Fayazuddin M *et al*. / *International Journal of Phytopharmacology*. 5(6), 2014, 423-426.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

EVALUATION OF CENTRAL ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF SEEDS OF *MORINGA OLIEFERA* IN EXPERIMENTAL ANIMALS

Fayazuddin M*, Ahmad F, Haseeb A, Kumar A

Department of Pharmacology, J.N. Medical College, Aligarh Muslim University, Aligarh 202002, India.

ABSTRACT

Evaluation of central analgesic activity of aqueous extract of seeds of *Moringa oliefera* in experimental animals. Albino wistar rats of either sex weighing 150-200g were used. Aqueous extract of *Moringa oliefera* seeds (AEMO) was prepared with the help of Soxhlet's apparatus. The analgesic activity was studied using tail immersion method and Eddy's hot plate method. Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by post hoc dunnett's test. $P < 0.05$ was considered statistically significant. In Tail immersion test all the groups treated with AEMO demonstrated dose dependent increase in reaction time but in AEMO 500 mg/kg and 750mg/kg treated groups there was significant increase in the reaction time when compared to control group ($p<0.001$) Similarly in Eddy's hot plate method, AEMO treated groups at the dose of 500mg/kg and 750mg/kg demonstrated dose dependent significant increase in reaction time when compared to control group ($p<0.001$). Thus it can be concluded from our study that the aqueous extract of *Moringa oleifera* seeds possesses central analgesic activity.

Key words: Moringa oleifera, Tail immersion method, Eddy's hot plate method, Analgesic activity.

INTRODUCTION

Pain is one of the most common reasons for patients to seek medical advice. Pain is defined by the International Association for the Study of Pain (IASP) as, "*an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*" (Bonica JJ, 1990). There is an increase in the incidence of diseases with chronic pain such as osteoarthritis, back pain etc. which generally require prolonged administration of analgesics. Both NSAIDs and opioids are associated with adverse effects like gastritis, nephrotoxicity and dependence with prolonged use (Rang HP *et al*., 2009). There is an exponential growth in the field of traditional and alternative medicines in last few years and these drugs are becoming popular because of their natural origin and lesser side effects in both in developing and developed countries. *Moringa oleifera* Lam. belongs to the family *Moringaceae* and is its best- known and most widely distributed species (Ramachandran C *et al*., 1980). It is commonly known as the drumstick tree or horseradish tree in English and shahjan in hindi. Various parts of *Moringa oleifera* have long been used in traditional medicine for their medicinal values (Nadkarni KM and Nadkarni AK, 1976). The leaves are reported to have antioxidant, diuretic, antispasmodic, anti-inflammatory and hypotensive activity (Caceres A *et al*., 1992; Faizi S *et al*., 1992).

The roots are reported to have hepatoprotective, antispasmodic, and anticonvulsant activity (Ruckmani K *et al*., 1998; Ray R *et al*., 1998). The seeds are reported to have anti-inflammatory, antiarthritic and antitumour activities (Fayazuddin M *et al*., 2013; Guenera PA *et al*., 1998; Hamza AA, 2010). However there are very few studies reporting the analgesic activity of *Moringa oleifera* seeds and to our best knowledge no study has been reported on aqueous extract of *Moringa oleifera*

Corresponding Author

Fayazuddin M
Email: drmdfayazuddin@yahoo.com

seeds. Hence this study was conducted for the experimental evaluation of central analgesic activity of aqueous extract of seeds of *Moringa oliefera* in rats.

MATERIAL AND METHODS

Plant Material and Extraction

The pods of *Moringa oliefera* were collected from University campus and seeds were separated from them and shade dried. The seeds were identified and authenticated by the Department of Botany of the University. A sample specimen was deposited, bearing voucher number SC-0130/11**.** Seeds were powdered with the help of a mechanical grinder and 100 gm of seed powder was extracted separately with 500ml of distilled water for aqueous extract with the help of Soxhlet's apparatus. The extract was collected in Petri dishes and evaporated till dryness in an incubator. The extract was stored at 4 ºC for further experimental work.

Drugs and Chemicals used

Pentazocine (Ranbaxy, India), Propylene glycol (BDH, Mumbai) were used in the study. The other chemicals used were of analytical grades manufactured by Merck Laboratories (Mumbai, India), BDH Laboratories (Mumbai, India) and CDH laboratories (New Delhi, India).

Animals

Albino wistar rats (150-200g) were procured from the Central Animal House of the college. They were housed in polypropylene cages at ambient temperature (25± 2◦C), relative humidity (55 ± 5%) and 12-hr light-dark cycle. Animals had free access to standard pellet diet and water ad *libitum*. The study protocol was approved by the Institutional Animal Ethics Committee.

Experimental design

Animals were divided into five groups of six animals each. Group-1 served as control and was given propylene glycol 2ml/kg orally, group-2 served as standard and was given pentazocin 30mg/kg, i.p., groups 3, 4 and 5 were given aqueous extract of *Moringa oleifera*(AEMO) seeds orally at the dose of 250, 500 and 750mg/kg respectively.

Analgesic activity by Tail immersion method

Rats were placed into restraining cages with their tail hanging out freely. The animals were allowed to adapt to the cages before testing. The distal 5 cm portion of the tail was marked and immersed in a water bath maintained at 55 °C. The time taken to withdraw the tail clearly out of the water was taken as the reaction time. It was recorded in seconds by a stopwatch. The reaction time was measured before and after 30, 60, 90, 120, 150 and 180 minutes after administration of the control and the test drugs. The cut off time for the reaction was 15 seconds (Sewell RDE, Spencer PSJ, 1976).

Analgesic activity by Eddy's hot plate method

It was performed by using analgesiometer (Orchid Scientifics, India). The paws of rats are very sensitive to heat at temperatures which are not damaging the skin. The hot plate consists of an electrically heated surface. The temperature is controlled in the range of 55° to 56 °C. The responses noted were jumping, withdrawal of the paws and licking of the paws. The animals were placed on the hot plate and the time until response occurs was recorded by a stop-watch. The reaction time was measured before and after 30, 60, 90, 120, 150 and 180 minutes after administration of the control and the test drugs. The cut off time for the reaction was 15 seconds (Eddy NB & Leimbach DJ, 1953).

Statistical analysis

All the values are expressed as Mean ± SEM (n=6). Statistical significance was calculated by one way ANOVA followed by post hoc Dunnett's multiple comparison test. $p < 0.05$ was considered to be statistically significant.

RESULTS

In Tail immersion method all the groups treated with AEMO demonstrated dose dependent increase in reaction time (Table1). But in AEMO 250 mg/kg treated group there was no significant change in the reaction time when compared to control group whereas in AEMO 500 mg/kg and 750mg/kg treated groups there was significant increase in the reaction time when compared to control group with onset of action at 60minutes ($p<0.001$) and their peak effect was seen at 90 minutes ($p<0.001$) when compared to control group and their analgesic effect lasted for more than 180 minutes following its administration (Figure1).

Similarly in Eddy's hot plate method, AEMO treated groups at the dose of 500mg/kg and 750mg/kg demonstrated dose dependent significant increase in reaction time (Table2). In AEMO 500 mg/kg and 750mg/kg treated groups onset of action was at 60minutes ($p<0.001$) and their peak was at 90 minutes ($p<0.001$) when compared to control group and their analgesic effect lasted for more than 180 minutes ($p<0.001$) following its administration (Figure 2).

DISCUSSION AND CONCLUSION

The *Moringa oleifera* seeds were screened for the central analgesic activity by two well known models, tail immersion method and Eddy's hot plate method. The paws of rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws.

Table 1. Effect of aqueous extract of *Moringa oleifera* seeds on Tail immersion method

Time interval→ Groups↓	Reaction Time in Seconds						
	Predrug	**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 30mg/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*
AEMO 250mg/kg	2.87±0.03	2.89±0.02	2.90±0.02	2.85±0.02	2.90±0.02	2.84±0.02	2.80±0.02
AEMO 500mg/kg	2.69±0.01	2.70±0.00	3.16±0.03**	4.13±0.02**	3.82±0.04**	3.77±0.01**	3.12±0.03*
AEMO 750mg/kg	2.75±0.02	2.74±0.01	3.06±0.23**	4.61±0.04**	4.39±0.04**	3.75±0.03**	3.22±0.04**

AEMO: Aqueous extract of *Moringa oleifera* seeds, n = 6 in each group Values were expressed as Mean +/- SEM.
p < 0.05 - significant *indicates p<0.05, ** indicates p <0.001 when compared to the control group.

Table 2. Effect of aqueous extract of *Moringa oleifera* seeds on Eddy's hot plate method

Time interval→ Groups↓	Reaction Time in Seconds						
	Predrug	**30 min**	**60min**	**90min**	**120min**	**150min**	**180min**
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 30mg/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**
AEMO 250mg/kg	3.22±0.06	3.16±0.05	3.17±0.06	3.02±0.03	3.01±0.02	2.83±0.05	2.78±0.05
AEMO 500mg/kg	3.11±0.07	3.31±0.08	4.51±0.07**	5.12±0.04**	4.24±0.06**	3.93±0.08**	3.52±0.06**
AEMO 750mg/kg	3.03±0.05	3.42±0.08	4.43±0.10**	5.22±0.06**	5.13±0.05**	4.04±0.04**	3.84±0.05**

AEMO: Aqueous extract of *Moringa oleifera* seeds, n = 6 in each group Values were expressed as Mean +/- SEM. p < 0.05 - significant *indicates p<0.05, ** indicates p <0.001 when compared to the control group.

Figure 1. Analgesic effect of Aqueous extract of *Moringa oleifera* seeds (AEMO) by Tail immersion method

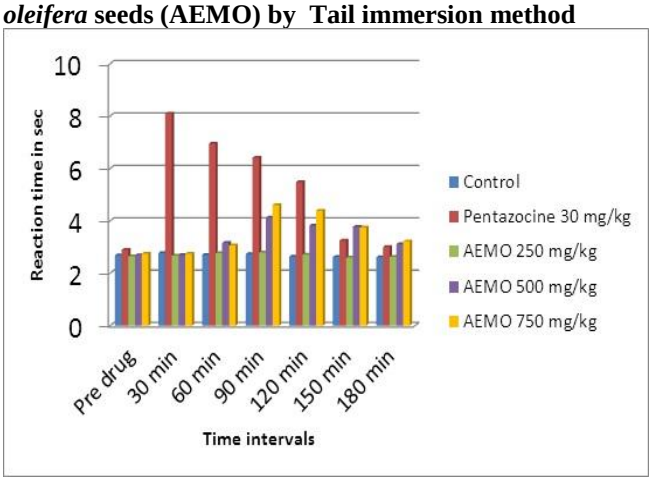
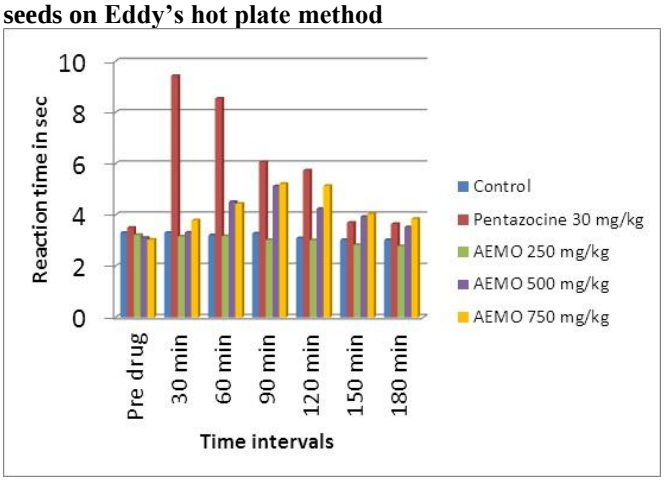


Figure 2. Effect of aqueous extract of *Moringa oleifera* seeds on Eddy's hot plate method



The time until these responses occur is prolonged after administration of centrally acting analgesics, similarly in the tail immersion method, the simple tail flick which is the endpoint of this test is mediated as a spinal reflex. This escape reaction can be regarded as a complex phenomenon mediated by the brain (Vogel HG, 2008). Therefore the observation of the escape reaction can be regarded as a true assessment of the influence of the drug on the brain. The test is very useful for discriminating between centrally acting morphine-like analgesics and

non-opiate analgesics. The aqueous extract of *Moringa oleifera* seeds exhibited significant ($p<0.001$) dose dependent increase in the reaction time in both the tests. It was also noticed that the analgesic effect of *Moringa oleifera* seeds persisted for a longer duration comparable to that of pentazocine. Pentazocine showed maximum effect at 30 minutes after its administration whereas aqueous extract of *Moringa oleifera* seeds showed maximum effect at 90 minutes and the effect lasted till 180 minutes after oral administration. The analgesia produced by *Moringa oleifera* is slow in onset and moderate in nature but of longer duration compared to pentazocine, which produces rapid onset and strong analgesia. The mechanism of central analgesic activity may be due to alteration of various neurotransmitters like serotonin, GABA and others in the pain pathway (Ganguly Guha D, 2008). The analgesic activity of *Moringa oleifera* seeds can be attributed to various phytochemicals like flavonoids, saponins, glycosides and alkaloids present in the seeds (Sanchez MD *et al*., 2006). Thus it can be concluded from our study that the aqueous extracts of Moringa *oleifera* seeds possesses central analgesic activity and further extensive studies are needed to elucidate the exact mechanisms and active principle responsible for analgesic activity of *Moringa oleifera* seeds.

REFERENCES

Bonica JJ. Definitions and taxonomy of pain. In The Management of Pain, 2nd ed, Philadelphia, Lea & Febiger, 1990, 18-27.

Caceres A, Saravia A, Rizzo S, Zabala L, De-Leon E, and Nave F. Pharmacological properties of M. oleifera 2. Screening for antispasmodic, anti-inflammatory and diuretic activity. *J Ethnopharmacol,* 36, 1992, 233–237.

Eddy NB, Leimbach DJ. Synthetic analgesics: II Dithienyl butenyl and Dithienyl butylamines. *J Pharmacol Therap,* 107(3), 1953, 385-393.

Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. *Phytochemistry,* 38, 1995, 957–963.

Fayazuddin M, Ahmad F, Kumar A, Yunus S. An experimental evaluation of anti inflammatory activity of *Moringa Oleifera* seeds. *Int J Pharm Pharm Sci,* 5(3), 2013, 717-721.

Ganguly, Guha D. Alteration of brain monoamines & EEG wave pattern in rat model of Alzheimer's disease & protection by *Moringa oleifera. Indian J Med Res,* 128, 2008, 744-751.

Guenera PA, Vergas C, Milagros UY. Anti-inflammatory and Anti-tumor activities of Seed extracts of Malunggay, *Moringa oleifera* L (Moringaceae). *Philli J Sc,* 125, 1996, 175-184.

Hamza AA. Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. *Food and Chemical Toxicology*, 48, 2010, 345-355.

Nadkarni KM and Nadkarni AK. *Indian Materia Medica.* Mumbai: Popular Prakashan, 1, 1976, 811–816.

Ramachandran C, Peter KV, Gopalakrishnan PK. Drumstick (*Moringa oleifera*). A multipurpose Indian vegetable. *Econ Bot,* 34,V1980, 276–283.

Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology, 6th ed, Churchill Livingstone Publications, 2009.

Ray R, Hazra and Guha D. Central inhibitory effect of *Moringa oleifera* root extract: possible role of neurotransmitters. *Indian J of Exp Biol,* 41, 2003, 1279-1284.

Ruckmani K, Kavimani S, Anandan R, Jaykar B. Effect of Moringa oleifera Lam on paracetamol-induced hepatoxicity. *Indian J Pharm Sci,* 60, 1998, 33- 35.

Sanchez MD, Lopez CJ Rios V. High-performance liquid chromatography method to measure α and γ-tocopherol in leaves and fresh beans from Moringa oleifera. *J. Chromatogr A,* 1105, 2006, 111-114.

Sewell RDE, Spencer PSJ. Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail immersion test in mice and rats. *Neuropharmacology,*15, 1976, 683-688.

Vogel HG. Drug Discovery and Evaluation. 2nd edition. Springerlink publications, 2008, 696-97.