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Analgesic, anti-inflammatory and antimicrobial effects of ethanol extracts of mango leaves

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Abstract

The present study was carried out to investigate the analgesic, anti-inflammatory, antibacterial and antifungal properties of ethanol leave extract of *Mangifera indica*. For evaluation of analgesic and anti-inflammatory properties, acetic acid induced writhing response model and carrageenan induced paw edema model were used in Swiss albino mice and Wistar albino rats, respectively. In both cases, leaves extract were administered and the obtained effects were compared with commercially available analgesic and anti-inflammatory drug, Diclofenac Sodium. In analgesic bioassay, oral administration of the ethanol leaves extract significantly ($P<0.01$) reduced the writhing response. The degree of inhibition of leaves extract was 55.8% compared to the effect of standard analgesic drug, Diclofenac Sodium (75.28%). On the other hand, though leaves extract reduce paw edema but they did not show any significant effect. The antibacterial and antifungal activity of leaves extract also carried out by disc diffusion technique and poisoned food technique. In antibacterial experiment, leaves extract showed 7.0 mm to 11.5 mm in diameter of zone of inhibition against six Gram positive bacteria (such as *Staphylococcus aureus, Streptococcus agalactiae, Bacillus cereus, Bacillus megaterium, Bacillus subtilis* and *Lactobacillus vulgaricus*) and two Gram negative bacteria (such as *Shigella flexneri* and *Shigella sonei*). But *Salmonella typhi* and *Proteus sp* had negative activity on leaves extract. The leaves extract of *Mangifera indica* showed antifungal activity against three fungal species (such as *Aspergillus ustus*, *Aspergillus niger* and *Aspergillus ochraceus*).

Keywords: *Mangifera indica,* Analgesic, Anti-inflammatory, Antibacterial, Antifungal

Introduction

Bangladesh owing to its favorable climatic influences has been blessed with immense natural resources including explored and unexplored herbal medicinal plants. About 5000 species of phanerogams and pteridophytes grow in Bangladesh. More than a thousand of these have been claimed to possess medicinal or curative properties. Recently, 546 species have been identified as having medicinal properties and therapeutic use (Ghani, 1998). Medicinal plants are the great importance to the health of individual and communities. The medicinal values of those plants possess some chemical active substances that produce a definite physiological action on the human body and animal health. The most important bioactive substances are alkaloid, tannin, flavonoid and phenolic compounds (Edeogra, 2005). Drugs which are used presently for the management of pain and inflammatory conditions are either steroidal like corticosteroids or non steroidal like aspirin. All of these drugs possess more or less side and toxic effects like renal failure, allergic reactions, hearing loss or they may increase the risk of hemorrhage by affecting platelet function (Thomas, 2000). Moreover, synthetic drugs are very expensive (Ahmad *et al.* 1992). On the contrary, many plant origin drugs had been used since long time without any adverse effects. The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Sharif and Banik, 2006). Plants are the cheapest and safer alternative sources of antimicrobials (Doughari *et al.,* 2007). In Bangladesh, very few works have been done to explore the possibilities of utilizing the local herbs and shrubs in veterinary practice. Therefore, the present research has been designed to investigate the analgesic, anti-inflammatory and antimicrobial effects of leaves extract of *Mangifera indica* (Mango) available in Bangladesh.

Materials and Methods

Sample collection

The leaves of *Mangifera indica* was collected from Barisal, Chittagong and Mymensingh district of Bangladesh. The plant was taxonomically identified by a Taxonomist, Industrial Botany Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong. All the research work was carried out according to the guidelines of Institutional Animal Ethics Committee (IAEC), BCSIR.

Preparation of plant extract

The fresh leaves of *Mangifera indica* were washed with water immediately after collection. The collected leaves were chopped into small pieces, air dried at room temperature for about 10 days and ground into powder form and stored in an airtight container. 650 gm powder was macerated in 5 litre pure methanol for 7 days at room temperature with occasional stirring. After 7 days, methanol extract was filtered off through cotton plug and finally with a Whatman no. 1 filter paper. The extract was concentrated under reduced pressure below 50º C temperature through rotatory vacoum evaporator. The concentrated extracts were collected in a Petri dish and allowed it to air dry for complete evaporation of methanol. The whole process was repeated three times and finally, 45 gm, 50 gm and 55 gm greenish colored concentrated leaves extract was obtained (yield 5.3 % w/w) which was kept in refrigerator at 4º C.

Experimental animals and diets

Swiss albino mice of both sexes weighing between 25 gm and 30 gm and Wistar Albino rats of either sex weighing between 150 gm and 200 gm were taken from animal house of BCSIR laboratories, Chittagong. The animals were acclimatized to room temperature (28±5°C) with a relative humidity of 55 ± 5 % in a standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study the animals were supplied with standard pellet diet and adlibitum water.

Screening of analgesic activity of plant extract

Acetic acid induced writhing test model as described by Koster *et al.* (1959) was performed to evaluate the antinocipeptive activities of *Mangifera indica* leaves extracts. 1 % (v/v) acetic acid solution, 2 mg/ml Diclofenac Sodium, 200 mg/ml *Mangifera indica*, and fifteen (15) Swiss Albino mice with average body weight of 25 gm to 30 gm and 3 months of age in both sexes were taken during experiment. Finally, percentage (%) analgesic activity was calculated using the following formula-

$$\%\,\text{analgesic activity} = \frac{\text{Mean writhing count (Control group} - \text{Treated group)}}{\text{Mean writhing count of control group}} \times 100$$

Assay of anti-inflammatory activity of plant extract

Carrageenan induced paw edema model described by (Olajide *et al.,* 2000) was used for evaluating potential *Mangifera indica* leaves extract on inflammation. 1 % w/v carrageenan, 200 mg/ml *Mangifera indica* leaves extract solution, 2 mg/ml Diclofenac Sodium, Fifteen Wistar Albino Rats and Plethesmometer were listed for this assay. The inhibitory activity was calculated according to the following formula –

$$\%\,\text{inhibition (I)} = \frac{(\text{Ct} - \text{Co})\,\text{control} - (\text{Ct} - \text{Co})\,\text{treated}}{(\text{Ct - Co})\,\text{control}} \times 100$$

Where Ct is the right hind paw thickness volume (in mm^3) at time t, Co is the right hind paw thickness volume (in mm^3) before carrageenan injection, Ct -Co is paw edema, (Ct - Co) control is edema or paw size after carrageenan injection to control rats at time t. (Ct - Co) treated is edema or paw size after carrageenan injection to treated rats at time t.

Antimicrobial screening

a) Test organisms

10 bacteria and 4 fungi were used as test organisms to screen the anti-microbial activity of leaves extracts. 6 were Gram positive and 4 were Gram negative among the 10 bacteria. Bacterial and fungal cultures were collected from Nutrition and Food Science Institute of Dhaka University.

b) Antibacterial activity

Disc diffusion methods

The disc diffusion technique (Bauer *et al*, 1996) is a widely accepted in vitro investigation for primary screening of agents which may possess any antibacterial activity. It is a qualitative or quantitative test indicating the sensitivity or resistance of the microorganism to the test materials. Mueller-Hinton agar media was considered to be the best for routine susceptibility testing of non-fastidious bacteria.

c) Antifungal activity

Poisoned food methods

The poisoned food technique (Grover and Moore, 1962) was used to screen the anti-fungal activity of the herbal products. Potato dextrose agar was used as a culture medium. The percentage inhibition of mycelial growth of the test fungus was calculated by the following formula:

$$\%\,\text{inhibition (I)} = \frac{\text{Diameter of the fungal colony in C} - \text{Diameter of the fungal colony in T}}{\text{Diameter of the fungal colony in C}} \times 100$$

Here, C= control group, T= treatment group.

Statistical analysis

The results were presented as the Mean ± SEM (Standard Error) and statistical significance between the groups was determined by means of one-way analysis of variance (ANOVA) followed by unpaired Student's *t*-test to determine statistical significance. SPSS for WINDOWS$^{@TM}$ was applied for analysis of data. Probability (P) value of 0.05 or less (P≤0.05) was considered as significant.

Results and Discussion

Analgesic activity

When 1 % (v/v) acetic acid solution (3.3 ml/kg body weight) was injected intraperitoneally in mice, the control animal showed 66.75±2.28 writhing count / 20 minutes. Diclofenac Sodium caused significant reduction of writhing count from 66.75±2.28 to 16.5± 1.71/20 minutes. On the other hand, *Mangifera indica* leaves extract reduced the writhing count from 66.75±2.28 to 29.5±2.72/20 minutes. *Mangifera indica* and Diclofenac Sodium showed significant (P<0.01) reduction of pain in comparison with control group (Table 1).

Table 1. Effect of leaves extract of *Mangifera indica* and Diclofenac Sodium on acetic acid induced writhing response in Swiss albino mice

Group	Treatment	Dose	Writhing count	% Activity
Control	Distilled water	2 ml	66.75±2.28	-
Positive control	Diclofenac Sodium	40 mg/kg	16.5± 1.71	75.28%
Treated	*Mangifera indica* extract	2 gm/kg	29.5±2.72	55.8%

Myorelaxant, anti-inflammatory substances (Koyama *et al.,* 1997) and opoid such as codeine (Aydin *et al.,* 1999) were able to reduced pain produced by acetic acid. Therefore, it was suggested that the inhibition of prostaglandin synthesis was remarkably efficient as an anti-nociceptive mechanism in visceral pain (Franzotti *et al.,* 2002). Since leaves extract of *Mangifera indica (*2 gm/kg) showed significant inhibition ($P<0.01$) of acetic acid induced writhing response of mice, so it could be suggested that *Mangifera indica* leaves extract had potential analgesic activity.

Anti-inflammatory activity

Anti-inflammatory activity of *Mangifera indica* leaves extract was assessed and showed in Table 2. Diclofenac Sodium produced 38.7%, 45.67%, 58.32% and 60.88% anti-inflammatory effect at 1st hour, 2nd hour, 3rd hour and 4th hour after carrageenan injection, respectively.

Table 2. Effect of leaves extract of *Mangifera indica* and Diclofenac Sodium on carrageenan induced paw edema Wistar albino rat

Group	Treatment	Dose	Paw edema (mm^3)				% Inhibition			
			1st hr	2nd hr	3rd hr	4th hr	1st hr	2nd hr	3rd hr	4th hr
Control	Distilled Water	2 ml	0.372± 0.048	0.635± 0.057	0.763± 0.069	0.882± 0.111	-	-	-	-
Positive Control	Diclofenac Sodium	40 mg/kg	0.228± 0.041	0.345± 0.071	0.318± 0.030	0.345± 0.044	38.7%	45.67%	58.32%	60.88%
Treated	Leaves extract of *Mangifera indica*	2 gm/kg	0.244± 0.043	0.390± 0.060	0.486± 0.061	0.628± 0.050	21.0%	34.4 %	36.3%	28.7%

The *Mangifera indica* leaves extract produced 21.0%, 34.4%, 36.4% and 28.7% effect at 1st hour, 2nd hour, 3rd hour and 4th hour after carrageenan injection respectively. Development of edema in the paw of rat after injection of carrageenen was a biphasic event (Vinger *et al.,* 1969). This study had showed the leaves extract of *Mangifera indica* possessed no significant anti-edematogenic effect compared to Diclofenac Sodium on paw edema in rats. The result of the study indicated that probably *Mangifera indica* had no significant anti-inflammatory activity.

Antimicrobial activity

Antibacterial activity of *Mangifera indica* leaves extract was found moderate activity against Gram positive bacteria and poor activity against Gram negative bacteria. More specifically *Mangifera indica leaves* extract showed 7.0 mm, 7.5 mm, 11.5 mm, 8.5 mm, 9.0 mm and 9.5 mm in diameter of zone of inhibition against six Gram positive bacteria like *Staphylococcus aureus, Streptococcus agalactiae, Bacillus cereus, Bacillus megaterium, Bacillus subtilis* and *Lactobacillus vulgaricus,* respectively and 9.5 mm and 9.5 mm in diameter of zone of inhibition was observed against two Gram negative bacteria like *Shigella flexneri* and *Shigella sonnei* but no activity was observed against *Salmonella typhi* and *Proteus sp* . On the other hand, standard antibiotic, tetracycline (30 µg/disc) showed significant antibacterial activity against all tested bacteria (Table 3). The bioactive component, mangiferin isolated from *Mangifera indica* was reported to possess remarkable anti-influenza activity (Neon, 1984). Some of this compound had been reported to possess antibacterial activity (Dweck, 2001). This result indicated that the leaves extract of *Mangifera indica* possessed certain phytochemicals which had moderate activity against Gram positive bacteria and low activity against Gram negative bacteria.

Table 3. Anti-bacterial activity of leaves extract of *Mangifera indica* and Tetracycline

Types of the Bacteria	Name of the Bacteria	Tetracycline (30µg/disc)	*Mangifera indica* (5mg/disc)
Gram positive	*Staphylococcus aureus*	12.5	7.0
	Streptococcus agalactiae	22.0	7.5
	Bacillus cereus	19.5	11.5
	Bacillus megaterium	7.0	8.5
	Bacillus subtilis	20.0	9.0
	Lactobacillus vulgaricus	11.5	9.5
Gram negative	*Salmonella typhi*	14.5	-
	Shigella flexneri	23.0	9.5
	Shigella sonei	22.5	9.5
	Proteus sp	11.0	-

Table 4. Anti-fungal activity of leaves extract of *Mangifera indica*

Name of the fungus	Percentage of inhibition (%)
Aspergillus ustus	29
Aspergillus niger	21
Aspergillus ochraceus	19

Mangifera indica leaves extracts showed antifungal activity that was satisfactory. Leaves extract (4mg/20ml) of *Mangifera indica* showed a prominent significant degree of anti-fungal activity (Table 4) against *Aspergillus ustus* (29%), *Aspergillus niger* (21%) and *Aspergillus ochraceus* (19%).

In a word, the resistance of the various types of organisms increased due to indiscriminate use of commercial anti-microbial drugs (WHO, 2002). This situation forced the researchers to search the new anti-microbial substances from different sources including medicinal plant and leaves extract of *Mangifera indica* might be considered as effective herbal drugs against pain, inflammation, bacterial infection and some fungal infection. Further investigation was required for isolation, identification and characterization of different active compounds of leaves extract, their mode of action and therapeutic ranges.

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