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In vivo anti-inflammatory activity of *Garcinia indica* fruit rind (Kokum) in rats

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Abstract

The aqueous extract of Garcinia indica fruit rind (GIE) was studied for anti-inflammatory activity in carrageenan induced paw edema and cotton pellet induced granuloma in rats. Wistar rats were orally administered GIE (400 mg/kg and 800 mg/kg) and the standard drug diclofenac sodium (10 mg/kg) 60 min prior to a subcutaneous injection of carrageenan (0.1 ml of 1% w/v) into their right hind paws to produce edema. The paw volumes were measured at various time intervals to assess the effect of drug treatment. In the granuloma model, 4 sterile cotton pellets were implanted in the ventral region in each rat. GIE (400 mg/kg and 800 mg/kg) and the standard drug diclofenac sodium (10 mg/kg) were administered orally for 8 days to the pellet implanted rats. The granuloma tissue formation was calculated from the dissected pellets and the activities of the marker enzymes AST, ALT and ALP were assayed from the serum. A significant reduction in paw edema and cotton pellet granuloma was observed with GIE treatment when compared with the carrageenan treated and cotton pellet implanted animals respectively. GIE treatment significantly attenuated the AST, ALT & ALP activities elevated by foreign body granulomas provoked in rats by the subcutaneous implantation of cotton pellets. It may be concluded that GIE possesses anti-inflammatory activity which may be due to an underlying antioxidant activity and/ or lysosomal membrane stabilization by virtue of its phenolic constituents.

Keywords: *Garcinia indica* fruit rind, Anti-inflammatory activity, Carrageenan, Cotton pellet.

Introduction

Inflammation is the body's defence reaction to injury in order to eliminate or limit the spread of injurious agents as well as remove consequent necrosed cells and tissues. It can be evoked by a wide variety of noxious agents such as infections, antibodies or physical injuries.

The main features of the inflammatory response are vasodilation, increased vascular permeability, and cellular infiltration by chemotaxis, granuloma formation and activation of cells of the immune system as well as of complex enzymatic systems of blood plasma.[1] The degree to which these occur is normally proportional to the severity of the injury and the extent of infection. During the different phases of inflammation, several mediators are released such as histamine, serotonin, chemotactic factors, bradykinins and prostaglandins which are responsible for vasodilation and increased capillary permeability due to alterations in the vascular endothelium, which leads to increased blood flow (hyperaemia) that causes redness (erythema) into the tissues.[2]

Garcinia indica Choisy (kokum) (Family: Guttiferae; Clusiaceae) is a slender evergreen tree endemic to the west coast of India. It has many culinary, pharmaceutical and industrial uses. The dried rind of the fruit of *Garcinia indica* known as "kokum" is an Indian spice and condiment used in many parts of the country for making several "curry" preparations. Syrup made from the fruits of *Garcinia indica* is a healthy soft drink used during summer to relieve sun stroke and gastric discomfort. Many therapeutic effects of the fruit have been described in Ayurveda, which include its usefulness as an infusion, in skin ailments such as rashes caused by allergies; in treatment of burns, scalds and chaffed skin; as a remedy for dysentery and mucous diarrhoea and a good liver tonic; as a cardiotonic and for bleeding, piles, dysentery, tumors and heart diseases.[3]

The major phytoconstituents present in *G. indica* are anthocyanins, fatty acids, hydroxycitric acid, garcinol, isogarcinol, citric acid and polyphenols.[4] Preclinical studies have shown that kokum and some of its phytoconstituents possess antibacterial, antifungal, cardioprotective, anticancer, chemopreventive, free radical scavenging, antioxidant, antiglycation and anti-obesity effects.[5-8]

Anthocyanins and polyphenols which are potent antioxidants have been evaluated for their anti-inflammatory activity.[9] *G. indica* fruits contain these active constituents. Therefore, the present study was designed to evaluate the anti-inflammatory activity of the *Garcinia indica* fruit rind.

Materials and methods

Plant material

The fruits of *G. indica* were collected from the Konkan region of Maharashtra, India, air dried under shade, powdered mechanically and stored in air tight containers. The powder was extracted using soxhlet apparatus and water as solvent and stored in a refrigerator for further use. The plant was authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai after matching with the existing specimen (accession no. 03587).

Drugs and chemicals

Carrageenan was purchased from Sigma Chemical Co., St Louis, MO, USA. Absorbent cotton wool was obtained from Jaycot industries, Hyderabad. All other chemicals were obtained from local sources and were of analytical grade.

Experimental animals

Wistar albino rats (180-200g) of either sex were used. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5 %), temperature (25±2°C) and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed, Pune, India) and water ad libitum. All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No.25/1999/CPCSEA) and conform to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

Acute toxicity study (ALD50)

Acute toxicity studies were carried out on Wistar rats by the oral route at different doses up to 2000 mg/kg of the aqueous extract of *Garcinia indica* fruit rind (GIE) as per the OECD guideline No.402.

Preparation of test and reference drug solutions

An aqueous solution of carrageenan (1% w/v) was prepared and used immediately. GIE was dissolved in distilled water and the aqueous solution was used for administration.

Diclofenac sodium was suspended in 1% (w/v) aqueous sodium carboxymethyl cellulose (CMC) solution and used immediately.

Anti-inflammatory activity

The effects of GIE were evaluated on carrageenan induced hind paw edema and cotton pellet granuloma models in rats. Diclofenac sodium was used as a standard drug in both the models for comparing the anti-inflammatory potential of GIE.

In vivo acute model of inflammation

Carrageenan induced hind paw edema in rat

Albino Wistar rats were randomly divided into four groups of 6 animals each and treated in the following way:

Group-1: Served as Toxicant control, which received orally 1ml/kg of 1% sodium CMC solution + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-2: Served as Standard and received diclofenac sodium 10 mg/kg orally (1 hr prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-3: Received GIE 400mg/kg orally (1 hr prior to carageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-4: Received GIE 800mg/kg orally (1 hr prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Groups 2, 3 & 4 received their respective drugs 1 h prior to the carrageenan injection. Carrageenan solution (0.1 ml) of 1% w/v was injected subcutaneously into the plantar region of the right hind paw of the rats of all groups to produce edema. Paw edema volumes were measured using plethysmometer (IITC 520) at various time intervals like 0, 1, 2, 3, 4, 6 & 24 hr after the carrageenan injection. The paw edema inhibition of the standard and test drugs was calculated by comparing with the Toxicant control group rats in the following way:

$$\% \text{ inhibition of paw edema} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc is the rat paw edema volume of the Toxicant control group; Vt is the rat paw edema volume of the drug treatment group.[10, 11]

In vivo chronic model of inflammation

Cotton pellet induced granuloma in rat

Albino Wistar rats were randomly divided into four groups of 6 animals each and treated in the following way:

Group-1: Served as Toxicant control, which received orally 1ml/kg of 1% sodium CMC solution daily for 8 days following subcutaneous implantation of cotton pellets.

Group-2: Termed as Standard and received diclofenac sodium 10mg/kg p.o. once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-3: Received GIE 400mg/kg once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-4: Received GIE 800mg/kg once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Four sterile cotton pellets (10 mg) were implanted subcutaneously in the ventral region 2 on either side, in each rat under light ether anesthesia. Groups 2, 3 and 4 received their respective drug treatments once daily for 8 days following implantation of cotton pellets. On the 9th day, the animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture. The cotton pellets along with granuloma tissue were removed and weighed immediately for wet weight. The pellets were dried in an oven at 60°C until a constant weight was obtained. The granuloma tissue formation and exudate formation was calculated using the following formulae:

Measure of granuloma tissue formation = constant dry weight – initial weight of pellet

Measure of exudate formation = wet weight of pellet - constant dry weight of pellet

The level of inhibition of granuloma tissue development was calculated using the expression:

$$\% \text{ inhibition of granuloma tissue formation} = \frac{WgrC-WgrT}{WgrC} \times 100$$

Where, WgrC = weight of granuloma tissue of the Toxicant control group; WgrT = weight of granuloma tissue of the treatment group

Blood collected by cardiac puncture was allowed to coagulate at room temperature for 30 min and serum was separated by centrifugation at 2500 rpm for 15 min. The separated serum was analyzed for the activities of the marker enzymes ALT, AST and ALP.

Marker enzyme assays

The lysosomal enzymes ALT, AST and ALP were assayed in serum using standard kits supplied from Accurex Biomedical Pvt Ltd (Mumbai, India). The results were expressed as IU/L.

Results

In vivo acute model of inflammation

Carrageenan induced hind paw edema in rat

In the acute anti-inflammatory model i.e. carrageenan induced hind paw edema in rats; carrageenan treatment caused an increase in paw volumes. Treatment with GIE at doses of 400 mg/kg and 800 mg/kg caused a significant inhibition of paw edema every hour up to 24 hours when

compared with the carrageenan treated group of rats. There was significant difference everywhere at all hours and all treatment groups. (Table 1)

Table 1: Effect of GIE in Carrageenan induced hind paw edema model in rats

Treatment group and dose (mg/kg)	Mean Edema volume (ml) Time interval in hours						
	0	**1**	**2**	**3**	**4**	**6**	**24**
Toxicant (Carrageenan) Control	0.88 ±0.006	1.24 ±0.005	1.36 ± 0.008	1.62 ± 0.01	1.59 ± 0.009	1.52 ± 0.006	1.20 ± 0.006
Standard Diclofenac sodium (10mg/kg)	0.86 ± 0.007 (2.27)	1.12 ± 0.006** (9.67)	1.18 ± 0.007** (13.23)	1.04 ± 0.008** (35.80)	0.97 ± 0.007** (38.99)	0.91 ± 0.006 ** (40.13)	0.84 ± 0.007** (30)
GIE (400mg/kg)	0.88 ± 0.006 (0)	1.18 ± 0.012* (4.83)	1.28 ± 0.009** (5.88)	1.16 ± 0.009** (28.39)	1.12 ± 0.006** (29.55)	1.02 ± 0.008 ** (32.89)	0.92 ± 0.006** (23.33)
GIE (800mg/kg)	0.86 ± 0.010 (2.27)	1.14 ± 0.006** (8.06)	1.20 ± 0.005** (11.76)	1.10 ± 0.007** (32.09)	1.05 ± 0.006** (33.96)	0.96 ± 0.008** (36.84)	0.87 ± 0.006** (27.5)

Values are mean ± SEM; N = 6 in each group.
One-way ANOVA followed by Dunnett's test is applied for statistical analysis
P values :**< 0.01, *< 0.05 when Drug treated groups were compared with the Toxicant control group. The values in the brackets indicate % inhibition

In vivo chronic model of inflammation

Cotton Pellet granuloma formation model in rats

In the cotton pellet induced granuloma, GIE 400 mg/kg as well as 800 mg/kg was found to be effective at the exudatory and granulatory phases of inflammation. Both, GIE 400 mg/kg and GIE 800 mg/kg were found to inhibit exudate formation by 27.43 and 52.25 % respectively and inhibit granuloma formation by 26.06 and 56.17% respectively. (Table 2)

Table 2: Effect of GIE on exudates and granuloma formation in cotton pellet granuloma

Treatment group and dose	Weight of exudate (in mg) (Wet - Dry)	Weight of granuloma (in mg) (Dry - Initial)
Toxicant Control (cotton pellet inserted)	98.56 ± 1.713	86.30 ± 1.473
Standard Diclofenac sodium (10mg/kg)	40.22 ± 1.571* (59.19)	31.49 ± 0.5174* (63.51)
GIE (400mg/kg)	71.52 ± 4.451* (27.43)	63.81 ± 2.289* (26.06)
GIE (800mg/kg)	47.06 ± 2.376 * (52.25)	37.82± 1.390* (56.17)

Values are mean ± SEM; N = 6 in each group
One-way ANOVA followed by Dunnett's test is applied for statistical analysis
P value :*< 0.01 when Drug treated groups were compared with the Toxicant control group; The values in the brackets indicate % inhibition

The effects of GIE on the serum marker enzymes-ALT, AST & ALP are summarized in Table 3. There was a marked increase in the serum activities of ALT, AST & ALP in the cotton pellet inserted group of animals. Treatments with GIE 400 mg/kg, 800 mg/kg as well as diclofenac sodium (10 mg/kg) significantly attenuated the ALT, AST & ALP activities elevated by foreign body granulomas provoked by the implanted pellets. GIE 800 mg/kg was comparable to the reference standard diclofenac sodium in reducing the elevated activities. GIE 800 mg/kg significantly attenuated the elevated activities of lysosomal SGOT, SGPT and ALP by 47.12, 58.31 and 55.93% respectively, while GIE 400 mg/kg showed 18.73, 28.88 and 25.98% attenuation when compared with the pellet inserted control group.

Table 3: Effect of GIE on biochemical parameters in cotton pellet granuloma

Treatment group and dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Toxicant Control	175.1 ± 3.67	95.47 ± 2.95	318.6 ± 5.78
Standard Diclofenac sodium (10mg/kg)	67.25 ± 1.30** (61.59)	33.49 ± 0.66** (64.92)	114.7 ± 2.47 ** (63.99)
GIE (400mg/kg)	142.3 ± 14.12* (18.73)	67.89 ± 2.80** (28.88)	235.8 ± 10.59** (25.98)
GIE (800mg/kg)	92.58 ± 1.31 ** (47.12)	39.8 ± 0.53** (58.31)	140.4 ± 4.50 ** (55.93)

Values are mean ± SEM; N = 6 in each group
One-way ANOVA followed by Dunnett's test is applied for statistical analysis
P values :**< 0.01, *< 0.05 when Drug treated groups were compared with the Toxicant control group.
The values in the brackets indicate % inhibition

Discussion

The aqueous extract of the fruit rind of *Garcinia indica* did not show any toxic or deleterious effects by oral route up to 2000 mg/kg indicating low toxicity of the fruit at high doses. The LD50 value could not be determined as no mortality was observed until a dose of 2000 mg/kg.

The results of the present investigation revealed that the fruit rind of *Garcinia indica* possesses a moderate anti-inflammatory effect that was evidenced by the significant reduction in paw edema and cotton pellet granuloma.
Most of the models used for evaluating anti-inflammatory activity in laboratory animals involve acute inflammation produced by injection of materials such as formalin, 5-hydroxytryptamine and dextran into the hind paw of rats. The material used in the present study to produce acute inflammation is carrageenan, which is a sulphated polysaccharide obtained from red green algae (Rhodophyceae). Carrageenan-induced rat paw oedema is a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw.[13]

Carrageenan induced rat paw edema has been described as a biphasic event in which various mediators operate in sequence to produce the inflammatory response.[14] Histamine, serotonin and bradykinins are the first detectable mediators in the early phase of carrageenan-induced inflammation; prostaglandins (PGs) are involved in the increased vascular permeability and are detectable in the late phase of inflammation.[15] Local and/or systemic inflammation is associated with enhanced levels of the pro-inflammatory cytokines TNF-α, IL-1, and IL-6. Kinins, once released, are able to activate B1 and/or B2 receptors, releasing other inflammatory mediators, such as prostaglandins (PGs), leukotrienes (LTs), histamine, nitric oxide (NO), platelet activating factor (PAF) and cytokines, among others derived mainly from leucocytes, mast cells, macrophages and endothelial cells, causing either cell influx and plasma extravasations. It has been reported that the second phase of edema is sensitive to most of the clinically effective anti-inflammatory drugs.[16] It is this phase which has been frequently used to access the anti-edematous effect of natural products. GIE showed dose dependent inhibition of paw edema in the first and the second phase. However the effect was more significant in the second phase and maximum inhibition was observed during the 3rd hour after carrageenan injection. The anti-inflammatory effect of GIE may be due to inhibition of kinin release and also inhibition of prostaglandin synthesis.

This activity probably will be due to its polyphenolic constituents.

The cotton pellet granuloma model has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation. There are three phases in the inflammatory response in the cotton pellet induced granuloma.[17] In the first phase imbibition of fluid containing low protein takes place at the site of cotton pellet implantation. In the second phase after 2-3 days of pellet implantation, exudation of fluid containing the protein takes place. In the 3rd phase, i.e. the proliferative phase, appearance of collagen, mucopolysaccharide synthesis, and increase in the number of fibroblasts around the cotton pellets occurs.[18] The amount of newly formed connective tissue can be measured after removing and weighing the dried pellets. GIE significantly decreased the final dry weight of the cotton pellets, i.e. it decreased the amount of granulomatous tissue, suggesting that it has the capability of reducing the synthesis of mucopolysaccharides and collagen and the number of fibroblasts, which are natural proliferative events of granulation in tissue formation. GIE decreased the weight of granuloma tissue in a dose-dependent manner, confirming its activity in the chronic phase of inflammation.

Lysosomal enzymes used to determine the degree of inflammation, include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP), which are altered during inflammation. During inflammation there is an increase in the serum level of these enzymes. This is because the chronic phase of inflammation involves damage to the lysosomal membrane.[19] As a result these lysosomal enzymes leak in to the blood stream. GIE attenuated the granuloma elevated serum ALT, AST and ALP activities which are increased during the inflammatory process. By stabilizing the lysosomal membrane, the anti-inflammatory drugs may cause interference with synthesis of lysosomal enzymes which participate in the process of inflammation.[20] The inhibition of lysosomal marker enzymes may be largely due to the membrane stabilizing property of GIE. GIE phytoconstituents, viz., anthocyanins and polyphenols are known to be potent antioxidants. It is likely that both, the antioxidant activity as well as a membrane stabilizing effect of these constituents might be contributing to the anti-inflammatory activity observed in different types of inflammation in this study.[21]

Conclusion

Thus, to summarize, the aqueous extract of *Garcinia indica* fruit rind showed significant anti-inflammatory activity probably by virtue of an underlying antioxidant activity and/ or lysosomal membrane stabilization.

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