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Research Paper

Evaluation of Analgesic Activity of Moringa Oleifera Lam. Stem Bark Extract by Acid Induced Writhing Response Method

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Abstract: Moringa oleifera *Lam. is also known as 'Miracle tree' because of its uses of all parts particularly for their great potential in pharmacological, nutritional and water purification aspects. The study has been done for the phytochemical screening and analysis of analgesic potential of Moringa olifiera Lam. methanolic stem bark extract using Acetic acid induced Writhing method. Qualitative chemical analysis was carried out through phytochemical investigation which indicated the presence of carbohydrates, glycosides, saponins, flavonois, tannins, proteins, alkaloids etc. in the extracts. To study analgesic activity Acetic acid induced Writhing test was used, where Methanolic stem bark extract was introduced intraperitonially at doses of 150 mg/kg and 300 mg/kg to Swiss-albino mice. The dose of 300 mg/kg showed significant inhibition of Writhing response created by acetic acid in a dose dependent manner when compared to the standard control drug Diclofenac Sodium. Those two different doses exhibited 5% and 80% inhibition in writhing response respectively while the Diclofenac Na inhibited about 46.25% of writhing response at a dose of 40 mg/kg of body weight. The results of this study support the potential pain management therapy using this crude extract.*

Keywords: Moringa oleifera, *Phytochemical investigation, Analgesic potential, stem bark extract, Acetic acid induced Writhing method.*

I. INTRODUCTION

Moringa oleifera Lam is one of the famous and most commonly found naturalized species of a monogeneric family Moringaceae. Moringa oleifera, indigenous of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia is now also available in the Philippines, Cambodia, Central America, North and South America and the Caribbean Islands M. oleifera is commonly known as the 'drumstick tree' or the 'horse radish tree', while in the Nile valley, the name is named as 'Shagara al Rauwaq', which means 'tree for purifying'. In Bangladesh, Moringa oleifera is locally known as 'Sajna' and is grown and cultivated all over the country[1]. It has been named as'the miracle tree' due to its amazing healing abilities for various sickness and even some chronic ailments. Moringa oleifera is a great food staple which has had enormous attention as the 'natural nutrition of the tropics' and can be used as an potential remedy for Malnutrition, specially for lactating mothers and infants. Indigenous medicines,specially used in South asia to treat various conditions including inflammation, infections along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders contain various plant parts of t Moringa oleifera, such as root,bark,gum, leaf,fruit(pods), flowers, seed and seed oil. This wonder plant has been used for long time and in many culture to treat various ailments such as asthma, blackheads, blood impurities, bronchitis, chest congestion, cholera and also have therapeutic uses like anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering, renal, anti-diabetic and hepatoprotective activities[2]. Pain is a very uncomfortable feeling, one may feel being conscious. There has been a great progress made in recent years in the development of pain therapy, still there is needed for effective, safe and potent analgesic, particularly which can be used for chronic pain. The analgesics used to alleviate chronic pain are related to various serious complications, such as liver dysfunction, kidney damage etc which necessitate the finding of safe option from nature as many plant derived compounds present potential analgesic effects. For this reason, they can be used as promising mother molecules for the development of new drugs, specifically designated to be designed for the treatment or control of chronic inflammatory and painful states. These

potential antinociceptive substances include alkaloids, terpenoids, flavonoids and others, which are present in the sample plant of this study, Moringa oleifera.

In this study we had attempted to analyze the analgesic potential of the methanolic extract (ME) of Moringa oleifera in animal models of nociception in mice using Acetic acid induced Writhing method [3,4].

II. MATERIALS AND METHODS

2.1 Sample collection followed by proper identification of the plant

The barks of Moringa oleifera were collected during the month of April 28, 2016, from the area of Mirpur, Dhaka, Bangladesh. The plant barks and leaves were authenticated from the Bangladesh National Herbarium, Mirpur, Dhaka 1216, Bangladesh. It was identified by the experts of the Bangladesh National Herbarium. (Accession number: DACB 43200).

2.2 Preparation of plant material

The barks were dried for 20 days using the method of shade drying. Dry coarse powder weighing 160 g was obtained from grounding with the help of a suitable grinder. The powder was stored in an air tight container and until analysis commenced was kept in a cool, dark and dry place.

2.3 Preparation of Bark Extract

The dried bark powder was put in a clean, flat bottom glass container, which was soaked in methanol. The containers were sealed with its contents and kept for a period of 10 days with concurrent occasional shaking and stirring. The mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was finally filtered by Whatmann filter paper (no. 02). The crude extract was obtained with the use of Rotary evaporator [5]. Mass of the extract was determined and the yield was calculated and expressed in percentage. And formula is

Yield (%) = Mass of crude extract x100/Total mass of dry powder.

= {(38x100)/80} %

= 47.5%

2.4 Phytochemical screening:

The extract was tested for detection of phytochemicals such as carbohydrates, alkaloids, glycosides, anthraquinone glycosides, glucosides, tannins, saponins, flavonoids and steroids by following different standard procedures [7].

2.5 Method:

(i) Preparation of dose and administration

100 mg of crude extract and small amount of Tween 80 were taken in mortar and triturated. The volume was made up to 5 ml by adding water and the concentration was maintained according to dose 150 mg/kg and 300 mg/kg. Body weight for each mouse was determined by using balance. These doses were then administered orally to the Mice.

Table 1: Volume of adjusted drug and acetic acid administration according to weight of Mice

Dose of Extract/(mg/kg)	150			300		
Weight of mice(gm)	23	22	21	23	33	23
Volume of acetic acid/ml	0.25	0.24	0.23	0.25	0.35	0.25
Volume of Extract/ml	3.45	3.30	3.15	6.9	9.9	6.9

(ii) Study of Acetic acid induced writhing response

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice. In this method, acetic acid was administered intraperitoneally (i.p) to the experimental animals to create pain sensation. In this study Diclofenac sodium was used as the positive control drug. The plant extracts were administered orally in two different doses (150 mg/kg and 300 mg/kg body weight) to the Swiss Albino mice after an overnight fasting. About 30 minutes prior of intraperitoneal administration of 0.7% v/v acetic acid solution (10ml/kg), test samples and vehicles were administered orally to whereas Diclofenac Sodium was administered 15 minutes before acetic acid administration. After placing on an observation cage, each mouse of all groups were observed individually for counting the number of writhing response they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the writhing was initiated by the animals but did not complete. This response was considered as a half-writhing and two half-writhing were taken as one full writhing

response. The number of writhes produced in each treated group was compared to that of a control group while Diclofenac sodium (40 mg/kg) was used as a reference substance (positive control).
Percentage inhibition was calculated by using the following formula:
% inhibition produced= { $(W_c - W_t) \times 100$ } / Wc
Where, W_c = No. of writhes in control group, W_t = No. of writhes in test group
Compounds with less than 70% inhibition of writhing responses were considered to have minimal analgesic activity [8, 9].

III. RESULTS

Essential phytochemical constituents were present in the alcoholic bark extract of Moringa oleifera, which may attributed to its' great medicinal value. The results are given in Table 2.

Table 2: Phytochemical screening of the alcoholic stem bark extract of Moringa oleifera.

Chemical Groups	Tests	Findings
Carbohydrates	Molishs test	+
	Benedicts test	+
	Fehling's test	+
Glycosides	General Laboratory test	+
Saponins	Frothing test	+
Flavonoids	HCl test	+
Tannins	Ferric chloride test	+
Proteins	Millon's test	+
Alkaloids	Mayer's reagent	+
	Hager's reagent	+
	Wagner's reagent	+
	Dragendorff's reagent	+
Coumarin	NaOH Test	+
Phenol	Ferric chloride test	+

'[+]' indicates Presence

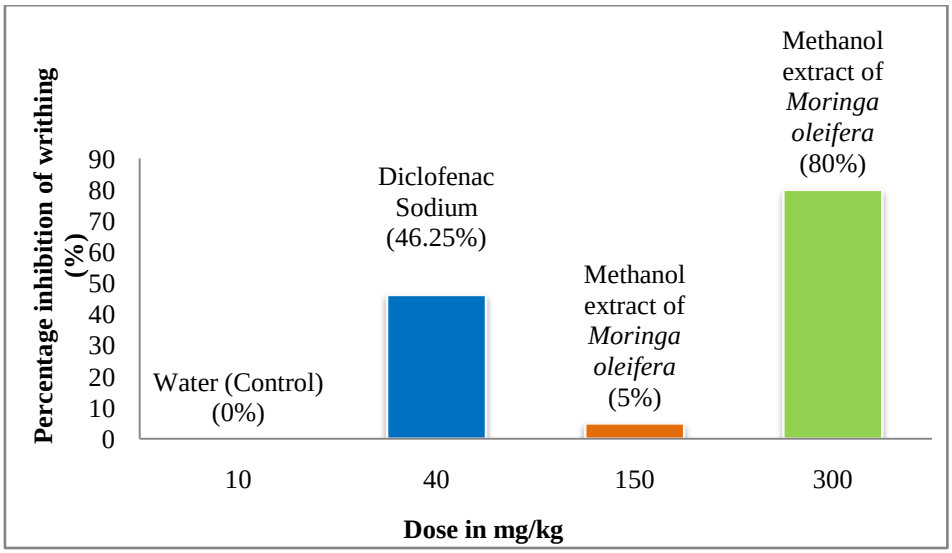
Table 3: The primary analysis after administration of different doses of Moringa oleifera Lam methanolic stem bark extract after acetic acid induced writhing production in mice.

Group	Average weight of Mice (gm)	No of writhing			Mean	SD	SEM
		Mouse 1	Mouse 2	Mouse 3			
Group-1	27	37	41	42	40.00	2.16	1.25
Group-2	25	22	21.5	21	21.50	0.41	0.24
Group-3	22	37	38	39	38.00	0.82	0.47
Group-4	26	6.5	8.5	9	8.00	1.08	0.62

SD=Standard Deviation SEM=Standard Error of Mean

Table 4: Observation of analgesic effect of Moringa oleifera Lam. methanolic stem bark extract on acetic acid induced writhing test in mice.

Group	Drug treatment	Dose, route	Mean	No. of writhing produced	Percentage of Writhing produced	Percentage inhibition of writhing (%)
Group-1	Water (Control)	10ml/kg oral	40.00	40.00±1.25	100	0
Group-2	Diclofenac sodium (Standard)	40mg/kg, p.o.	21.50	21.50±0.24	53.75	46.25
Group-3	Methanol extract of Moringa olifera	150 mg/kg, p.o.	38.00	38.00±0.47	95.00	05.00
Group-4	Methanol extract of Moringa olifera	300 mg/kg, p.o.	08.00	08.00±0.62	20.00	80.00



Graph 1: Bar graph represent the percentage inhibition of writhing response plotted against doses of 150 and 300mg/kg of methanol extract of Moringa oleifera stem bark.

IV. DISCUSSION

Analgesics are drugs that help to reduce pain. There are a lot of analgesics available, but the side effects they produce, always emphasizes the necessity to find safer options from nature. For this purpose methanolic bark extract of Moringa oleifera was analyzed to study its' analgesic activity. The methanolic extract of Moringa oleifera showed potential analgesic property in the model of acetic acid induced writhing in Mice compared to the standard drug (Diclofenac sodium). The percentage inhibitions of writhing responses at 150 mg/kg and 300 mg/kg dose of methanol extract were 5 % and 80% respectively which ensures strong analgesic potential. The extracts showed effective analgesic property when compared to the standard drug Diclofenac Sodium, which has only 46.25% inhibition of writhing at 40 mg/kg dose. The highest inhibition of pain occurred at the dose of 300mg/kg and the lowest at 150mg/kg in case of extract. The best analgesic activity was exhibited by 300mg/kg dose of the extract, which showed around 80% inhibition of acetic acid induced writhing response in mice. The result of analysis of analgesic potential of the extract of Moringa oleifera bark using acetic acid induced writhing counting method at different doses is shown in Table 3 and Table 4. The bar graph shows the comparison of analgesic potential among four treatment groups.

V. CONCLUSION

The result shows that the methanolic bark extract of Moringa oleifera Lam.Contains important phytochemical constituents and also possess good analgesic potential which was observed in animal model. Therefore, this plant may be acts as the source of pain killing compounds and can be investigated for future potential source of analgesic drug. This study also indicates that there is a scope for further investigation of this plant extract in order to identify the active principles responsible for the analgesic property.

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