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RESEARCH ARTICLE

Central analgesic activity of ethanolic extract of *Moringa oleifera* seeds

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ABSTRACT

Background: *Moringa oleifera* Lam. commonly known as the drumstick tree is an exceptionally nutritious vegetable tree with a variety of potential medicinal uses. **Aims and Objectives:** The objective of this study was to evaluate the central analgesic activity of ethanolic extract of *M. oleifera* (EEMO) seeds. **Materials and Methods:** The central analgesic activity was studied using Eddy's hot plate method and tail immersion method in Albino Wistar rats of either sex weighing 150–200 g at doses of EEMO 50 mg/kg, 100 mg/kg, and 200 mg/kg. Statistical significance was calculated using one-way analysis of variance followed by *post hoc* Dunnett's test. **Results:** EEMO at all three doses 50 mg/kg, 100 mg/kg, and 200 mg/kg exhibited central analgesic activity by significantly increasing the reaction time in Eddy's hot plate method and tail immersion method when compared to control group. **Conclusion:** EEMO seeds exhibit central analgesic activity in experimental animals.

KEY WORDS: *Moringa oleifera*; Eddy's Hot Plate Method; Tail Immersion Method; Central Analgesic Activity

INTRODUCTION

Moringa oleifera Lam. belongs to the family *Moringaceae* (genus *Moringa*) and most widely distributed species.[1] The whole plant possesses antimicrobial activity[2] and is also used for the treatment of rheumatic conditions, ascites, and venomous bites and for enhancing cardiac function.[3,4] The leaves exhibit strong hypotensive, diuretic, and spasmolytic effects and have been seen to be useful against inflammation and scurvy.[5,6] Plant roots have been used as carminatives, anthelminthics, and diuretics and for treating intermittent fever, epilepsy, and chronic rheumatism.[7] The plant's seeds have been used for purgation, in fever and against inflammatory conditions.[8]

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Pain is a condition that is associated with most of the diseases that a person encounters in once life and seeks medical advice. The major classes of drugs available for the alleviation of pain include non-steroidal anti-inflammatory drugs and opioids.[9] Even though some of these drugs exhibit excellent ability in relieving pain, many of the drugs which are used commonly have serious adverse effects such as peptic ulcer, hepatotoxicity, and nephrotoxicity which limit their use.[10] Analgesic activity of the *M. oleifera* seeds is least evaluated.[11] This study was designed to evaluate central analgesic activity of *M. oleifera* seeds using *in vivo* analgesic models.

MATERIALS AND METHODS

Plant Material

The *Moringa oleifera* pods were procured, seeds were separated, and shade dried. The seeds specimen was authenticated by Dr Athar Ahmad Khan, Assistant Professor, Department of Botany, AMU. Voucher number (SC-0130/11) was obtained for deposited specimen**.** The seeds were finely powdered, 100 g of it was extracted with 200 ml of ethanol

in Soxhlet's apparatus. The ethanolic extract obtained was filtered, and filtrate was evaporated till dryness at 40°C with the help of autoclave. The semisolid ethanolic extract was weighed, sealed with aluminum foils (airtight), and stored at 4°C after calculating its yield.

Drugs and Chemicals Used

- Pentazocine (Ranbaxy, India)
- Propylene glycol (BDH, Mumbai).

IAEC Approval

The study was carried out after taking due approval by the Institutional Animal Ethics Committee (IAEC) on November 15, 2011. All animal experiments were done as per the directives and regulations of IAEC and Committee for the Purpose of Control and Supervision of Experiments on Animals.

Animals Used

Wistar albino rats of both sex (150–200 g)

Rats were procured from the Central Animal House, JNMC, Aligarh Muslim University. They were kept in polypropylene cages in pharmacology section. The rats were given standard rodent diet and water ad libitum. Rats were kept in well-ventilated room and maintained under standard experimental conditions during the course of experiment (temperature 27 ± 2C°, 12 h light/dark cycles). They were habituated to the laboratory condition for 1 week before conducting experiments.

Screening of the Analgesic Activity

Analgesic activity by Eddy's hot plate method[12]

It was done using Analgesiometer (Orchid Scientifics, India). The hot plate consists of an electrically heated surface. The temperature of hot plate was maintained at 55°C –56°C. Rats were placed on the hot plate, and the response such as jumping, withdrawal of the paws, and licking of the paws was noted. Reaction time was taken as time when rat was put on hot plate to the time it showed the response. It was recorded by a stop watch. The reaction time was measured before and at intervals of 30, 60, 90, 120, 150, and 180 min after control/test drugs administration. The cutoff time for the reaction was 15 s. Propylene glycol served as the control while pentazocine served as the standard drug.

Analgesic activity by tail immersion method[13]

Rats were placed in restraining cages exposing the distal part of tail. They were allowed to habituate to the cages before carrying out experiment. The temperature in the water bath was maintained at 55°C. Reaction time was recorded as the time between immersion to withdrawal of tail out of the water bath. It was recorded in seconds by a stopwatch. The reaction time was measured before and at intervals of 30, 60, 90, 120, 150, and 180 min after control/test drugs administration. The cutoff time for the reaction was 15 s. Propylene glycol was taken as control while pentazocine was used as the standard drug.

Experimental Design: Grouping of Animals

Rats were assigned into five groups. Each group consisted of 6 animals of either sex (n=6). Fresh animals were taken for each group in each screening method [Table 1].

Statistical Analysis

Values were recorded as mean ± standard error of mean (SEM). SPSS-17 software was used to carry out statistical tests. One-way analysis of variance followed by *post hoc* Dunnett's multiple comparison test was employed to assess statistical significance. $P < 0.05$ was considered as statistical significance.

RESULTS

Ethanolic extract – It was a green-colored semisolid mass of oily consistency. Yield was 7.54%.

Analgesic Effect of *M. oleifera* Seeds by Eddy's Hot Plate Method

The analgesic activity was evaluated by noting down the reaction time in seconds at various intervals for each group. Values are recorded as mean ± SEM in Table 2. Fig. 1 shows the changes occurring in the reaction time in various groups after administration of the Etanolic extract and control drugs at different time intervals.

In control group, there was no significant change in the reaction time at different time intervals. In pentazocine

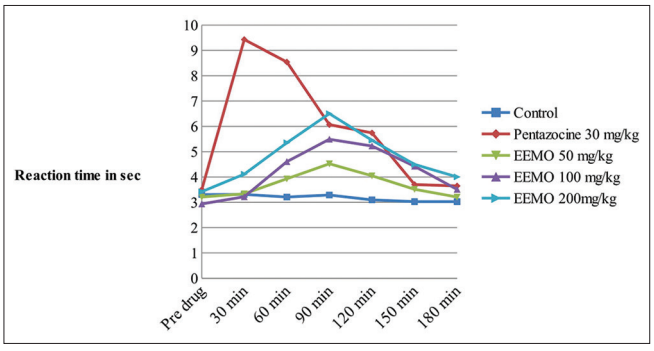

Figure 1: Analgesic effect of EEMO by Eddy's hot plate method

Table 1: Different groups and treatment received

Groups	Name	Treatment received
I.	Normal control	Propylene glycol 2 ml/kg, Orally
II.	Standard control	Pentazocine 30 mg/kg, Orally
III.	Test group- 1	Ethanolic extract 50 mg/kg, Orally
IV.	Test group-2	Ethanolic extract 100 mg/kg, Orally
V.	Test group-3	Ethanolic extract 200 mg/kg, Orally

30 mg/kg group, the maximum analgesic activity was recorded at 30 min ($P < 0.001$) and its effect lasted beyond 180 min ($P < 0.001$).

All EEMO-treated groups demonstrated dose-dependent increase in reaction time. In EEMO 50 mg/kg and 100 mg/kg groups, significant increase in reaction time was started at 60 min ($P < 0.001$) and was maximum at 90 min ($P < 0.001$) compared to control group. Analgesic effect lasted till 150 min ($P < 0.001$) in EEMO 50 mg/kg group and beyond 180 min ($P < 0.001$) in EEMO 100 mg/kg group.

While in EEMO 200 mg/kg treated group, significant increase in reaction time was started at 30 min ($P < 0.001$) and was maximum at 90 min ($P < 0.001$) compared to control group. Analgesic effect lasted beyond 180 min ($P < 0.001$).

Analgesic Effect of *M. oleifera* Seeds by Tail Immersion Method

The analgesic activity was evaluated by noting down the change in reaction time in at various intervals for each group. All the values are shown as mean ± SEM in Table 3. Fig. 2 shows the changes occurring in the reaction time in various groups after administration of the Etanolic extract and control drugs at different time intervals.

In control group, there was no significant change of reaction time at different time intervals. In pentazocine 30 mg/kg group, the maximum analgesic activity was recorded at 30 min ($P < 0.001$) and its effect lasted beyond 180 min ($P < 0.001$).

In all EEMO-treated groups, increase in reaction time was noted. In EEMO 50 mg/kg group, significant increase in reaction time was started at 60 min ($P < 0.001$) and was maximum at 90 min ($P < 0.001$) compared to control group. Analgesic effect lasted till 150 min ($P < 0.001$).

In EEMO 100 mg/kg group, significant increase in reaction time was started at 30 min ($P < 0.05$) and was maximum at 90 min ($P < 0.001$) compared to control group. Analgesic effect lasted beyond 180 min ($P < 0.001$).

In EEMO 200 mg/kg group, significant increase in reaction time was started at 30 min ($P < 0.001$) and was maximum at 90 min ($P < 0.001$) compared to control group. Analgesic effect lasted beyond 180 min ($P < 0.001$).

DISCUSSION

Eddy's hot plate method and tail immersion method were conducted to screen the central analgesic activity of *M. oleifera* seeds. The Eddy's hot plate method is used to screen the central analgesic activity of drugs. The paws

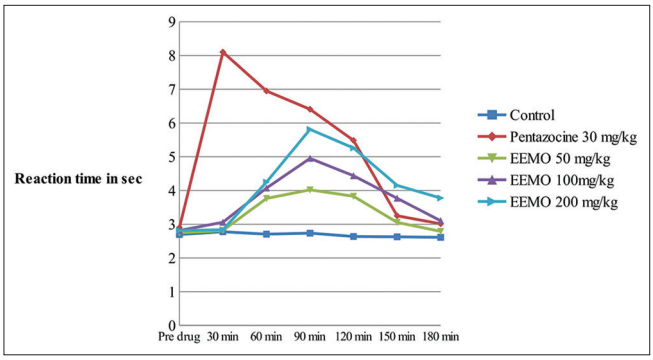

Figure 2: Analgesic effect of EEMO by Tail immersion method

Table 2: Analgesic effect of EEMO seeds on Eddy's hot plate method

Groups	Time interval						
	Pre-drug	30 min	60 min	90 min	120 min	150 min	180 min
Control	3.30±0.1	3.31±0.10	3.21±0.09	3.28±0.09	3.09±0.05	3.02±0.02	3.02±0.02
Pentazocine 30 mg/kg	3.50±0.08	9.43±0.18**	8.54±0.10**	6.06±0.04**	5.74±0.09**	3.7±0.1**	3.65±0.08**
EEMO 50 mg/kg	3.20±0.06	3.33±0.06	3.92±0.10**	4.52±0.13**	4.04±0.05**	3.51±0.06**	3.20±0.06
EEMO 100 mg/kg	2.93±0.05	3.21±0.07	4.60±0.14**	5.49±0.07**	5.22±0.10**	4.42±0.10**	3.51±0.05**
EEMO 200 mg/kg	3.42±0.09	4.11±0.05**	5.35±0.09**	6.50±0.03**	5.44±0.11**	4.49±0.09**	4.00±0.03**

EEMO: Ethanolic extract of *Moringa oleifera* seeds, n=6 in each group. *P<0.05, **P<0.001 when compared to the control group

Table 3: Analgesic effect of EEMO seeds on tail immersion test

Groups	Time interval						
	Pre-drug	30 min	60 min	90 min	120 min	150 min	180 min
Control	2.69±0.03	2.77±0.07	2.70±0.06	2.73±0.05	2.63±0.03	2.62±0.03	2.61±0.03
Pentazocine 30 mg/kg	2.89±0.03	8.10±0.15**	6.95±0.11**	6.40±0.18**	5.48±0.13**	3.25±0.06**	3.01±0.02**
EEMO 50 mg/kg	2.74±0.03	2.81±0.02	3.76±0.02**	4.02±0.04**	3.82±0.02**	3.05±0.02**	2.70±0.02
EEMO 100 mg/kg	2.81±0.03	3.05±0.03*	4.0±0.02**	4.95±0.04**	4.43±0.03**	3.76±0.04**	3.10±0.02**
EEMO 200 mg/kg	2.81±0.02	3.53±0.04**	4.24±0.06**	5.81±0.07**	5.25±0.05**	4.15±0.04**	3.77±0.04**

EEMO: Ethanolic extract of *Moringa oleifera* seeds, n=6 in each group. *P<0.05, **P<0.001 when compared to the control group

of rats are very sensitive to hot temperature. Rats respond to heat by jumping, withdrawal, or licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics.[14] Similarly, in the tail immersion method, the simple tail flick which is the endpoint of this test is mediated as a spinal reflex in response to heat. The test is used to differentiate between central analgesics and peripheral analgesics. It is based on the hypothesis that central analgesics are capable of increasing the reaction time of tail withdrawal reflex in rats when immersed in warm water of 55°C. This escape reaction is a complex phenomenon mediated by the brain.[14]

Ethanolic extract of the *M. oleifera* (EEMO) seeds exhibited significant dose-dependent increase in the reaction time at the doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg in both experimental models. It was also noticed that the analgesic effect of *M. oleifera* seeds persisted for a longer duration than that of pentazocine. Pentazocine showed maximum effect at 30 min and the effect lasted till 120 min after its administration, whereas EEMO 200 mg/kg showed maximum effect at 90 min and the effect lasted beyond 180 min after oral administration. Pentazocine showed a maximum increase in reaction time of 9.43±0.18 s at 30 min while a maximum delay in reaction time by EEMO 200 mg/kg was 6.50±0.03 s at 90 min in the Eddy's hot plate method. In tail immersion method, maximum increase in reaction time in pentazocine group was 8.10 ± 0.15 s, whereas in EEMO 200 mg/kg group, maximum response of 5.81 ± 0.07 s was noted at 90 min. The analgesia produced by *M. oleifera* is moderate in nature while pentazocine produced stronger analgesia.

Results of this study are similar to that of aqueous extract of *M. oleifera* seeds.[11] It is also in coherence with the effect of different alcoholic extracts of *M. oleifera* seeds in similar experimental models.[15]

M. oleifera seeds demonstrated central analgesic activity in well-known experimental models in rats. Phytochemicals responsible for demonstrating central analgesic activity in EEMO seeds need to be isolated and identified for further studies.

CONCLUSION

In the present study, *M. oleifera* seeds exhibited central analgesic activity. This can be attributed to various phytochemicals such as flavonoids, saponins, and alkaloids present in the seeds which may cause alteration of various neurotransmitters such as serotonin, gamma-aminobutyric acid, and others in the pain pathway.[16] There is a need of further studies to identify the mechanisms and phytochemicals responsible for exhibiting its analgesic activity.

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