decreased this locomotor stimulant effect induced by MK 801, thereby indicating that it may be acting as a serotonin receptor antagonist. This observation however is in contradiction to what has been reported earlier by some workers. Ray *et al*. reported the central inhibitory effect of *Moringa oleifera* root extract. They found that at higher concentrations (>300 mg/kg) there was a decrease in the locomotor activity and an increase in the serum serotonin levels.[23] Also the root extract significantly increased the pentobarbitone induced hypnosis which was attributed to increase in the serum serotonin levels again at higher doses (>300 mg/kg)[24] whereas the aqueous extract of *Moringa oleifera* leaves in higher doses (>300 mg/kg) was found to release serotonin from enterochromaffin cells of gastric mucosa via 5HT3 receptors.[25] Our observations might be due to the lower dose of MOA (25, 50 and 100 mg/kg p.o.).

Many theories have been established for understanding the mechanism of migraine, of these, one of the theories suggest the involvement of serotonergic and dopaminergic system in migraine. According to the vascular theory, initial trigger of migraine is due to an increased release of serotonin, a neurotransmitter, which being a potent vasoconstrictor causes localised ischemia. The vasoconstriction causes shunting of blood causing increased intracranial pressure resulting in pulsatile headache and vasodilation which in turn causes depletion of serotonin in the later stages.[1] There is also an increase in dopamine levels as implicated by associated symptoms such as yawning, nausea, vomiting and GI disturbances, as a result of which dopamine receptor antagonists are effective therapeutic agents in migraine.[26]

In the present investigation it was observed that MOA inhibited behavioral effects induced by dopamine and serotonin agonists in all the mentioned models. Hence this action of MOA may be through the dopamine and serotonin receptors. The above findings indicate that the leaf juice of *Moringa oleifera* and its extracts could be effectively used for the treatment of migraine.

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