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Evaluation of the analgesic and anti-inflammatory activities of methanolic extracts of the leaves of *Averrhoa bilimbi* leaves

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ABSTRACT

Objectives: This study was aimed to investigate the analgesic and anti-inflammatory activities of crude methanolic extract *Averrhoa bilimbi* leaves.

Materials and Methods: Methanolic extracts of *Averrhoa bilimbi* leaves with different concentration were tested for analgesic activity in mouse model by acetic acid induced writhing and anti-inflammatory effect was tested by carrageenan induced paw edema model

Results: The extract, at 400 mg/kg, showed higher analgesic activity (67.51%) against acetic acid induced pain in mice while the standard reference drug Diclofenac sodium exhibited 64.33% activity at 10 mg/kg dose. The anti-inflammatory effect of the extract was comparable to reference drug Ibuprofen and the effect was sustained for 2-4 hr.

Conclusion: Methanolic extractof *Averrhoa bilimbi* leaves have moderate analgesic and anti-inflammatory properties.

Key words: *Averrhoa bilimbi,* analgesic activity, anti-inflammatory activity, crude extract.

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INTRODUCTION

Herbal medicines have been used to treat different diseases all across the world for centuries. Application of herbal medicines is growing in developing as well as developed countries, due to their wide biological and medicinal activities, higher safety margins and affordability. According to WHO, the market for traditional medicine has observed a significant growth over the past few years.[1] All these reasons make investigations into the medicinal use of plants and their parts all the more important.

Averrrhoa bilimbi (A. bilimbi) (common name: Bilimbi) is a medicinal plant belonging to the family *Oxalidaceae*. *A. bilimbi* Linn. is one of the important medicinal plants of many tropical and subtropical countries of the world which has been widely used in the traditional system of medicines for the treatment of a variety of ailments, particularly as an antidiabetic, antihypertensive, and antimicrobial agent[2,3] Bilimbi fruits are very sour in taste and are used to manufacture vinegar, wine and pickles. The fruit is an excellent source of vitamin C. It is an important medicinal tree in the tropical and sub-tropical regions especially in Asia and has been used to treat different medical conditions like diabetes, hypertension and infections. Its leaves, fruits and fruit juice are cited to possess antimicrobial activities.[4,5] The leaves and fruits have been traditionally used to control hypertension. Studies have found its leaves extract to show significant antihypertensive effects[6] Tropical application of its leaves extracts has shown wound healing potential.[7] Antidiabetic properties of its leaves extract has also been reported.[8,9] In this study we tried to evaluate the analgesic and anti-inflammatory activity of methanol extracts of the leaves of *Averrhoa bilimbi*.

MATERIALS AND METHODS

Chemicals

Diclofenac Na, Ibuprofen and Diazepam were obtained from Square Pharmaceuticals Ltd., Bangladesh, Acetic acid was collected from Merck, Germany. Normal saline water (0.9%) NaCl was brought from Beximco Infusion Ltd. Bangladesh. Formalin, carrageenan and all other chemicals were of analytical grade.

Animal

Swiss albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting

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of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh. Animal treatment and maintenance for acute toxicity and analgesic effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

Collection of Plant Materials

The leaves of the *Averrhoa bilimbi* Linn were collected from Brahmanbaria district, Bangladesh during September 2017. The plant material was taxonomically identified by the National Herbarium of Bangladesh-Dhaka (Accession number-43898).

Preparation of Plant Extract

The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The dried powder material (1.2 kg) was refluxed with methanol for three hours. The total filtrate was concentrated to dryness, in vacuum at 40°C to render the methanol extract (310 g). The obtained methanolic extract was concentrated deep green colored. The extract was preserved in an air-tight container as a crude extract sample.

Analgesic Activity

Acetic Acid-induced Writhing Test

The analgesic activity of the samples was also studied using acetic acid- induced writhing model in mice.[10] The animals were divided into six groups with five mice in each group. Group I animals received vehicle (Water), Group II received Diclofenac Na at 10 mg/kg body weight while animals of groups-III and IV were treated with 200 mg/kg and 400 mg/kg body weight, per oral of the methanolic extract of *Averrhoa bilimbi* Linn leaves. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 1.0% v/v acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min.

Anti-inflammatory Activity

Carrageenan Induced Paw Edema Test in Mice

Swiss albino mice (25-30g) were divided into four groups of five animals each. The test groups (Group III & Group IV) received 200 mg/kg and 400 mg/kg body weight, per oral of the methanolic extract of *Averrhoa bilimbi* Linn leaves. The reference group (Group II) received Ibuprofen (10 mg/kg body weight, per oral) while the control group (Group I) received 1 ml/kg body weight normal saline. After 1 h, 0.1 ml 1% carrageenan suspension in normal saline was injected into the subplanatar tissue of the right hind paw. The paw volume was measured at 1, 2, 3 and 4 hrs after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

% inhibition = (1-Dt/Do) x 100

Where, Do was the average inflammation (hind paw edema) of the control group of mice at a given time, Dt was the average inflammation of the drug treated mice at the same time.[11]

Statistical Analysis:

All values were expressed as the mean ± SEM of three replicate experiments.

RESULTS

Analgesic Activity

The effect of methanlic extract of leaves of *Averrhoea bilimbi* investigated against acetic acid induced writhing in mice is represented in Table 1. About 64.33% inhibition of writhing was observed in mice treated with Diclofenac sodium (10 mg/kg), the reference drug. The methanol extract of leaves of *Averrhoea bilimbi* significantly reduced the acetic acid induced abdominal constrictions and stretching in a dose dependent manner (Group-III, IV) compared to that of control (Group-I). The analgesic effect of the extract at a dose of 400mg/kg was comparable to that of a dose 10 mg/kg of Diclofenac sodium.

Anti-inflammatory Activity:

The extract exerted anti-inflammatory effect at the test dose of 200 and 400 mg/kg body weight were comparable to that of the reference group (Group-II) (Table 2). The percent inhibition of carrageenan-induced inflammation at those doses were relatively low for initial1 hr period but had more pronounced effect subsequently at 2-3 hr andwas comparable to that of standard drug Ibuprofen at 10 mg/kg dose.

DISCUSSION

Acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from

Table 1 **Effects of the methanolic extract of *Averrhoa bilimbi* Linn leaves on acetic acid-induced writhing in mice**

Groups	Treatment	Dose (mg/kg)	No. of writhing (Mean±SEM)	% inhibition
Group I	Water	Vehicle	26.16±1.71	
Group II	Diclofenac Na	10	9.33±1.46	64.33
Group III	Methanol Extract	200	10.16±1.21	61.14
Group IV	Methanol Extract	400	8.5±1.17	67.51

Table 2 **Effects of the methanolic extract of *Averrhoa bilimbi* Linn leaves on carrageenan induced paw edema in mice**

		Oedema diameter (mm)				Inhibition (%)			
Group	Dose (mg/kg)	1h	2h	3h	4h	1h	2h	3h	4h
Group I	Vehicle	4.6±0.907	4.35±0.71	4.25±0.56	3.78±0.44				
Group II	10	2.48±0.454	1.9±0.46	1.4±0.38	.98±0.31	47.06	56.32	67.06	74.17
Group III	200mg/kg	4.28±0.486	2.45±0.56	2.2±0.44	1.7±0.6	8.566	43.68	48.24	54.97
Group IV	400mg/kg	3.88±0.816	2.5±0.82	2.3±0.77	1.43±0.72	17.11	42.52	45.29	62.25

the tissue phospholipid. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways.[12] The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics.[12] It is well known that non- steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process.[13] Therefore, it is likely that the methanolic extract of *Averrhoa bilimbi* Linn leaves might have exerted its peripheral antinociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonizing the action of pain mediators at the target sites (Table 1). The above findings clearly demonstrated that both central and peripheral mechanisms are involved in the antinociceptive action of the methanolic extract of *Averrhoa bilimbi* Linn leaves. Interestingly, compounds like flavonoids and steroids, triterpenes in part, have been shown to possess anti- inflammatory and analgesic activity.[14]

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2hr) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages.[15] Since the extract significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as ibuprofen, whose mechanism of action is inhibition of the cyclooxygenase enzyme (Table 2). Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation. This hypothesis is strongly supported by the previous study, which has shown that methanolic extract of *Averrhoa bilimbi* Linn leaves possesses anti-inflammatory activity due to the presence of flavonoid content.[14]

CONCLUSION

The results of the experiments suggest that the methanolic extract of *Averrhoa bilimbi* Linn leaves may be used as an alternative or supplementary herbal remedy for relieving pain and inflammation. Because the methanolic extract of *Averrhoa bilimbi* Linn leaves posses a remarkable analgesic and moderate anti-inflammatory effects. Thus the present study warrants further investigation involving components of the methanolic extract of *Averrhoa bilimbi* Linn leaves for possible development of new class of analgesic and anti- inflammatory drugs.

CONFLICT OF INTEREST

Authors has no conflict of interest

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