e-ISSN: 2349-2678, p-ISSN:2961-6050

available online on www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

NLM (National Library of Medicine ID: 101732687)

Index Copernicus Value 2022: 72.59

Volume 12 Issue 6; 2024; Page No. 59-64

Anti-Inflammatory potential of Moringa oleifera Leaves in Wistar rats.

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Article Info: Received: 11-09-2024 / Revised: 18-10-2024 / Accepted: 17-11-2024

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Conflict of interest statement: No conflict of interest

Abstract

(Moringaceae) is a bush of African savannah, commonly known as Drum stick used in folk Medicine for the treatment of rheumatic and articulanry pain. Its seeds shown analgesic activity [7], Antipyretic activity [8]. Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported [23] hence the analgesic and anti-inflammatory effects produced by these extracts may be attributed due to the flavonoids and steroids. However, its pharmacological actions and mechanisms have not been precisely documented in spite of its increasing usage recently. Present work reported the potential effects of the stem bark of Moringa oleifera, as an anti-inflammatory agent using both in vivo and in vitro models. Carrageenan-induced paw edema and cotton pellet granuloma formation in rats reflect the edematous stages during acute and chronic inflammation. [24,25] In the present study, two different extracts of leaves of Moringa oleifera were tested. Carrageenan induced rat paw edema has been a popular inflammatory model to investigate nonsteroidal anti-inflammatory effect of compounds [26] Serotonin, histamine, bradykinin, and prostaglandin have been identified as a mediators for carrageenan induced rat paw edema. [27] Petroleum ether and methanolic extracts were found to possess a prominent anti-inflammatory activity, showing inhibition to the paw edema induced by carrageenan during the three time points from 1 to 3 h. In cotton pellet granuloma model Petroleum ether and methanolic extracts showed significant inhibition.

Key Words: Moringa oleifera, Anti-inflammatory, Carrageenan, cotton pellet

Introduction

M. oleifera is a fast-growing, deciduous tree [7] that can reach a height of 10–12 m (33–39 ft) and trunk diameter of 46 cm (18 in).[8] The bark has a whitish-gray color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches, and the leaves build up a feathery foliage of tripinnate leaves. The flowers are fragrant and hermaphroditic, surrounded by five unequal, thinly veined, yellowish-white petals. The flowers are about 1–1.5 cm (3⁄8–5⁄8 in) long and 2 cm (3⁄4 in) broad. They grow on slender, hairy stalks in spreading or drooping flower clusters, which have a length of 10–25 cm (4–10 in).[8] Flowering begins within the first six months of planting. In seasonally cool regions, flowering only occurs once a year in late spring and early summer (Northern Hemisphere between April and June, Southern Hemisphere between October and December). In more constant seasonal temperatures and with constant rainfall, flowering can happen twice or even all year-round.[8] The fruit is a hanging, three-sided, brown, 20–45 cm (8–17+1⁄2

in) capsule, which holds dark brown, globular seeds with a diameter around 1 cm. The seeds have three whitish, papery wings and are dispersed by wind and water.[8]In cultivation, it is often cut back annually to 1–2 m (3–6 ft) and allowed to regrow so the pods and leaves remain within arm's reach.[8]

Materials and Methods

Plant Material leaves of Moringa oleifera Fam Moringaceae was collected from local region of Mirzapur in September 2024. The plant material was identified and authenticated by Dr. Ashwini Kumar Kushwaha Assistant Professor, Department of Dravyaguna faculty of ayurveda. IMS, RGSC, Banaras Hindu University. Barkachha, Mirzapur (Ref no. RSC/PID-AY/2024/03).

Preparation of Extract

The plant material leaves were cleaned, dried under shade and pulverized by using grinder. 500g of the powder of plant was successively extracted with Petroleum ether, chloroform, and methanol in order of increase their polarity using Soxhlet apparatus. The yield of extracts obtained as Petroleum ether as 0.89 %, Chloroform as 3.6 % , Methanol as 16.63 %. From the Pharmacology online 3: 641-650 (2011) Kumbhare et al. 643

Preliminary Phytochemical study revealed that presence of sterols, glycosides, Alkaloids , Triterpenoids, Flavonoids and tannins in the extracts.

Experimental Animals:

Albino rats of Wistar strain (150-200 g) of either sex were used in the entire study and were procured from Bilwal medichem and research laboratory pvt.ltd. Jaipur . They were housed in standard polypropylene cages and kept under control room temperature (24 ± 2 ºC; relative humidity 60%-70%) in a 12 h light-dark cycle. The animals were fed with standard laboratory diet of Pranav agro pvt .Ltd. and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethical Committee.

Anti-Inflammatory Activity:

Carrageenan induced rat paw edema

The anti-inflammatory activity using carrageenan induced hind paw edema was carried out as described by Winter et al.[18] Anti-inflammatory activity was evaluated using the Carrageenan induced rat paw edema according to the technique of Winter et al. . After 16h of fasting, the rats of 150-200 gm were divided into eight groups of six each.

Group I served as control group and received distilled water (DW), orally.
Group II received Diclofenac as standard at a dose of 5 mg/Kg.
Group III, IV and V animals received Pet Ether extract of Moringa oleifera at a dose of 100, 200 and 400 mg/kg ;
Group VI, VII, VIII received methanol extract of Moringa oleifera at a dose of 100, 200 and 400 mg/kg; After 1 h, 0.1 ml of 1% w/v Carrageenan suspension was injected subcutaneously in to the plantar surface of the right hind paw. The paw volume was measured using a Digital plethysmometer PLM-01 (Orchid Scientifics, India) immediately and 3 h after carrageenan injection. [19]

Cotton pellet induced granuloma formation in rats

After 16h of fasting, the rats of 150-200 gm were divided into eight groups of six each.

Group I served as control group and received distilled water (DW), orally.
Group II received Diclofenac as standard at a dose of 5 mg/Kg.
Group III, IV and V animals received Pet Ether extract of Moringa oleifera at a dose of 100, 200 and 400 mg/kg ;
Group VI, VII, VIII received methanol extract of Moringa oleifera at a dose of 100, 200 and 400 mg/kg; orally for consecutive six days [20,21]. The cotton

pellet weighing 50±1 mg was sterilized in an autoclave (Lab hosp, Mumbai, India) handled with sterile instrument. The pellet was inserted in each animal on the back. Control group received vehicle. The animals were sacrificed on seventh day and cotton pellet along with granuloma mass was collected, it was weighted and dried at 60ºc.Results of the assay were calculated as % inhibition of dry weight of granuloma formation by using the formula: 100 (A-B)/A, where, A= gain in dry weight of control pellet (mg), B= gain in dry weight of drug treated (mg).

Statistical Analysis

Results of all the above estimations have been indicated in terms of mean ± SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's test multiple comparisons test using GraphPad InStat version 5.00, GraphPad Software, CA, USA. The level of significance was set at $P < 0.05$.

Result

Effect of various extracts of Moringa oleifera on carrageenan induced rat paw edema.

Effect of Moringa oleifera leaves extracts on rat paw edema induced by carrageenan In the present study two different extracts were evaluated for anti-inflammatory activity using carrageenan-induced rat paw edema and the data was compared with that of control. [Table-1] Vehicle treated rats and Diclofenac (5 mg/kg p.o.) treated rats showed an increase of paw volume as 2.4± 0.01 ml and 1.45 ± 0.002 ml respectively after 3h. Treatment with Petroleum Ether extract of Moringa oleifera (100, 200, and 400 mg/kg, p.o.) showed a significant inhibition of paw volume after 1h, 2 h and 3 h. ($p<0.01$). Treatment with Methanol extract (100, 200, and 400 mg/kg, p.o.) showed significant ($p<0.01$) inhibition of carrageenan induced rat paw edema. Maximum inhibition was observed at 400 mg/kg dose as compared to the control. It was observed that the methanolic extracts of Moringa oleifera (400 mg/kg, p.o.) exhibits maxim Manti inflammatory activity against carrageenan induced hind paw edema. The inhibition obtained with Moringa oleifera and was 61.33%

TABLE 1: Effect of various extract of Moringa olifera on carrageenan induced rat paw edema.

Group	Treatment (mg/kg)	Mean increase in paw volume (ml)				% Decrease in paw volume at 3h
		0h	1h	2h	3h	
I	Control	0.90± 0.024*	1.49±0.01*	1.90±0.03*	2.40±0.014*	
II	Diclofenac (5)	0.89±0.008*	1.01±0.01*	1.29±0.01*	1.45±0.028**	62.66
III	P.E (100)	0.87±0.033**	1.49±0.02*	1.77±0.02**	1.89± 0.022*	32.09
IV	PE (200)	0.88 ±0.026*	1.33±0.032**	1.55±0.038**	1.75±0.031**	42.05
VI	P.E(400)	0.89±0.029**	1.39± 0.019*	1.49± 0.017*	1.70± 0.021*	46.12
VII	M.E (100)	0.90±0.038**	1.56±0.039**	1.79± 0.022*	1.80± 0.027*	40.08
VIII	M.E (200)	0.91±0.037**	1.28±0.045**	1.39± 0.018*	1.65±0.037**	50.66
IX	M.E (400)	0.87±0.031**	1.21± 0.023*	1.35±0.029**	1.61± 0.019*	61.33

Data were analyzed using ANOVA and expressed as Mean ± SEM (=5) followed by Dunnett's test and differences between means were regarded significant at * ($P < 0.05$), ** ($P < 0.01$)

Effect of Moringa oleifera leaves extracts on cotton pellet granuloma formation in rats

The Effect of Moringa oleifera extracts on cotton pellet granuloma formation is shown in Table 2. . The extracts significantly inhibited cotton pellet granuloma. The percent inhibition for diclofenac Standard was found to be 44%. The percent inhibition for Petroleum ether extract was 19% ,28% , 32 % at doses of 100,200, and 400 mg/kg, respectively. The percent inhibition for Methanol extract was 17% ,29.6% , 36.8 % at doses of 100,200 and 400 mg/kg, respectively.

Table 2: Effect of various extracts of Moringa oleifera in cotton pellet induced granuloma formation

Group	Treatment (mg/kg)	Average weight of cotton pellet	Average weight of cotton pellet with granuloma	% Inhibition
I	Control	50 ± 0.01	125.10 ± 5.91**	
II	Diclofenac (5)	50 ± 0.01	70.52 ±3.21*	44.13
III	P.E (100)	50 ± 0.01	101.38±6.18**	19.07
IV	PE (200)	50 ± 0.01	90.23±5.41*	28.11
VI	P.E(400)	50 ± 0.01	85.16±2.16*	32.19
VII	C.E (100)	50 ± 0.01	103.58±5.71**	17.25
VIII	C.E (200)	50 ± 0.01	88.08±6.75**	29.68
IX	C.E (400)	50 ± 0.01	79.67±3.69*	36.87

Data were analyzed using ANOVA and expressed as Mean ± SEM (= 5) followed by Dunnett's test and differences between means were regarded significant at * ($P < 0.05$), ** ($P < 0.01$).

DISCUSSION

Moringa oleifera (Moringaceae) is a bush of African savannah, commonly known as Drumstick used in folk Medicine for the treatment of rheumatic and articulanry pain. Its seeds shown analgesic activity [7], Antipyretic activity [8]. Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported [23] hence the analgesic and anti-inflammatory effects produced by these extracts may be attributed due to the flavonoids and steroids. However, its pharmacological actions and mechanisms have not been precisely documented in spite of its increasing usage recently. Present work reported the potential effects of the stem bark of Moringa oleifera, as an anti-inflammatory agent using both in vivo and in vitro models. Carrageenan-induced paw edema and cotton pellet granuloma formation in rats reflect the edematous stages during acute and chronic inflammation. [24,25] In the present study, two different extracts of leaves of Moringa

oleifera were tested. Carrageenan induced rat paw edema has been a popular inflammatory model to investigate nonsteroidal anti-inflammatory effect of compounds [26] Serotonin, histamine, bradykinin, and prostaglandin have been identified as a mediators for carrageenan induced rat paw edema. [27] Petroleum ether and methanolic extracts were found to possess a prominent anti-inflammatory activity, showing inhibition to the paw edema induced by carrageenan during the three time points from 1 to 3 h. In cotton pellet granuloma model Petroleum ether and methanolic extracts showed significant inhibition. The effectiveness of these extracts at 1 and 3 h in carrageenan induced paw edema indicates their antagonist effect at Serotonin, histamine, bradykinin and prostaglandin. Because the release of serotonin and histamine occurs 1 h after carrageenan whereas bradykinin and prostaglandin are released 2 and 3 h, respectively, after carrageenan injection. [28] The cotton pellet granuloma is a model of chronic inflammation, and dry weight has been shown to correlate with the amount of granulomatous tissue formed. [29] In the present study animals treated with Petroleum ether and methanolic extracts showed significant inhibition of granuloma formation. Diclofenac was found to be more effective in preventing granuloma formation compared to extracts respectively. Flavonoids isolated from some medicinal plants have been proven to posses antinociceptive and/ or anti-inflammatory effects. [32] It has been shown by Meli et al and Dicarlo et al. [33,34] that flavonoids also inhibit gastric motility in a dose - dependent, manner. It is therefore possible that the inhibitory effects on anti-nociceptive and anti-inflammatory effects observed in these extracts may be attributed in part to its flavonoid content. Flavonoids also inhibit the phosphodiesterases involved in cell activation. [33] Much of this effect is upon the biosynthesis of protein cytokines that mediates adhesion of circulating leukocytes to sites of injury. Flavonoids inhibit biosynthesis of prostaglandins, which are involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase parthways. [35] Protein Kinases are another class of regulatory enzymes affected by flavonoids. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes. [36,37] Flavonoids [36] potently inhibit prostaglandins, a group of powerful proinflammatory signaling molecules. Studies have shown that this effect is due to flavonoid inhibition of key enzymes involved in prostaglandin biosynthesis (i.e., lipoxygenase, phospholipase, and cyclooxygenase). Flavonoids also inhibit phosphodiesterase's involved in cell activation. Much of this effect is upon the biosynthesis of protein cytokines that mediate adhesion of circulating leukocytes to sites of injury. Protein kinases are another class of regulatory enzymes affected by flavonoids. Inhibition of these key enzymes provides the mechanism by which flavonoids inhibit inflammatory processes [36]. anti-inflammatory effects have already been observed in flavonoids as well as in tannins .[38,39].

Conclusion

From the present study, it was concluded that extracts of stem bark of Moringa oleifera is capable of inhibiting inflammatory reactions as well as pain. The results provided experimental evidence for its traditional use in treating various diseases associated with inflammation and pain.

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