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Research Article

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Phytochemical and pharmacological screening of *in vivo* anti-inflammatory activity of *Aegle marmelos*(L.) Corr. Serr.

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ABSTRACT

The aim of this study is to evaluate anti-inflammatory activity and to determine phytochemical constituent of ethanolic leaf extract of Aegle marmelos(L.) Corr. Serr.. Plant materials are collected from Punjab. Ethanolic extract was screened for different phytochemical constituents. Acute toxicity studies are done according to OECD guidelines. Ethanolic extracts were screened for anti-inflammatory activity (induced by Carrageenan) in Wistar Albino rats. Ethanolic extract Aegle marmelos(L.) Corr. Serr. Shows significant anti-inflammatory activity compared to control. Presence of alkaloid, saponins and steroids were identified in the extract. The result suggests that the ethanolic extract of Aegle marmelos(L.) Corr. Serr. contains some active principles which may possess significant anti-inflammatory activity.

Key words: *Aegle marmelos, EAM-ethanolic extract of Aegle marmelos,* Carrageenan

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INTRODUCTION

The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation. The factors that can stimulate inflammation include microorganisms, physical agents, chemicals, inappropriate immunological responses, and tissue death. The inflammatory process involves a complex biological cascade of molecular and cellular signals that alter physiological responses, ultimately resulting in the familiar clinical symptoms of pain, swelling, heat, and redness. Currently using anti-inflammatory drugs are associated with some severe side effects.

In many countries, herbal therapies are among the most popular of all “alternative treatments”. *Aegle marmelos(L.) Corr. Serr.* has been used for centuries as an herbal medicine. It is commonly known as Bael, is indigenous to India and is one of the most useful medicinal plants in India. Its stem, bark, root, leaves and fruits have medicinal value. The ancient systems of medicine, including Roman, Ayurveda, Greek, Siddha and Unani have mentioned its therapeutic applications in cardiovascular disorders, diabetes, diarrhoea and dysentery. Other actions like antifungal, antibacterial, antiprotozoal, hypoglycemic, antioxidant, antiviral and cardioprotective effects have been studied using various parts of the plant[1],[2],[3]. Besides its antioxidant properties, *Aegle marmelos*(L.) Corr. Serr. unripe fruit aqueous extract interacts by various other mechanisms in a complex way to elicit its therapeutic effects.

Aegle marmelos(L.) Corr. Serr. Belonging to the family *Rutaceae*, is found abundantly throughout India. Physicochemical studies proved that bael fruit is rich in nutritional value, and this is being used from several years ago. Bael pulp is a rich source of glucose, sugar, and fiber. In the traditional medicine system the pulp of bael is used as an energy drink with milk. That drinks is very useful to excrete the hair from the stomach. Other nutritive elements of bael are-protein, fat, minerals, fibers, carbohydrates, calcium, phosphate, potassium, iron, vitamins A, vitamin B1, nicotinic acid, riboflavin, vitamin C .

Aegle marmelos(L.) Corr. Serr.is reported to have number of coumarins, alkaloids, steroids, and essential oils[7],[8]. Root and fruits contain coumarins such as scoparone, scopoletin, umbellliferone, marmesin and skimming. Fruits in addition contain xanthotoxol, imperatorin and alloimperatorin and alkaloids like aegeline and marmelline. It also contains polysaccharides like galactose, arabinose, uronic acid and L-rahaminose, which may obtain after hydrolysis. Different types of carotenoids have been reported in the fruit, which is responsible for the yellow pale colour of fruit. Marmelosin, skimmianine and umbelliferone are the therapeutically active principles of bael plant. Minor constituents are like ascorbic acid, sitosterol, crude fibers, tannins, α amyrin, carotenoids, and crude proteins are also resent. Apart from these chemical constituents more than 100 compounds have been isolated these are skimminine, aegelin, lupeol, cineole, citral, citronellal, cuminaldehyde, eugenol, marmesinin, marmelosine, luvangetin, aurapten, psoralen, marmelide, fagarine, marmin, and tennins have been proved to be biologically active against various major and minor disease.

From the previous studies aqueous root bark extract, aqueous extract of the dried flowers and methanolic leaf exctract of *aegles marmeloes*(L.) Corr. Serr. shows significant anti-inflammatory activity in addition to this unripe fruit pulpwas reported to possess anti-inflammatory activity. Total alcoholic and aqueous extracts of leaves were evaluated for its toxic effect in experimental rats by veerappan et al in 2007.Taking into consideration the above facts, an attempt has been made to evaluate anti-inflammatory activity of ethanolic leaf extract of *aegles marmeloes*(L.) Corr. Serr.

EXPERIMENTAL SECTION

1.1. Preliminary phytochemical screening

The leaf specimens were collected in the month of April from punjab, India and authenticated by Professor Dr. Girish, Herbal Science Laboratory, Centre for Advanced Studies in Botany, University of horticulture and forestry, Nauni, Solan (H.P) India and voucher specimen number 5067. After a thorough investigation leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water subjected for drying for 15-20 days.

Dried leaves were powdered in a mechanical grinder. The powdered plant sample was packed into a Soxhlet apparatus and extracted exhaustively with ethanol(95%) for 24h. The ethanolic extract was concentrated using a rotary evaporator at 40°C. Then extract was kept in refrigerator at 5^0C for experimentation.

The different qualitative chemical tests were performed for establishing the profile of the ethanolic leaf extract for its chemical composition.

- **Test for alkaloids**

A small portion of the extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidal reagents such as Mayer's reagent, Dragondroff's reagent, Hager's reagent and Wagner's reagent.

- **Test for carbohydrates**

The minimum amount of the extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates.

- **Saponin glycosides**

Foam test – The extract and powder were mixed vigorously with water.

- **Test for flavonoids**

With aqueous solution of sodium hydroxide blue to violet colour (Anthrocyanins), yellow colour (Flavones), yellow to orange (Flavonones)

- **Test for tannins and phenolic compounds**

Small quantity of various extracts were taken separately in water tested for the presence of phenolic compounds and tannins with

(a) Dilute ferric chloride solution (5%) - violet colour
(b) 1% solution of gelatin with 10%NaCl - white precipitate
(c) 10% lead acetate solution - white precipitate

- **Test for steroids**

Libermann – Burchard Reaction: 2 ml extract was mixed with chloroform. To this 1-2 ml acetic anhydride and 2 drops concentrated sulphuric acid were added from the side of test tube. First red, then blue and finally green colour appears.

1.2. Acute toxicity study

Swis Albino mice of either sex (20 - 25 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423(Organization for Economic Co-operation and Development) and animals were observed for mortality and behavioral changes. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

1.3. Pharmacological screening of anti-inflammatory activity

***Carrageenan Induced Rat Paw Edema Animal Mode*l [6]**

Adult albino rats of either sex weighing between 150 to 250 grams were randomly selected from central animal facility. Animals were housed in groups of 3, at an ambient temperature of 25±1°C with ad libitum access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.

Animals were randomly divided into 3 groups of 6 rats each; I group: Control (1ml distilled water); II group: Standard drug (Indomethacin 10 mg/kg); III group: Test drug (400 mg/kg). Following models were used to screen the anti-inflammatory activity.

In this method, rats were divided in 3 groups of six animals each. The animals were pretreated with drugs orally 1 hr before the experiment. 0.1 ml of 1% carrageenan was injected aseptically into the subplantar surface of right hind paw of each rat. Paw edema was measured by Mercury Plethysmograph at '0'hour and at the end of '4' hours. The difference between the zero and 4 hours gives the actual edema. Percentage inhibition (protection) against edema formation was taken as an index of acute anti-inflammatory activity.

It was calculated by:

The percent inhibition of edema = $100 \times (1- Vt / Vc)$

Where, Vc = mean paw edema volume in the control group.
Vt = mean paw edema volume in the drug treated group.

1.4. Statistical Analysis

Results were expressed as mean ± Standard deviation (SD). Statistical analysis was performed using One-way analysis of variance (ANOVA).

RESULTS

2.1. Preliminary phytochemical screening

Preliminary phytochemical screening of ethanolic extract of *Aegle marmelos*(L.) Corr. Serr. leaf revealed the presence of alkaloids, saponins, steroids.

2.2. Acute toxicity study

Acute toxicity studyshowed that the extract have high safety profile as no death was observed at a dose of 2000mg/Kg in mice. Some behavioral changes were observed such as reduced motor activity, ataxia etc.

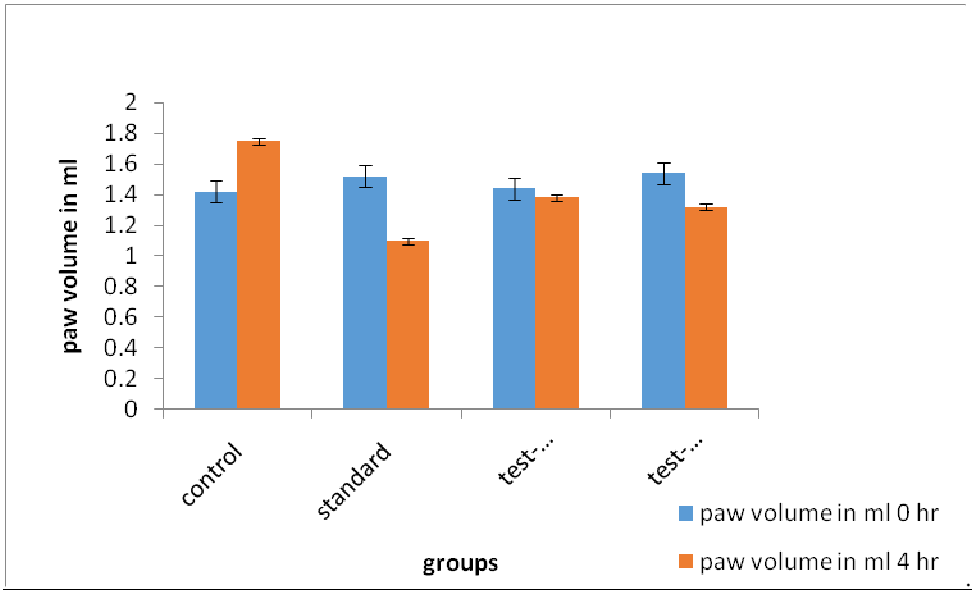
2.3. Carrageenan-Induced Rat Paw Edema

The result presented in table 1 and figure 1 showed that the extracts of *Aegle marmelos*(L.) Corr. Serr. leaves was found to be the significant ($P < 0.001$) anti-inflammatory activity at a dose of 400 mg/ kg. All test samples at the dose of 400 mg/kg was comparable with indomethacin 10 mg/kg. The ethanolic extract of *Aegle marmelos*(L.) Corr. Serr.(400mg/kg) and indomethacin (10 mg/kg) both significantly inhibited carrageenan induced rat paw edema ($P < 0.001$). The maximum inhibition of paw edema was observed in both indomethacin and ethanolic extract of *Aegle marmelos*(L.) Corr. Serr. at the end of four hours when compared to the control group.

Graph I: Effect of EAM on carrageenan induced rat paw edema

Groups	Paw volume in ml	
	0 hr	4 hr
Control(vehicle)	1.32±0.031	1.75±0.083
Standard(indomethacin 10mg/kg)	1.42±0.072	1.1±0.075**
test-200mg/kg	1.44±0.0993	1.38±0.059
test-400mg/kg	1.48±0.059	1.22±0.064*

Figure: 1 Effect of EAM on carrageenan induced rat paw edema



DISCUSSION

Carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by mediators of acute inflammation. Development of edema induced by carrageenan is commonly correlated with early the exudative stage of inflammation, involving release of histamine and serotonin from mast and basophil cells, and is characterized by increase in vascular permeability. Later there is release of bradykinins (an important chemical mediator of both pain and inflammation), prostaglandins, and cyclooxygenases products, and marked increase in cellular infiltration and subsequent release of acute inflammatory mediators such as myeloperoxidase and cytokines (interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, etc.) at the inflammatory site. Further, neutrophils, macrophages, endothelial, and other cells at the site of inflammation may produce reactive oxygen species (ROS) and reactive nitrogen species, which play a modulating role in the inflammatory response Carrageenan is regarded as an established phlogistic agent/oedemogen and edema induced by the subplantar injection of carrageenan in the rat hind paw is reported to have been inhibited by a number of steroidal and non-steroidal anti-inflammatory drug. In our study the aqueous extract of *Aegle marmelos*(L.) Corr. Serr400 mg/kg, p.o. significantly reduced edema induced by the carrageenan. The percent inhibition of paw edema by indomethacin was 52.5% while that of *Aegle marmelos*(L.) Corr. Serris 30.5 %(figure I). The probable mechanism of the acute anti-inflammatory activity might be due to the inhibition of release of mediators like histamine, serotonin and prostaglandins. This activity probably will be due to the chemical constituents like saponins**,** steroids etc.

CONCLUSION

This study demonstrated that ethanolic extract of *Aegle marmelos*(L.) Corr. Serr**.** shows significant anti-inflammatory activity. Further studies were needed to understand actual mechanism of action.

REFERENCES

[1] Udupa SL; Udupa AL; Kulkarni DR, *Fitoterapia*., **1994**, 65,119–23.
[2] Mazumder R; Bhattacharya S;Majumder A; Pattnaik AK; Tiwari PM,Chaudhary S, *Phytother Res*., **2006**, 20, 82–4.
[3] Chauhan A; Agarwal M; Kwhwaha S; Mutresa A, *Contraception*.,**2007**, 76,474–81.
[4] Jagetia GC; Venkatesh P; Balinga MS, *Int J Radiat Biol.*, **2004**, 80,281–90.

[5] Narendra T ;Sweta S; Tiwari P; Papi Reddy K; Kholiq T; Prathipati P; *Bioorg Med Chem*., **2007**, 17, 1808–11.
[6] Jyoti M; Benn M.K;Jayanthi; R.N. Suresha, *Indian J Pharmacol*., **2011**, 43(4),393–397.
[7] AnsariS.H;Naved, *journal of pharmaceutical research*.,.**2006**,5(2),46-59.
[8] Sharma BR;Rattan RK; Sharma P, *Phytochemistry*.,**1981**,(20)11,2606-2607.
[9] Mishra BB; Singh DD; Kishore N; Tiwari VK; TripathiV, *Phytochemistry*., **2010**,71(2-3),230-234.
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