EVALUATION OF ANTIINFLAMMATORY AND ANTIPYRETIC ACTIVITY OF *AEGLE MARMELOS* LEAVES IN RATS

Ramachandra Y L, Shruthi S D, Gavimath C.C. and Sujan Ganapathy P.S.

Department of P G Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Shankaraghatta - 577 451, Karnataka, India

*Corresponding author: ylrpub@gmail.com

Summary

The plants belonging to family Rutaceae are found to be a rich source of substances of phytochemical interest**.** *Aegle marmelos* is one member of this family which is traditionally used as folk medicine since ancient times. The present study was aimed at testing the anti-inflammatory and antipyretic properties of leaf extracts of *Aegle marmelos* in rats at a dose of 200mg/kg body weight. Petroleum ether, ethanol and aqueous extracts of the leaves were chosen for above pharmacological screening. The experimental models used were carrageenan induced paw edema for antiinflammatory activity and yeast induced pyrexia for antipyretic activity. Petroleum ether and ethanol extracts of leaves of *Aegle marmelos* exhibited more significant antiinflammatory in acute inflammation model. Significant antipyretic activity was observed only with leaves of Petroleum ether extract. These results demonstrated that the leaves of *Aegle marmelos* exhibit beneficial effects for inhibiting inflammation and fever.

***Keywords*:** *Aegle marmelos*; Antiinflammatory; Carrageenan; Paw edema; Antipyretic; Brewer's yeast; Pyrexia.

Introduction

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans (1). The research into plants with alleged folkloric use as pain relievers, antiinflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new antipyretic and antiinflammatory drugs (2).

Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules (3). Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent (4). It is a pathophysiological response of living tissue to injuries that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defensive mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (5). Drugs show to a greater or lesser degree, the therapeutic properties of reducing pain, inflammation and fever and the side effects of causing gastrointestinal irritation and renal pathology (6). Therefore herbal medicines are in line with nature having new and more powerful drugs with little or no side effects (7).

Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defence to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's which increase the synthesis of prostaglandin E2 near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature (8). Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects. A natural antipyretic agent with reduced or no toxicity is therefore essential (9).

Aegle marmelos Corr. is a medium sized, deciduous tree bearing strong axillary thorns. Plant is considered to be very auspicious as its leaves are used to worship Shiva. It has many Indian names among which commonly called as bilwa or bael. In English it is called as Bengal quince, golden apple, stone apple etc. (10). More than thirty compounds have been reported from leaves of *Aegle marmelos* (11). The essential oil obtained from the leaves has shown a broad spectrum of antibacterial and antifungal activities (12; 13). The aqueous extract of the leaves has been reported to have hypoglyceamic effect (14; 15). No scientific work is available in the literature regarding antiinflammatory and antipyretic properties of *Aegle marmelos.* Hence an attempt has been made to investigate antiinflammatory and antipyretic properties of different extracts of leaves of *Aegle marmelos.*

Materials and Methods

Chemicals and Drugs

Lambda Carrageenan (Sigma Aldrich, Bangalore), Diclofenac sodium (Dr. Reddy Labs, Hyderabad), Paracetamol (Pure Pharma Pvt. Ltd., Mumbai), Brewer's yeast, Petroleum ether (Merck, India), Ethanol (AR grade, s.d. fine chem., Mumbai, India) and double distilled water.

Plant material

The leaves of the plant *Aegle marmelos* was freshly collected in and around the local areas of Belgaum in October 2005. The plant material was taxonomically identified by Prof. N. A. Jadhav, Dept. of Botany, B.K. College, Belgaum. A voucher specimen (No. BT. AM. 302) has been preserved in Dept. of Biotechnology, Kuvempu University, Shankaraghatta for future references. The shade dried leaves were pulverised and subjected for extraction using petroleum ether, ethanol and aqueous separately using soxhlet apparatus. Then the extracts were concentrated using rotary flash evaporator (Buchi Flawil, Switzerland). The suspension of extracts were prepared using 2% gum acacia and used for the study.

Animals

Studies were carried out using Wistar strain albino male rats (150-200 g), which were procured from Venkateshwara enterprises Bangalore. They were housed under standard laboratory conditions (25 ± 2^0C) with dark light circle (14/10hr). Animals were allowed free access to standard pellet diet (Sai Durga Feeds, Bangalore) and water *ad libitum*. Food was withdrawn 2 hrs before and during experimental duration.

All experimental protocols were prepared and performed based on ethical guidelines of Institutional Animal Ethics Committee (No. SETCP/IAEC/200 -2007/462).

Acute toxicity study

This study was carried out to determine the therapeutic dose of extract (16). The animals were divided into six groups containing eight animals per each group. Experiment was conducted for the petroleum ether, ethanol and aqueous extracts of leaves at different concentrations by stair case method (17). 200 mg/kg b.w was taken as the therapeutic oral dose for all the extracts.

Antiinflammatory activity

Carrageenan induced rat paw edema

The rats were divided into 5 groups of 6 animals each and initial paw volume was measured at 0 hr. Further paw edema was induced by injecting 0.1ml of 1% carrageenan in physiological saline into the subplantar tissue of the left hind paw of each rat (18). The test groups were treated orally with all the three extracts (200 mg/kg b.w), control group received normal saline (1 ml/kg b.w) orally and standard group animals received Diclofenac sodium (10 mg/kg b.w) by intraperitoneal injection 30 min. prior to carrageenan administration. The paw volume was measured at an interval of 30 min. up to 3 hrs by mercury displacement method using plethysmograph. The percentage inhibition of edema in the test drug treated group was calculated by using the formula (19).

$$\% \text{ Inhibition} = 1-(Vt/Vc) \times 100$$

Where Vt = Edema volume in the test drug treated animals.

Vc = Edema volume in the control group animals.

Antipyretic activity

Yeast induced pyrexia

Rats were taken and divided into into 5 groups of 6 animals each. The normal body temperature of each rat was measured rectally at predetermined time intervals. Hyperplasia was induced in rats by subcutaneous injection of 20% suspension of Brewer's yeast in normal saline (10 ml/kg b.w) in the back below the nape of the rat (20; 21). The site was massaged to spread the suspension beneath the skin. The rectal temperature was recorded using digital thermometer lubricated with glycerin into the rectum before (-18h) and 18 hr after (0h) Brewer's yeast injection. The test group animals were treated orally with all the three extracts (200 mg/kg b.w) where as control group animals received normal saline (1 ml/kg b.w) and standard group animals received paracetomol (100 mg/kg b.w) by intraperitoneal injection. The rectal temperatures of all the animals were recorded at 30 min. of intervals till 3 hrs. All the temperatures recorded were tabulated and difference in the rise of temperature from that of normal rat temperature was computed statistically.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis of data was performed using ANOVA followed by student t-test to study the differences amongst the means (23). Values of $P < 0.05$ were considered as statistically significant.

Results

Antiinflammatory effect

The antiinflammatory activity of leaf extracts were measured at an interval of 30 min. up to 3 hrs at a dose of 200 mg/kg b.w against the acute paw edema induced by carrageenan. Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness (0.92 ± 0.02) up to an interval of 3hrs. Whereas petroleum ether (0.39 ± 0.01) and ethanol extracts (0.40 ± 0.01) showed significant ($P<0.05$) antiinflammatory activity with percent inhibition of 57.7% and 56.6% respectively. Standard drug Diclofenac sodium showed more significant ($P<0.01$) value (0.24 ± 0.01) with percent inhibition of 74%. However the aqueous extract (0.80 ± 0.02) of leaf was devoid of this activity with less inhibition of 13.1% which is shown in Table I.

Antipyretic effect

The effect of leaf extracts on body temperature in rats is presented in Table II. The results showed that the 200 mg/kg b.w caused significant lowering of the body temperature up to 3hrs. Prominent increase in temperature was observed in pyrexia induced control group (38.76±0.15). The experimental rats showed a mean increase of about 1.672^0C in rectal temperature, 18h after Brewer's yeast injection. The petroleum ether extract of leaf showed significant antipyretic activity ($P<0.05$) at 30 min. (38.72±0.15) and more significant antipyretic activity ($P<0.01$) at 90 min. (37.51±0.20) after drug administration. At an interval of 3hrs petroleum ether extract (37.16±0.14) of leaf showed significant activity whereas the ethanol extract (39.80±0.18) and aqueous extract (39.64±0.30) of leaf failed to exhibit this property. However, the standard drug paracetamol (37.31±0.22) treated animals showed more significant ($P<0.05$ and $P<0.01$) antipyretic activity throughout 3 hrs observation period.

Table I: Effect of leaf extracts of *Aegle marmelos* on carrageenan-induced rat paw edema

Group	Paw edema volume									
	30 (mins)		60 (mins)		90 (mins)		120 (mins)		180 (mins)	
	Mean±SEM	%PEI	Mean±SEM	%PEI	Mean±SEM	%PEI	Mean±SEM	%PEI	Mean±SEM	%PEI
Control	0.68 ±0.02	----	0.74 ±0.01	-----	0.86 ±0.02	-----	0.90 ±0.02	-----	0.92 ±0.02	----
Standard	0.42 ±0.01	38.3**	0.38 ±0.02	48.7**	0.36 ±0.01	56.7**	0.32 ±0.01	64.5**	0.24 ±0.01	74.0**
Petroleum ether extract	0.56 ±0.02	17.7	0.50 ±0.02	32.4*	0.44 ±0.02	48.9**	0.42 ±0.01	53.4**	0.39 ±0.01	57.7*
Ethanol extract	0.50 ±0.02	26.5*	0.48 ±0.02	35.2*	0.45 ±0.02	47.7**	0.42 ±0.01	53.2**	0.40±0.01	56.6*
Aqueous extract	0.66 ±0.01	3.0	0.70 ±0.02	5.5	0.78 ±0.01	9.4	0.80 ±0.02	11.2	0.80 ±0.02	13.1

n = 6 in each group, * P<0.05, ** P<0.01 compared to control

Table II: Effect of leaf extracts of *Aegle marmelos* on Brewer's yeast induced pyrexia in rats.

Group	Rectal temperature in ^{0}C						
	-18[a] (h)	0[b] (h)	30 (mins)	60 (mins)	90 (mins)	120 (mins)	180 (mins)
Control	36.80±0.20	39.40±0.20 (+0.78)[c]	39.62±0.14	39.85±0.11	39.97±0.13	40.25±0.37	40.32±0.41
Standard	37.10±0.18	38.93±0.30 (+1.83)[c]	38.36±0.24*	38.22±0.12**	37.44±0.15**	37.38±0.18*	37.31±0.22*
Petroleum ether extract	37.10±0.12	39.68±0.11 (+2.58)[c]	38.72±0.15*	38.46±0.13*	37.51±0.20**	37.33±0.14*	37.16±0.14*
Ethanol extract	36.92±0.14	38.66±0.20 (+1.74)[c]	39.25±0.35	39.33±0.20	39.68±0.22	39.73±0.35	39.80±0.18
Aqueous extract	37.16±0.10	38.59±0.15 (+1.43)[c]	38.94±0.37	39.20±0.18	39.36±0.15	39.52±0.24	39.64±0.30

n = 6 in each group, * P<0.05, ** P<0.01 compared to control

a: temperature before yeast injection.

b: temperature just before drug administration.

c: change in temperature following yeast injection.

Discussion

The present study establishes the antiinflammatory and antipyretic activity of petroleum ether extract of the leaves of *Aegle marmelos* and the ethanol extract antiinflammatory activity in the experimental models. Carrageenan-induced acute inflammation and Brewer's yeast induced pyrexia are the most suitable test procedures to screen antiinflammatory and antipyretic agents respectively. Development of carrageenan-induced edema is biphasic (23). Early phase of acute inflammation is due to release of histamine and serotonin stores in the cells and late response are due to stimulating effect on the synthesis of prostaglandins. Petroleum ether and ethanol extracts showed antiinflammatory activity throughout 3 hrs observation period. Hence it is possible that the antiinflammatory effect of leaf extracts of *Aegle marmelos* is due to its effect on synthesis of prostaglandins. However slow absorption from gastrointestinal tract or other factors which affect bioavailability, could not be ruled out and require further studies to know the exact mechanism of antiinflammatory activity of *Aegle marmelos.*

It is well known that most of the antiinflammatory drugs possess antipyretic activity. The petroleum ether extract of leaf showed significant antipyretic effect in rats. In general non-steroidal antiinflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus (24; 25). Although there is no direct evidence of *Aegle marmelos* to interfere with prostaglandin synthesis in hypothalamus but it can be supported by a related study in which *Dalbergia odorifera* extract was found to inhibit prostaglandin biosynthesis (26). Therefore it appears that antipyretic action of *Aegle marmelos* may be related to the inhibition of prostaglandin synthesis in hypothalamus.

Conclusion

In conclusion, the pharmacological activities of *Aegle marmelos* support its traditional use in folk medicine to reduce the inflammation and fever but further studies are needed to elucidate the exact mechanism by which the *Aegle marmelos* inhibits inflammation and fever.

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