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Research Article

Phytochemical screening and studies of analgesic potential of *Moringa oleifera* Lam. stem bark extract on experimental animal model

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Abstract

The work has been done for the phytochemical investigation and study of analgesic activity of *Moringa oleifera* Lam. ethanolic stem bark extract using Acetic Acid Induced Writhing method. The effect of extract was tested for qualitative chemical analysis which reveals the presence of alkaloid, glycosides, flavonoids, tannins, saponin, carbohydrate etc. For peripheral analgesic effect acetic acid induced writhing test was used and for this stem bark extract was administered intraperitoneally at doses of 250 mg/kg and 500 mg/kg of body weight to young Swiss-albino mice. Both doses of the extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner which is comparable to the positive control drug Diclofenac Na. These two different doses were found to exhibit 13.22% and 28.94% writhing inhibitory response respectively where the Diclofenac Na inhibited about 42.15% of writhes at a dose of 100mg/kg of body weight. The obtained results provide promising baseline information for the potential use of these crude extract in the treatment of pain.

Keywords: *Moringa oleifera Lam., Phytochemical screening, Analgesic activity, Acetic acid induced writhing test, Swiss albino mice.*

1. Introduction

Algesia (pain) is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus. An analgesic selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So analgesic activity means capacity of a substance to neutralize the pain sensation[1]. The nature has provided a complete storehouse of remedies to cure all ailments of mankind. Since the dawn of civilization, in addition to food crops, man cultivated herbs for his medicinal needs. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature, so that today we possess many effective means of ensuring health-care. In the past, almost all the medicines used were from plants. Today there has accumulated a vast store of knowledge concerning the therapeutic properties of different plants. The opioid analgesics and their derivative have never been surpassed as painkillers in efficacy or patient acceptability despite their disadvantages[2]. Therefore, man has been on hunt since ages for suitable alternatives to NSAIDs and opioid analgesics.

Moringa oleifera Lam. belonging to the family Moringaceae is commonly known as Sajna gachh, Sojne (Bengali); Horse-Radish tree, Drumstick tree (English). A small to medium-sized deciduas tree with long strangling branches, large imparipinnate compound leaves of small oblong-obovate leaflets, fragrant pinkish white flowers in loose axillary panicles and long, narrow and ridged cylindrical fruits, planted commonly all over Bangladesh, India, Pakistan, Central America, Afghanistan, and Africa. Root is used as stimulant in paralytic affections, intermittent fever, epilepsy and carminative, stomachic, diuretic, cardiac and circulatory tonic, rubefacient in palsy and chronic rheumatism and also an abortifacient. The gum is used in dental caries and mixed with sesame oil in ear diseases. Fruits are used in diseases of liver and spleen, articular pains, tetanus and paralysis. Seed oil is used externally in rheumatism[3-5]. Flowers are stimulant, tonic and diuretic and increase the flow of bile. The leaves are tasty, cooling; removes all kinds of excessive pain & inflammation. The roots and seeds are prescribed for treatment of snake bite (Charaka, Sushruta) and scorpion-sting (Sushruta)[6]. In recent decades, many scientific studies using the extracts of leaves, barks, seeds and roots of *Moringa oleifera* Lam. are being carried out to confirm many potential uses including anti-tumour[7], antihepatotoxic[8], antiulcer[9], hypotensive[10], hypolipidemic[11], antiurolithiatic[12] acute anti-inflammatory[13,14], analgesic[15], antipyretic[4], antibacterial activities[16] etc. Taking this background into consideration this study was done to investigate the presence of phytochemical constituents and to evaluate the analgesic activity of *Moringa oleifera* Lam. stem bark extract.

2. Materials and methods

2.1 Plant material collection

The fresh stem bark of *M. oleifera* Lam. were collected from Natore district of Bangladesh, in April, 2013 and identified by Mr. Md. Habibur Rahman, taxonomist, National Herbarium, Mirpur, Dhaka-1216, Bangladesh where a voucher specimen DACB32494 has been deposited. The stem bark was cut, air dried for several days and then oven dried for 24hours below the temperature 60^0C. The bark was then pulverized into coarse powder using a grinding machine.

2.2 Plant material extraction

The powdered dried stem bark (1.07 kg) was subjected to cold 95% ethanol (4 L) extraction in flat bottom glass container through occasional shaking and stirring for 10 days[17,18]. The whole extract was filtered and the solvent was evaporated to dryness in vacuum with rotary evaporator at 40^oC to 50^oC to afford a semisolid mass (60 gm) which was used for further investigation.

2.3 Preliminary phytochemical screening

The alcoholic stem barks extract of *M. Oleifera Lam*. was subjected to qualitative chemical investigation for the identification of the active principles by using the following reagents and chemicals[19,20], shown in Table 1. In each test 10% (w/v) solution of extract in water was taken unless otherwise mentioned in individual test.

2.4 Collection and maintenance of animals

Young healthy Swiss Albino mice of either sex weighing around 22-30gm, 3-4 weeks of age, were procured from the International Centre for Diarrhoeal Disease Research of Bangladesh (ICDDRB), Dhaka, Bangladesh. The animals were housed in plastic cages having a dimension of $71\times35cm^2$ under standard laboratory conditions (relative humidity 55-65%, temperature: $24.0\pm1.0^{\circ}$C and 12 h dark/light cycles). They were fed with standard 'Mouse-pellets' diet and distilled water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to the experiment.

2.5 Preparation of sample

To prepare suspension of the test sample at the dose 250 and 500 mg/kg body weight, 0.5 and 0.25 mg of the sample was measured respectively and were triturated separately by the addition of small amount of Tween-80. After proper mixing distilled water was slowly added to make the final volume of the suspension (10 ml). For the preparation of standard drug Diclofenac-Na at the dose of 100mg/kg body weight, 0.1 gm of Diclofenac-Na was taken and a suspension of 10 ml was made.

2.6 Acetic acid induced writhing method

Swiss Albino mice were randomly divided into 4 groups with 3 mice each; Group I: Control (1% tween80 in saline at the dose of 10ml/kg body weight); Group II: Positive control (standard drug diclofenac 100 mg/kg body weight); Group III and IV (*Moringa oleifera* Lam. ethanolic stem bark extract 250 and 500 mg/kg body weight respectively). Test sample was given orally 1 hour before, and the standard drug diclofenac (100 mg/kg) was given intraperitoneally half-an-hour before the administration of acetic acid [21]. Next acetic acid 1%, v/v; 10 ml/kg was injected intraperitoneally in albino mice and the writhing movements were recorded after 5 min for a total of 30 min. Writhing movements were characterized by specific abdominal contractions accompanied by elongation of body with arching of back, belly touching the ground and dragging of hind limbs. Sometimes the mouse started to give writhing but they did not complete it, so two half writhing was counted as one full writhing.

3. Results and Discussion

The result of qualitative chemical investigation of *Moringa oleifera* Lam. ethanolic stem bark extract has indicated the presence of following compounds shown in Table 1.

Table 1: Showing phytochemical screening results of the alcoholic stem bark extract of *M. oleifera* Lam.

Chemical Groups	Tests	Results
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Glycosides	Legal's test	+
	Bromine water test	+
	Keller-Kiliani test	+
Flavonoids	Ferric Chloride test	+
	Lead Acetate test	+
Steroids	Salkowski's test	+
	Liebermann Burchards's test	+
	Sulphur test	+
Carbohydrates	Molish's Test	+
	Fehling's test	+
Tannins	Ferric Chloride test	+
Saponins	Frothing test	+

'(+)' indicates Present

Analgesic potential of *Moringa oleifera* Lam. ethanolic stem bark extract in mice were measured using the acetic acid induced writhing test. In this test acetic acid 1%, v/v; 10 ml/kg produced a substantial number of writhes in control mice pre-treated with (1% tween80 in saline at the dose of 10ml/kg body weight). Dose-dependent increase in the percentage inhibition of writhes was noted with plant extract 250 and 500 mg/kg body weight showing 13.22% and 28.94% respectively. The lowest doses of treatment failed to inhibit significantly the acetic acid-induced abdominal writhing. The dose of 500 mg/kg seems to be the maximal effective dose in this model since it profoundly reduced the number of writhes. The effect of the maximal doses of plant extract (500 mg/kg) was comparable to that of standard reference drug diclofenac (100 mg/kg) showing 42.15% inhibition of writhing. The results of the acetic acid-induced writhing test along with the percentage inhibition of writhes have been showed in Table 2, 3.

Table 2: Showing primary data of acetic acid induced writhing test for ethanolic stem bark extract of *Moringa oleifera* Lam. in mice.

Group	Average body weight of mice (gm)	No. of writhing			Mean	SD	SEM
		M-1	M-2	M-3			
Group-1	30	39	40	42	40.33	1.24	0.88
Group-2	30	25	20	25	23.33	2.35	1.66
Group-3	30	32	38.5	34.5	35	2.67	1.89
Group-4	30	27	29	30	28.66	1.24	0.88

SD - Standard Deviation, SEM - Standard Error of Mean

Table 3: Showing effect of ethanolic stem bark extract of *Moringa oleifera* Lam. on acetic acid induced writhing test in mice.

Treatment	Dose (mg/kg)	Mean	No. of writhing	%Writhing	% of Inhibition
Group-1	10	40.33	40.33±.88	100	0
Group-2	100	23.33	23.33±1.66	57.85	42.15
Group-3	250	35	35±1.89	86.78	13.22
Group-4	500	28.66	28.66±.88	71.06	28.94

Pain is an unpleasant sensation but a protective mechanism of our body. Analgesics are defined as substances, which decrease pain sensation by increasing pain threshold to external stimuli without altering consciousness. All the currently available analgesic drugs such as NSAIDs and opioid analgesics are subjected to their own side effects such as gastric erosions with NSAIDs and tolerance, dependence with opioid analgesics. Therefore, analgesic drugs lacking the side effect as alternative to nonsteroidal anti-inflammatory drugs (NSAIDs) and opiates are in demand for the society[22]. Analgesic activity using petroleum ether and methanolic extract of *Moringa oleifera* Lam. stem bark [23] and the hydro ethanol extract of pods have already been done[24]. In these studies, flavonoids, tannin, saponins, and alkaloids like ingredients were claimed to possess analgesic activity. The mechanism of action postulated in these studies was due to prostaglandin inhibition. Present work reported the potential effects of the alcoholic stem bark extract of *Moringa oleifera* Lam. as an analgesic agent using acetic acid induced writhing method in a dose-dependent manner. It is a suitable method widely used to screen and study the antinociceptive activity of different compounds[25.] Although this test is relatively simple, it presents great sensitivity to various analgesics, nonsteroidal and steroidal anti-inflammatory drugs as well as morphine-like compounds and other analgesic substances that act to relieve pain generated from peripheral origin. The manifestations of abdominal writhings are due to sensitization of chemosensitive nociceptors by prostaglandins. Increased level of prostanoids, particularly prostaglandin E2 (PGE_2) and prostaglandin F2 (PGF_2), as well as lipoxygenase products have been found in the peritoneal injections of acetic acid[26]. Acetic acid-induced writhing method also liberates endogenous substances like serotonin, bradykinin, histamine which stimulate the sensory nerve endings[27]. Recently, it was found that nociceptive activity of acetic acid may be due to release of cytokines like tumor necrosis factor alpha (TNFα), interleukin 1β, interleukin-8 by resident macrophages and mast cells[28]. Anti-nociceptive action of alcoholic stem bark extract of *Moringa oleifera* Lam. could be due to inhibition of these mediators.

The phytochemical ingredients found in stem bark extract of this plant like flavonoids[29] which potently inhibit prostaglandins, especially the endoperoxidase, tannins, alkaloids[30] could also contribute to the anti-nociceptive action of this plant. The results therefore suggest that *Moringa oleifera* Lam. stem bark extract could become promising natural analgesic agents with potential applications in pharmaceutical field for controlling pain.

4. Conclusion

From the present study, it was concluded that stem bark extracts of *Moringa oleifera* is capable of producing significant anti-nociceptive action. The results provided experimental evidence for its traditional use in treating various diseases associated with pain. Further studies are in progress to isolate the active constituents responsible for the observed effect.

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