

Effects of aqueous and ethanolic extracts of *Aegle marmelos* (BAEL) leaves on chronic inflammation in rats

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Abstract

Aegle Marmelos Linn (Rutaceae) is used as ethno medicine against various human ailments. Several curde extracts from various parts (Leaves, flower, stem, root etc) of the plant *A. marmelos* Linn have shown variable anti-inflammatory effects in acute and chronic inflammation in animal models. The anti-inflammatory effects of *A. marmelos* linn may be of special advantage compared to conventional anti-inflammatory drugs. The present study has therefore been undertaken with the objective to evaluate the anti inflammatory effect of aqueous and ethanolic extracts of *A. marmelos* leaves, compared to a standard anti-inflammatory drug (indomethacin) in chronic inflammatory conditions. The anti-inflammatory effect was studied in rats using cotton pellet implantation, where granuloma formation was used as an index of chronic inflammation. Aqueous and ethanolic extracts of *A. marmelos* leaves were given orally for 7 days daily at doses of 100 mg/kg body weight. The percent inhibition of granuloma formation following treatment with aqueous and ethanolic extracts of *A. marmelos* leaves, and indomethacin compared to control were 16.5%, 25.72%, and 39.37% respectively. The differences were statistically significant ($p < 0.05$ in case of aqueous and ethanolic extracts and $p < 0.001$ in case of indomethacin). The results suggest that in case of chronic inflammation, both aqueous and ethanolic extracts of *A. marmelos* have significant anti-inflammatory effect. The ethanolic extracts compared to aqueous extract produced greater anti-inflammatory effects.

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Introduction

Inflammation is normally a primary physiologic defense mechanism that helps body to protect itself from tissue injury caused by physical trauma, noxious chemicals or microbiological agents. It is the body's effort to inactivate or destroy the invading organisms, remove irritants and set the stage for tissue repair.¹ Though it is a defense mechanism, the complex events and mediators involved in inflammatory reactions induce, maintain or aggravate many disease processes. An uncontrolled and persistent inflammation may act as etiologic factor for many chronic illness.² The currently available antiinflammatory drugs are a heterogeneous group of compounds, often chemically unrelated, which nevertheless share certain unwanted effects. The most common adverse effect is a propensity to induce gastric

irritation, hyperacidity and other symptoms. Therefore, the present trend is to find out more acceptable agents which would be devoid of the potential adverse effect. Use of herbal medicine throughout the world is increasing. Plants are the primary source of supply of many important drugs used in modern medicine also.

Aegle Marmelos Linn, commonly known as 'Bael' in Bengali language is one such plant grown wildy in hilly forests and is also cultivated for its fruits all over Bangladesh and is also in many countries of South East Asia including India, Sri Lanka, Myanmar, Thailand and Indochina. Extensive chemical investigations on various parts of the tree have been carried out and over 100 compounds have been isolated, many of which have therapeutic potential.³ Several

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crude extracts of *A. marmelos* Linn have shown significant anti-inflammatory (in both acute and chronic inflammatory animal models) effect.^{4,5} Considering its reported anti-inflammatory properties and availability the present study was undertaken to evaluate the anti-inflammatory effect of aqueous and ethanolic extracts of *A. Marmelos* leaves compared to a standard antiinflammatory drug (indomethacin) in case of chronic inflammation in rats.

Materials and Methods

Plant material and preparation of extract

The leaves of *Aegle marmelos* were collected from Botanical garden, Mirpur and authenticated by the Bangladesh National Herbarium, Dhaka. Aqueous & ethanolic extract were prepared in Drug Research Laboratory of Center for Advanced Research of Science (CARS), Dhaka University following standard method.

Animals

Thirty two (32) Wister Albino Rats of either sex, weighting between 100-150g were kept under standard condition of light and temperature, fed with standard rat pellet diet and allowed to drink water *ad libitum*.

Experimental design

The antiinflammatory activity of extracts *A. marmelos* leaves was tested by cotton pellet induced granuloma in rats.^{6,7} Experimental rats were divided into four groups each consisting of eight. Chronic inflammation (granuloma) was induced by implanting one autoclaved cotton pellet (10mg) subcutaneously in one groin of each rat by making small subcutaneous incision on 1st day. They were treated as follows for 7 days: Group 1 served as control that received normal saline, Group 2 and Group 3 were given aqueous and ethanolic extracts of *A. marmelos* leaves respectively at a dose of 100mg/kg body weight, Group 4 were given indomethacin at a dose of 10mg/kg body weight orally. On the 8th day rats were sacrificed and implanted cotton pellets were dissected out, dried in a hot air oven at 60°C and the final weights were measured. The difference between the initial and final weight of cotton pellets were considered to be the weight of granulomatous tissue produced. The dry weight correlates well with the amount of granulomatous tissue formed. The percentage change of granuloma weight relative to vehicle control

group was taken as an index of chronic antiinflammatory activity.

Percent inhibition of granuloma formation was calculated in each case by using the formula: $100 \times (1 - W_t / W_c)$. Where, W_t = mean dry weight of granuloma in drug treated group;

W_c = mean dry weight of granuloma in drug untreated control group.

Statistical Analysis

All the results have been expressed as the mean \pm standard error of mean. The significance of the differences between treatment and control group were calculated using student's t-test.

Results

In the study, mean initial weight of cotton pellets in each group were 10.17 ± 0.02 mg. The increase in weight of cotton pellet following 7 days of treatment in control (Group 1), aqueous extract (Group 2), ethanolic extract (Group 3) and indomethacin (Group 4) groups were 62.56 ± 0.77 mg, 52.24 ± 0.78 mg, 46.47 ± 1.42 mg, 37.93 ± 0.88 mg respectively. The difference in cotton pellet weight (granuloma) of aqueous extract (Group 2), ethanolic extract (Group 3) and indomethacin (Group 4) treated groups compared to control (Group 1) were statistically significant ($p < 0.05$ in group 1 Vs Group 2, 3 and $p < 0.001$ in Group 1 Vs Group 4). The detail results have been shown in Table-1. The percent inhibition of granuloma formation following treatment with aqueous extract (Group 2), ethanolic extract (Group 3) and indomethacin (Group 4) compared to control (Group 1) were 16.5%, 25.72% and 39.37% respectively.

Discussion

Inflammation, though basically is a part of body's defense mechanism, the complex events and mediators involved in inflammatory reactions may induce, maintain or aggravate many disease processes. An uncontrolled and persistent inflammation may act as causative factor for many chronic illness. Commonly used antiinflammatory drugs such as non-steroidal antiinflammatory drugs (NSAIDs) and corticosteroids, besides being costly, have their own limitation and are frequently associated with unwanted side effects notably gastrointestinal disturbances including peptic

Table-1: Effects of aqueous and ethanolic extracts of *Aegle marmelos* leaves and Indomethacin on cotton pellet induced granuloma in rats

Group	Number of rats (n)	Initial weight of cotton pellet (mg) (Mean \pm SEM)	Final weight of cotton pellet (mg) (Mean \pm SEM)	Increase in weight of cotton pellet (mg) (Mean \pm SEM)	Inhibition of granuloma formation %
Group-1 (Control)	8	10.17 \pm 0.02	72.73 \pm 0.76	62.56 \pm 0.77	-
Group-2 (Aqueous extract 100mg/kg b.w)	8	10.17 \pm 0.02	62.41 \pm 0.77	52.24 \pm 0.78*	16.5 %
Group-3 (Ethanolic extract 100mg/kg b.w)	8	10.17 \pm 0.02	56.64 \pm 1.4	46.47 \pm 1.42*	25.72 %
Group-4 (Indomethacin 10mg/kg b.w)	8	10.17 \pm 0.02	48.10 \pm 0.88	37.93 \pm 0.88**	39.37 %

* $P < 0.05$ in a test of significance difference from control.

** $P < 0.001$ in a test of significance difference from control.

ulceration. In the present study, the percent inhibition of granuloma formation (indicative of chronic inflammatory process) following treatment with aqueous extract (100 mg/kg body weight in Group 2) and ethanolic extracts (100 mg/kg body weight in Group 3) of *Aegle marmelos* leaves, and standard drug, indomethacin (10 mg/kg body weight in Group 4) compared to control (Group 1) were 16.5%, 25.72%, 39.37% respectively. The differences were statistically significant ($P < 0.05$ in case of aqueous and ethanolic extracts and $P < 0.001$ in case of indomethacin). The ethanolic extracts compared to aqueous extract produced greater anti-inflammatory effects. The results are similar with those found in earlier studies.^{4,5}

The study showed that both aqueous and ethanolic extracts of *Aegle marmelos* leaves produced significant anti-inflammatory effect. The results of the present study provide a rationale for use of *A. marmelos* leaves as a herbal medicine in chronic inflammatory conditions. However, further studies is needed to provide evidences for the safety of the long term administration of the extracts, before it can be recommended as potential anti-inflammatory drug in the management of chronic inflammatory conditions. Then further studies may be undertaken to isolate and identify the active anti-inflammatory component from the studied crude extracts.

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