Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 1, 2011 ISSN - 0974-2441

Research Article

EVALUATION OF ANTINOCICEPTIVE ACTIVITIES OF FRESH LEAF JUICE AND ETHANOLIC EXTRACT OF *MORINGA OLEIFERA* LAM.

KANCHAN P UPADHYE[1]*, VINOD D RANGARI[1], VIJAY B MATHUR[2]

[1] J. L. Chaturvedi College of Pharmacy, Electronic Zone. MIDC Hingna Road, Nagpur 440009, India.
[2]Sharad Pawar College of Pharmacy, Hingna road, Nagpur, 440009, India
E-mail: kanchanupadhye@ yahoo.co.in

ABSTRACT

The fresh leaf juice and ethanolic extract of the leaves of *Moringa oleifera* (Family: Moringaceae) were administered orally at doses of 25, 50,100 mg/kg in mice and were tested for antinociceptive activities using three models: Acetic acid induced writhing, formalin induced paw licking and tail flick test using analgesiometer. Amongst all doses, a dose of 100mg/kg of both the administered extracts showed a significant antinociceptive activity in mice. The effect was significantly reversed by the opoid receptor antagonist naloxone indicating the role of both the central and peripheral opoid receptors in alleviating pain.

Key words: Antinociceptive, *Moringa oleifera,* writhing.

INTRODUCTION

Moringa oleifera Lamm belonging to the family Moringaceae is also called as Sajna, Drumstick and Shevga in regional languages. It is a medium sized tree with tripinnate leaves[1] and is cultivated throughout India. All parts of the plant are useful. The leaves of *Moringa oleifera* are reported to show antioxidant[2], antihypertensive[3] hypocholesteromic [4], antifungal [5], radioprotective [6] and wound healing activities[7]. The roots and barks of the plant possess central inhibitory effect[8], antispasmodic, diuretic[9] and antiepileptic[10] activities while the seeds possess antipyretic and anti-inflammatory activities[11]. The leaves are used in folklore medicine for the treatment of pain. Some previous reports indicate that the aqueous extract of the leaves possesses significant antinociceptive activities[12]. Thus the present study is planned to screen the usefulness of the leaf juice and ethanolic extract in the treatment of pain.

MATERIALS AND METHODS

Materials

Fresh leaves of *Moringa oleifera Lamm* (Moringaceae) were collected in February 2010 were authenticated at Department of Botany herberium, Nagpur University, Nagpur (voucher number 9116)

Preparation of the extracts

The fresh leaf juice extract was prepared by crushing the fresh leaves in minimum amount of distilled water followed by filtration to remove the leaves. The ethanolic extract was prepared by cold maceration of the fresh leaves with ethanol (99%) for 24 hours. All of these extracts were shade dried. The percentage yields of the three extracts were 3.33% and 1.66% respectively. At the time of use, the extracts were resuspended in distilled water at the desired concentrations.

Animals

Swiss albino mice (20-25 g) of either sex obtained from animal house of our institute were 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 (92/1999/CPSCEA).

Drugs

Both the extracts of *Moringa oliefera* were tested in three doses (25, 50,100mg/kg).Pentazocine (2mg/kg) was used as the standard drug in all the three models of nociception .Naloxone was used as opoid receptor antagonist.

EXPERIMENTAL PROTOCOL

Acute toxicity

Both the extracts were subjected to acute toxicity studies [13] by administering each of the extracts in increasing doses upto 5g/kg per orally to different groups of mice. Toxicity was compared with control group that received only distilled water. The animals were observed for 48 hours and mortality was recorded for each group at the end of this period.

Writhing test

Mice received oral doses of fresh leaf juice extract and ethanolic extract (25, 50, 100 mg/kg) or vehicle (water) 60minutes prior to the intraperitoneal injection of 0.6%v/v acetic acid[13]. The number of writhings of each mouse was counted for 20 minutes.Pentazocine (10mg/kg; i.p.) was administered as positive control. Naloxone (2mg/kg; s.c.) was administered 15 minutes prior to the extracts or pentazocine injection.

Formalin test

Mice were orally treated with fresh leaf juice extract and ethanolic extract (25, 50, 100 mg/kg).One hour after they were injected with 25 µl of 5% (v/v) formalin solution (s.c.) on the plantar surface of one rear paw and the duration of paw licking was determined 0-10 min (early phase) and 20-40 min (late phase) after formalin injection [14]. Pentazocine (10 mg/kg, i.p.) was administered as positive control. Naloxone (2mg/kg; s.c.) was administered 15 minutes prior to the extracts or pentazocine injection.

Tail flick test

Mice were orally treated with fresh leaf juice extract and ethanolic extract (25, 50, 100 mg/kg) and the tail flick latency was assessed with analgesiometer (Inco, India) over a period of 60, 90 and 120 minutes[15]. Pentazocine (10 mg/kg, i.p.) administered to one group served as the positive control. Strength of the current passing through the nichrome wire was kept constant at 6 amperes. The distance between the heat source and tail skin was 1.5 cm. The site of application of radiant heat in tail was measured from root to tail. The cut of reaction time was fixed at 10 seconds to avoid tissue damage. Naloxone (2mg/kg; s.c.) was administered 15 minutes prior to the extracts or pentazocine injection.

Statistical analysis:

The results are reported as mean ± S .E.M and analyzed with ANOVA followed by Dunetts multiple comparison test. $p<0.05$ are considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of both the extracts indicated

the presence of saponins, glycosides and some phenolics. The antinociceptive effect of orally administered fresh leaf juice and alcoholic extract was demonstrated in this study by three different nociceptive tests. Both the extracts given orally in doses of 100 mg/kg significantly and dose dependently reduced the number acetic acid induced writhes in mice [Table 1]. A similar effect was also observed in both early and late phases of formalin induced paw licking [Table 2]. In the tail flick method the tail flick latency was significantly and dose dependently prolonged by both the extracts in doses of 100 mg/kg [Table 3]. The antinociceptive response of the extracts was significantly antagonized by naloxone, an opoid receptor antagonist. The observed results indicate that the extracts posses both peripheral and central antinociceptive activities with the involvement of opoid receptors. Thus both the extracts can be further explored for the isolation of constituents responsible for such an activity. These observations may thus provide some pharmacological rationale for its use in the treatment of pain in folklore medicine.

ACKNOWLEDGEMENT

The authors are grateful to J.L. Chaturvedi college of Pharmacy for providing the facilities to carry out the present work.

Table -1: Acetic Acid Induced Writhing Test

S. No.	Treatment	Dose (mg/kg)	Writhings (Mean ± S.E.M)	Inhibition(%)
1.	Control (water)	10ml/kg	45.4± 10.69	
2.	Pentazocine (i.p)	10	7± 1.84	84.5
		25	20.8 ± 4.29*	54.2
3.	Leaf Juice	50	16.2 ± 4.49**	64.3
		100	4.2 ± 3.36*	85.02
		25	15.4± 4.11*	66
4.	Ethanolic extract	50	14.6 ± 1.96*	67.8
		100	8.2 ± 0.8*	81.9
5.	Pentazocine + Naloxone	10 + 2	39.3± 0.69*	13.6
6.	Leaf juice+ Naloxone [c]	100 +2	40.3± 0.56*	11.2
7.	Ethanolic extract + Naloxone [c]	100 +2	38.7 ± 1.23*	15.6

Values are mean ± S.E.M. (n=6); * p<0.01 vs., control, one- way ANOVA followed by Dunett's comparison test.

Table -2: Formalin induced paw licking

S.No.	Treatment	Dose (mg/kg)	Early phase[b]	% Inhibition	Late phase[b]	%Inhibition
1.	Control (water)	10ml/kg	132.8 ± 16.1		67.2 ± 14.3	
2.	Pentazocine (i.p)	10	62.8 ± 5.4*	52.71	27 ± 2.8*	59.8
		25	57.8± 5.8*	66.05	28.2± 3.3*	58.0
3.	Leaf Juice	50	52± 8.8*	60.8	24± 4.4*	64.2
		100	48.4± 6.02*	63.4	22.6± 1.5*	66.3
		25	44± 1.14*	66.8	20± 1.14*	70.2
4.	Ethanolic extract	50	28.6± 2.63*	78.5	15.4± 2.50*	77.0
		100	18.2± 1.94*	86.3	8± 0.54*	88.0
5.	Pentazocine +Naloxone	10 + 2	115.2 ±1.84*	13.2	60 ±1.31*	10.72
6.	Leaf juice+ Naloxone [c]	100 +2	112± 1.23*	15.6	58.5± 0.66*	12.8
7.	Ethanolic extract + Naloxone [c]	100 +2	118.7± 0.98*	10.6	60.3± 0.67*	10.2

Values are mean ± S.E.M. (n=6); * p<0.01 vs., control, one- way ANOVA followed by Dunett's comparison test.

Table 3: Analgesic activity by tail flick method

S.No.	Treatment	Dose (mg/kg)	Tail flick latency		
			60 min	90min	120min
1.	Control (water)	10ml/kg	1.92±0.18	1.73± 0.27	1.81± 0.27
2.	Pentazocine (i.p)	10	6.01± 0.57*	5.87± 0.5*	5.63± 0.47*
		25	2.14±0.19**	2.06± .16**	1.94± 0.15*
3.	Leaf Juice	50	3.77± 0.29*	2.76± 0.07*	2.33± 0.09*
		100	5.39± 0.51*	4.41± 0.23*	3.99± 0.18*
		25	3.99±0.43**	3.53±0.37**	3±0.43**
4.	Ethanolic extract	50	5.18±0.78*	4.38±0.59*	3.59±0.55**
		100	6.05±0.9*	4.66±0.74*	3.69±0.49**
5.	Pentazocine + Naloxone	10 + 2	2.06± 0.98*	1.62± 0.89*	1.58± 0.78*
6.	Leaf juice+ Naloxone	100 +2	2.62± 0.5*	2.58± 0.72*	2.41 ±0.76*
7.	Ethanolic extract + Naloxone	100 +2	1.98± 0.9*	1.96± 0.78*	1.95± 0.8*

Values are mean ± S.E.M. (n=6); * p<0.01 vs., control, one- way ANOVA followed by Dunett's comparison test

REFERENCES

1. Kirtikar, K. R., Basu, B, D., Indian Medicinal Plants (M/S Bishen Singh, Mahendra Pal Singh, New Cannaught Place, Dehradun) 1975, Vol. 1, Edn 2, 676 – 680.
2. Siddharaju P, Becker K. Antioxidant properties of various solvent extracts of the total phenolic constituents J. Agric. Food .Chem 2003; 51(8): 2144-2150.
3. Dangi SY, Jolly C L, Narayanan S.Antihypertensive activity of total alkaloids from the leaves of *Moringa oliefera.* Pharm. Biology 2002; 40(2), 142-148
4. Ghasi S, Nwebodo E, Ofili J.O. Hypocholesteromic effect of crude extract of leaf of *Moringa oliefera* Lamm in high fat diet fed Wister rats. Journal of Ethnopharmacology 2000; 69(1),21-25.
5. Chuang PH, Lee CW, Chou JY, Murugan M, Sheih BJ, Chen H.M.Antifungal activity of crude extract and essential oil of *Moringa oliefera*Lamm. Bioresource technology 2007; 98 (1), 232-236.
6. Rao AV, Umadevi P, Kamath R, In vivo radioprotective effect of *Moringa oliefera* leaves. Ind .J .Exp. Bio 2001; 39: 858 -863.
7. Rathi BS, Bodhankar SL, Baheti AM,Evaluation of aqueous leaves

extract of *Moringa oliefera* Linn for wound healing in albino rats. Indian journal of experimental biology .2006;44: 898 –901.
8. Ray K, Zahra.R, Guha D, Central inhibitory effect of *Moringa oliefera* root extract: Possible role of neurotransmitters. Indian journal of experimental biology .2003; 36: 1279- 1284.
9. Cacares A, Saravia A, RizzoS, ZabalaL, DeLeon.E, Nave F, Screening for antispasmodic, anti-inflammatory and diuretic activity. Journal of Ethnopharmacology1992; 69(1), 21-25.
10. Abubaka Z, Dangoggo SM, Hassan LG, Biological activity and phytochemistr of *Moringa oliefera*. A review. Journal of Chemical Society Nigeria2002; 27(2), 123- 127.
11. Jadhav.SL, Sharma SR, Pal SC, Kasture SB, KastureVS, Chemistry and pharmacology of *Moringa oliefera* Lamm and *Moringa concanensis* Nimo. Indian Drugs 2000; 37(3), 139-144
12. Sulaiman. MR, Zakaria ZA, BujariminAS, Somchi MN ,Israf D A.,Moin.S.Evaluation of *Moringa oleifera* Aqueous Extract for Antinociceptive and Anti-Inflammatory Activities in Animal Models.Pharmaceutical Biology2008; 46(12), 838-845.
13. Hoeessin H, Hani MY. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacology 2002, 2-7.
14. Tjolsen A, Berge OG, Hunskaar S, Rosland, JS, Hole K.The formalin test: an evaluation of the method. Pain 1992 ;51, 5-17
15. Chakraborty A, Devi RKB, Rita S, Sharatchandra K, Singh T I, Preliminary studies on anti-inflammatory and analgesic activities of *Spillanthes acmella* in experimental animal model. Indian Journal of Pharmacology 2004; 36, 148-150.