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Analgesic, Antiinflammatory and Antiulcerogenic Activity of the Unripe Fruits of *Aegle marmelos*

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Abstract

Analgesic, antiinflammatory and antiulcer effects of the unripe fruits of 50% ethanol extract of *Aegle marmelos*, an Indian medicinal plant were examined. For analgesic activity, the methods involves the application of force to the paw of the mice was used using Analgesy-meter. Inflammation was induced by injecting 0.1ml of 1% λ carrageenan into the subplantar side of the left hind paw. Phenylbutazone ($100mgkg^{-1}$) was used as positive control. *Aegle marmelos* ($50\text{-}200mgkg^{-1}$) significantly ($P < 0.5$ to $P < 0.001$) increased the pain weight. The inflammation caused by λ carrageenan was reduced significantly ($P < 0.1$ to $P < 0.001$) at 3h equivalent to 37.14%-65.71% protection. *Aegle marmelos* ($50\text{-}200mgkg^{-1}$) showed a significant ($P < 0.1$ to $P < 0.001$) and dose dependent gastric ulcer protection (27.03% to 75.35%) in indomethacin induced ulcers. In acetic acid induced chronic ulcers, extract reduced the ulcer index significantly ($P < 0.5$ to $P < 0.05$) at 100 and $200mgkg^{-1}$ after 5 days treatment with decreased perforations at five and ten days treatment. The intensity of duodenal ulcers induced by cysteamine in control is 80%. *Aegle marmelos* (100 and $200mgkg^{-1}$) significantly ($P < 0.01$ to $P < 0.001$) reduced the severity and incidence of ulcers.

Key words: *Aegle marmelos*, Pain, Inflammation, Ulcer.

Introduction

Aegle marmelos (L.) Correa (Rutaceae), commonly known as Bael in Hindi and Bilva in Sanskrit. It is indigenous to India and is abundantly found in Himalayan tract, Bengal, Central and South India. It is one of the most important plants used in the indigenous system of medicine. The Ayurvedic practitioners use almost all of their parts but the greatest medicinal value ascribed to its fruits. Such studies appear to substantiate the clinical use of this plant in various gastrointestinal disfunctions and inflammation of the Ayurvedic system of medicine. The ripe fruit is aromatic, cooling, alternative, laxative and nutritive (Dikshit and Dutta, 1930). It is used as astringent and febrifuge and acts as a tonic for heart and brain. When taken fresh, it is valuable in habitual constipation, chronic dysentery and dyspepsia. It also relieves flatulent colic in patients suffering from a condition of chronic gastrointestinal catarrh (Nadkarni, 1954). Powder of the dried ripe pulp is used as febrifuge, anti-scorbutic, nauseant, stimulant and antipyretic (Satyavati *et al.*, 1976; Anonymous, 1985). In this study we aimed to provide more information on the unripe fruits of the plant by investigating some of its pharmacological effects related to its acclaimed medicinal uses.

Materials and Methods

Plant material: The plant materials used in this study, unripe fruits of *A. marmelos* were collected in the botanical garden of National Botanical Research Institute, India in July 2002.

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The plant material was identified and authenticated taxonomically by National Botanical Research Institute, Lucknow. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of extract: The outer hard shell was removed and the whole fruits were dried, powdered and passed through 10-mesh sieve. The coarsely powdered material (1000g) was first defatted with the n-hexane twice and then the marc was extracted thrice with ethanol (50% v/v) in the cold. The cold extracts were filtered, polled and first concentrated on rotavapour and then dried in "freeze dry system/Freezone® 4.5" at high vacuum and at temperature –40°C (yield 8.5% w/w). Preliminary qualitative phytochemical screening of unripe fruit gave the positive test for alkaloids, proteins, carbohydrates, and tannins (Anonymous, 1985). For the pharmacological tests the extract was suspended in double distilled water containing 1% carboxymethyl cellulose.

Animals: Sprague-Dawley rats (140-160g) and albino mice (18-24g) of either sex were purchased from the animal house of the Central Drug Research Institute, Lucknow, India. They were kept in departmental animal house in well cross ventilated room at 27 ± 2°C, and relative humidity 44-56%, light and dark cycles of 10 and 14h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India). Rearing of animals in the experimental period and their upkeep during the entire experimental span conformed to ethical guidelines laid down by Institutional Animal Ethics Committee (IAEC) of National Botanical Research Institute, India.

Analgesic activity: The analgesic effect of the extract was tested in mice of either sex using an Ugo Basile Analgesy meter (No. 32725) (21025 Comerio-varese, Italy) (Rodriguez, 1990). This method involves the application of force to the paw of the mice using the Analgesy-meter, which exerts a force that increases at a constant rate. The mice was gently placed between the plinth and plunger. The instrument was switched on and a constant motor rate was used to drive the plunger on to the paw of the mice. When the mice struggles, the instrument is switched off and the force at which the animal felt pain was read on a scale calibrated in grams X 10 by pointers. The pretreatment and the after treatment weight causing pain was determined for each mice. The extract was administered at a dose of 50, 100 and 200mgkg^{-1} respectively 30 minute before testing.

Antiinflammatory activity: The antiinflammatory activity was studied by using λ carrageenan induced edema in animal model (Winter *et al.*, 1962). Rats were injected with 0.1ml of 1% λ carrageenan into the subplantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and dipped in perspex cell up to this mark. The paw volume was measured with an Ugo Basile Plethysmometer (No: 61402) (7140 Comerio-varese, Italy) immediately and 3h after injecting the λ carrageenin suspension. The extract was administered at dose of 50, 100 and 200mgkg^{-1} respectively orally by gavage 1h before the λ carrageenin injection. Phenylbutazone (100mgkg^{-1}, p.o.) (Narayanan *et al.*, 1999) was used as a positive control.

Indomethacin induced acute ulcers: The ulcer was induced by the method of Urushidani *et al.*, (1979). The animals were fasted for 24h but had access of water. The extract was administered at dose levels of 50, 100, 200mgkg^{-1} and 1h later 20mgkg^{-1} of indomethacin as a 1% suspension in tween 80 was injected s.c. After 8h the animals were killed by cervical dislocation. The stomach of each rat was excised and cut along the greater curvature, washed carefully with 5.0ml of 0.9% NaCl and inflated on cork. The ulcers were examined with the help of a magnifying glass (10x) and scored by an observer unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was expressed by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows: 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1-2mm with more than two-thirds of the mucosal

thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and haemorrhage, muscularis still remaining unaffected. The curative ratio (%C) was determined as follows: %C = 100 – (Ulcer index treated X 100/ Ulcer index control) (Sairam *et al.*, 2001; Sanyal *et al.*, 1982).

Acetic acid induced chronic ulcers: The rats were anesthetized with pentobarbitone (35 $mgkg^{-1}$, i.p.). The abdomen was opened and the stomach was visualized. A cylindrical glass tube of 6mm in diameter was tightly placed upon the anterior serosal surface of the glandular portion of stomach 1cm away from the pyloric end. 50% acetic acid (0.06 $mlanimal^{-1}$) was instilled into the tube and allowed to remain 60 seconds on the gastric wall. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. Extract in dose of 100 and 200$mgkg^{-1}$, on day one p.o., 4h after the application of acetic acid and continued up to 5 or 10 days after induction of ulcers. The animals were then sacrificed after 18h of the last dose of drug either on 6th day or 11th day of experiment to access the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm^2rat^{-1}) of ulcer (Okabe *et al.*, 1971).

Cysteamine induced duodenal ulcers: The method described by Szabo (1978) was followed. Duodenal ulcers were induced by administrations of two doses of cysteamine hydrochloride, 400$mgkg^{-1}$, p.o. in 10% aqueous solution at an interval of 4h. Extract at dose levels of 100 and 200$mgkg^{-1}$ mg/kg, ranitidine (50 $mgkg^{-1}$, p.o.) were administered 30 minutes before each dose of cysteamine hydrochloride. All the animals were sacrificed 24h after the first dose of cysteamine and duodena were excised carefully and opened along the antimesentric side. The duodenal ulcers were scored for intensity, using a scale of 0 to 3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmular necrosis and 3 = perforated or penetrated ulcer (into the pancreas or liver).

Statistical analysis: All the data were presented as mean ± SEM and analyzed by Wilcoxon Sum Rank Test (Padmanabha pillai *et al.*, 1982) followed by unpaired Student's t-test for the possible significant interrelation between the various groups. A value of $P < 0.05$ was considered statistically significant.

Results

Analgesic activity: The experimental data of the present study indicate that the extract treated mice exhibited resistance against mechanical pain after 30 minutes. The weight that indicates the pain after the treatment was significantly increased ($P < 0.5$ to $P < 0.001$) dose dependently (Table1).

Table 1. Analgesic effect of *A.* marmelos extract in mice.

Treatment	Dose (mg/kg)	Weight causing pain (gm)	
		Before administration	After administration
A. marmelos	50	85.1 ± 5.9	91.3 ± 6.1
A. marmelos	100	82.5 ± 4.7	106.5 ± 5.4[a]
A. marmelos	200	84.5 ± 3.9	132.2 ± 7.2[b]
Phenylbutazone	100	81.4 ± 3.8	158.3 ± 8.3[b]

Values are mean ± SEM for six mice.
P: [a]<0.01 and [b]<0.001 compared to respective before administration group.

Antiinflammatory activity: Treatment with different doses of *A. marmelos* (50-200mgkg^{-1}) caused a significant ($P < 0.1$ to $P < 0.001$) and dose dependent inhibition of the swelling caused by the λ carrageenin at 3h equivalent to 37.14%-65.71% protection. Under same experimental conditions the antiinflammatory effect of phenylbutazone was 68.57% at the dose of 100mgkg^{-1} (Table 2).

Table 2. Effect of *A. marmelos* extract on λ carrageenan induced edema in rats.

Treatment	Dose (mg/kg)	Increase of paw volume after 3h	% Curative ratio
Control (1%CMC)	---	0.35 ± 0.03	---
A. marmelos	50	0.28 ± 0.02	37.14
A. marmelos	100	0.18 ± 0.02[a]	48.57
A. marmelos	200	0.12 ± 0.01[b]	65.71
Phenylbutazone	100	0.11 ± 0.02[b]	68.57

Values are mean ± SEM for six rats.
P: [a]<0.01 and [b]<0.001 compared to control group.

Indomethacin and acetic acid induced ulcers: The extract of *A. marmelos* showed a significant ($P < 0.1$ to $P < 0.001$) and dose dependent gastric ulcer protection (27.03% to 75.35%). The effect was similar to that produced by ranitidine and percent protection is 82.20 (Table 3).

Table 3. Effect of *A. marmelos* extract on indomethacin induced acute gastric ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer index	% Curative ratio
Control (1%CMC)	---	4.55 ± 0.50	---
A. marmelos	50	3.32 ± 0.22	27.03
A. marmelos	100	2.41 ± 0.02[a]	47.03
A. marmelos	200	1.12 ± 0.01[b]	75.38
Ranitidine	50	0.81 ± 0.01[b]	82.20

Values are mean ± SEM for six rats.
P: [a]<0.01 and [b]<0.001 compared to control group.

In chronic ulcer induced by 50% acetic acid, *A. marmelos* reduced ulcer index significantly at 100 and 200mgkg^{-1} after five days ($P < 0.5$ to $P < 0.05$) with decreased perforations observed after five and ten days treatment (Table 4).

Table 4. Effect of *A. marmelos* extract on 50% acetic acid induced chronic ulcer in rats.

Treatment	Dose ($mgkg^{-1}$)	Acetic acid induced ulcers (healing)			
		Five days treated		Ten days treated	
		Ulcer index	% Incidence of perforation	Ulcer index	% Incidence of perforation
Control (1%CMC)	---	27.8 ± 2.5	76.5	17.2 ± 2.3	35.6
A. marmelos	100	22.4 ± 2.1	43.1	8.2 ±1.0[b]	4.1
A. marmelos	200	15.3 ± 1.8[a]	31.2	1.1 ± 0.2[c]	0.0

Values are mean ± SEM for six rats.
P: [a]<0.05, [b]<0.01 and [c]<0.001 compared to respective control group.

Cysteamine induced duodenal ulcers: Cysteamine produced duodenal ulcers in 80% of the control rats. Usually two ulcers were produced close to the pylorus, the larger on the anterior and the smaller on the posterior wall of the duodenum. They were elongated extending longitudinally down the duodenum. Treatment with *A. marmelos* as (100 and 200$mgkg^{-1}$) produced a significant ($P < 0.01$ and $P < 0.001$) and dose dependent reduction in the severity and incidence of cysteamine induced duodenal ulcers. However, the H_2 receptor blocker ranitidine (50$mgkg^{-1}$) also produces a significant protective effect (Table 5).

Table 5. Effect of *A. marmelos* and ranitidine on cysteamine induced duodenal ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer incidence		Ulcer score	
		No	%	Total lesion area (mm^2)	% Protection
Control (1%CMC)	---	8/10	80	4.80 ± 0.37	---
A. marmelos	100	4/10	40	1.51 ± 0.31[a]	68.5
A. marmelos	200	2/10	20	0.47 ± 0.10[b]	90.2
Ranitidine	50	2/10	20	1.06 ± 0.17[c]	77.9

Values are mean ± SEM for ten rats.
P: [a]<0.05, [b]<0.01 and [c]<0.001 compared to respective control group.

Discussion

In order to provide a scientific explanation for the acclaimed medicinal uses of *A. marmelos*, we have investigated the various biological effects on the experimental animal models. Extract of *A. marmelos* contributes to its analgesic activity by the destruction of the mediators of inflammation such as 5-hydroxytryptamine, bradykinin, histamine, prostaglandins etc. A significant resistance against mechanical pain indicates the potent analgesic activity of *A. marmelos*. A potent anti-inflammatory activity was evidenced as the extract significantly antagonizes the λ carrageenan induced paw edema. The result obtained shows that the extract

possesses antiinflammatory activity, which may be consistent with its ability to inhibit prostaglandin synthesis (Di-Rosa *et al.*, 1971). Additionally, the extract of the *A. marmelos* exerted a significant protection against acute and chronic ulcers in rats. Synthetic NSAIDs like indomethacin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions (Rao *et al.*, 2000; 2001). Gastric ulcer is often a chronic disease and it may persist for 10 to 20 years characterized by repeated episode of healing and re-exacerbations. Acetic acid induced ulcer better resembles clinical ulcer in location, chronicity and severity and serves as the most reliable model to study healing process (Okabe and Pfeiffer, 1972). *A. marmelos* significantly healed the penetrating ulcers induced by acetic acid after 5 and 10 days treatment. Cysteamine ulcers are considered to be due to a long lasting hypersecretion of gastric acid (Szabo, 1978; Kirkegaard *et al.*, 1980), which may be due to partly to increased plasma level of gastrin (Lichtenberger *et al.*, 1977). In fact, hypersecretion of acid distributed gastric duodenal motility, hypergastrinaemia, and decreased mucosal resistance have all been implicated in the pathogenesis of cysteamine induced duodenal ulceration (Ishii *et al.*, 1976; Lichtenberger *et al.*, 1977a). The parallelisms between the antiulcer activity of *A. marmelos* against indomethacin, acetic acid induced gastric ulcers and cysteamine induced duodenal ulcers suggest that a number of predisposing factors are responsible for the beneficial effect. However, the role of the other defensive factors like bicarbonate, mucin secretion, glycoprotein, prostaglandins etc. stimulated by *A. marmelos* may not be ruled out. Moreover, the active compounds responsible for these pharmacological actions also remain to be identified. Thus, the results of the present study conformed the traditional claims of analgesic, anti-inflammatory and antiulcer activities of *A. marmelos.*

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References

Anonymous, (1985). Wealth of India: Raw materials, Vol.1A (Revised), Council of Scientific and Industrial Research Publication, CSIR, New Delhi, pp. 85-88.

Dikshit, B.B.L., Dutta, S. (1930). Preliminary chemical examination of *Aegle marmelos* or the Indian Bael. *J. Ind. Chem. Soc.* 7 : 759.

Di-Rosa, M., Giroud, J. P., Willoughby, D. A. (1971). Studies of the mediators of acute inflammatory response induced in rat in different site by carrageenan and turpentine. *J. Pathol.* 15 : 104.

Ishii, U., Fujii, Y., Homma, M. (1976). Gastric acid stimulating action of cysteamine in the rats. *Eur. J. Pharmacol.* 36 : 331-336.

Kirkegaard, P., Poulsen, S. S., Loud, F.B., Halse, C., Christiansen, J. (1980). Cysteamine induced duodenal ulcer and gastric secretion in the rat. *Scand. J. Gastroenterol.* 15 : 621-624.

Lichtenberger, L. M., Szabo, S., Reynolds, E. S. (1977a). Gastric emptying in rats is inhibited by duodenal ulcerogens, cysteamine and proprionitrile. *Gastroenterol.* 73 : 1072-1075.

Lichtenberger, L. M., Szabo, S., Trier, J. S. (1977). Duodenal ulcerogens, cysteamine and proprionitrile stimulate serum gastrin levels in the rat. *Gastroenterol.* 73 : 1304-1308.

Nadkarni, K.M. (1954). Indian Materia Medica, Vol.1, Ed.3, Karnataka Printing Press, Bombay, India. p. 45.

Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Vijayasekaran, V., Sukumar, E. (1999). Antinociceptive, anti-inflammatory and antipyretic effect of ethanol extract

of *Clerodendron serratum* roots in experimental animals. *J. Ethnopharmacol.* 65 : 237-241.

Okabe, S., Pfeiffer, C. J. (1972). Chronicity of acetic acid ulcer in the rat stomach. Digestive Diseases. 7 : 619-629.

Okabe, S., Roth, J. A., Pfeiffer, C. J. (1971). Differential healing periods of the acetic acid ulcer model in rats and cats. *Experientia* 27 : 146-148.

Padmanabha pillai, N., Ramaswamy, S., Gopalakrishinan, V., Ghosh, M. N. (1982). Effect of cholinergic drugs on acute and chronic morphine dependence. *Arch. Int. Pharmacodyn.* 257 : 147-154.

Rao, Ch, V., Sairam, K., Goel, R. K. (2000). Experimental evaluation of *Bacopa monniera* on rat gastric ulceration and secretion. *Indian J. Physicol. Pharmacol.* 44 : 435-439.

Rao, Ch. V., Sairam, K., Goel, R. K. (2001). Experimental evaluation of *Emblica officinalis* fruits in gastric ulceration and secretion. *Acta Pharm. Turcica* 43: 155-160.

Rodriguez alia, R. E. (1990). Antinociceptive activity of glycosidic enkephalin analogues. *Psychopharmacology.* 101 : 222-225.

Sairam, K., Rao, Ch, V., Goel, R. K. (2001). Effect of *Convolvulus pluricaulis* Choice on gastric ulceration and secretion in rats. *Indian J. Exp. Biol.* 39 : 350-354.

Sanyal, A. K., Panday, B. L., Goel, R. K. (1982). The effect of a traditional preparation of copper, tamrabhasma, on experimental ulcers and gastric secretion. *J. Ethnopharmacol.* 5: 79-89.

Satyavati, G. V., Raina, M. K., Sharma, M. (1976). Medicinal Plants of India, Vol.1, published by ICMR, New Delhi, p. 24.

Szabo, S. (1978). Animal model of human disease. Cysteamine induced acute and chronic duodenal ulcer in the rat. *Am. J. Pathol.* 93 : 311-323.

Urushidani, T., Kasuya, Y., Okabe, S. (1979). The mechanism of aggravation of indomethacin-induced gastric ulcers by adrenalectomy in the rat. *Jpn. J. Pharmacol.* 29 : 775-780.

Winter, C. A., Risley, E. A., Nuss, G. W. (1962). Carrageenan induced edema in hind paw of the rats as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111 : 544-547.

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