Research Article Available online through
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EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF *GARCINIA INDICA* FRUIT RIND EXTRACTS IN WISTAR RATS

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Received: 05-10-2010; Revised: 06-11-2010; Accepted: 13-11-2010

ABSTRACT
Garcinia indica choisy (Kokum) is known for its food, medicinal and commercial values. The present study was carried out to evaluate the effect of aqueous and ethanolic extract of *Garcinia indica* fruit rind (GIFR) for its anti inflammatory activity in rats. The inflammation was induced by carrageenan induced paw odema. The serum enzymes like Acid phoshatase(ACP) and Alkaline Phosphatase(ALP) were estimated. Both extracts at dose (200 & 400 mg/kg p.o single dose) shows significant (P<0.001) anti inflammatory activity in (Carrageenan induced paw odema) acute inflammation. The extracts treatment also showed significant (p<0.001) reduction in the levels of serum enzymes ACP & ALP. Similar results were obtained from aspirin (200mg/kg) treated group. The result obtained from the present study indicates both aqueous and ethanolic extracts possessing anti inflammatory activity and further study required to establish its mechanism of action.

KEYWORDS: *Garcinia indica*, anti inflammatory, carrageenan, Acid phosphatase, Alkaline Phosphatase.

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INTRODUCTION

Inflammation is a local protective response of the body to the tissue injury. Research in the last few decades demonstrated that inflammation is regulated by many pro and anti- inflammatory chemical mediators such a histamine, prostaglandins (PGE_2 & Prostacyclins), leukotrienes (LTB_4), serotonin, bradykinin, cytokines(IL-I, IL-6, IL-8, IL-11, TNF-α),reactive oxygen species, growth factors, lysosomal enzymes of neutrophils. The extent of involvement of these chemical mediators varies depending upon the nature of inflammation.[1, 2]

Garcinia indica choisy is commonly known as kokum, family (Clusiaceae) known for its food, medicinal and commercial values. The plant has got many medicinal activities like cardiotonic, cooling, emollient, demulcent, improve peristalsis, anthelmentic and antitumor. The major phytoconstituents present in the *Garcinia indica* are anthocynines, fatty acids (Palmitic, Steric, Oleic and linoleic acid), Hydroxycitric acid (HCA), Garcinol and Isogarcinol, citric acid and polyphenols.[3,4.]

Anthocynins, polyphenols and antioxidants have been evaluated for its anti inflammatory activity. Plants containing these phytoconstiuents showed anti inflammatory activity and reported in the literature e.g. *Ocimum sanctum*, *Pongamia pinnata*, *Michelia champaca*, Garlic etc.[5, 6] *Garcinia indica* fruit contains these active constituents. Therefore the present study was designed to evaluate anti-inflammatory activity in carrageenan induced paw edema in male Wistar rats.

MATERIAL METHODS

Collection and authentification of plant

The fruits of *Garcinia indi*ca were collected from the Sawantwadi, Maharashtra. The fruits were identified and authenticated by Dr. Harsha Hegde research Officer Regional Medical Research Centre Belgaum, where herbarium of the plant deposited.

Preparation of extracts

Fruits of *Garcinia indica* were shed dried, separated from the seed and pulverized to coarse powder. The powder material was passed through a NO.40 sieve to get uniform powder, which is then defatted by petroleum ether (40-80 ^{0}C) and filtered and subjected to solvent extraction by ethanol and water.

i) **Preparation of aqueous extract**

About 100g of dried defatted fruit rind powder was macerated with chloroform water IP for seven day at room temperature and extract was filtered, concentrated on rotary evaporator and dried in desicator over sodium sulphite.

ii) **Preparation of ethanolic extract**

About 100g of dried defatted fruit rind powder was taken in shoxlet extractor and extracted with 95% ethanol v/v at 60 ^{0}C. Appearance of colourless solvent in a siphen tube was taken as termination of extraction process. The extract was concentrated on rotary evaporator and dried in desicator over sodium sulphite. The resulting extract was stored at 4^{0}C, used for the activity.

Animals

Healthy male Wistar rats procured from Shri. Venkatesh Enterprises Bangalore, for the study. They were housed in polypropylene cages at room temperature with 12/12 h light dark cycle, free accesses to standard animal diet (Amrut laboratory animal feed, Sangali, Maharashtra) and clean drinking water *ad labium.*

The present study was dully approved by IAEC Reg. NO. 627/02/a/CPCSEA JN Medical College Belgaum.

Acute toxicity study

Acute toxicity study was carried out by using adult Male Wistar rats by “fixed dose” method of OECD (Organization for economic co-operative and development) guideline NO.420. Starting dose of 2000 mg/kg body weight was adopted. There was no toxic effects and mortality observed up to 14th days.

Selection of dose

The LD_{50} cut off value found to be 2000 mg/kg. For the assessment of anti-inflammatory activity two dose levels were selected i.e. first dose is one-tenth of LD_{50} cut off value and second dose was twice that off one-tenth dose (200mg/kg & 400mg/kg p.o single dose).

Anti-inflammatory study

Animals and experimental protocol

Male Wistar rats weighing 150-180 gm were taken acclimatized to normal laboratory conditions with 12 hours natural light dark cycle and were maintained on standard laboratory diet with free access to water *ad labium.*

Grouping Of animals

Group I: Normal control (normal saline 2ml/kg treated)
Group II: Treated with Aspirin 200mg/kg.
Group III: Treated with aqueous extract of GIFR 200mg/kg
Group VI: Treated with aqueous extract of GIFR 400mg/kg.
Group V: Treated with ethanolic extract of GIFR 200mg/kg
Group VI: Treated with ethanolic extract of GIFR 400mg/kg

Acute anti-inflammatory study

Carrageenan induced paw edema

The experiment was used to check the anti inflammatory activity of the extract by the method of Winter *et al* (1962). The overnight fasted (with water *ad labium*) animals were divided in to control and treatment groups. The control group I received normal saline 2ml/kg and five treatment groups received the dose of Aspirin 200mg/kg aqueous GIFR extract 200mg/kg & 400mg/kg, ethanolic GIFR 200mg/kg & 400mg/kg p.o single dose respectively. The animals pretreated with normal saline, extracts and standard drug aspirin half an hour before were injected with 0.1ml of 1% carragenan in to subplantar region of right hind paw. A mark was put on the leg at the malleolus to facilited the dipping of the leg to same level at the second and subsequent times. The paw volume was measured with the help of Plethysmograph (UGO Basile, Italy) by mercury displacement method at zero min, 30min, 1,2,3,4 and 5 hours. Reduction in the paw volume compared with the saline treated normal control animals was considered as anti-inflammatory activity.

Biochemical estimation

Biochemical estimation is carried out after 5 hour in carrageenan induced paw oedma acute study model. The animals were anaesthetized under light ether anesthesia and blood samples were collected by retro orbital plexus puncture, serum was separated after coagulating the blood at 37^0C for 30 minutes and centrifuge at 1200-1500 rpm for 15-20 minutes. Serum was analyzed for Acid phosphatase and alkaline phosphatase enzymes. The procedures were followed described in the literature supplied by the Calkine Acid Phosphatase Kit (Crest Biosystems), Erba Mannheim Alkaline Phosphatase Kit. Estimation was made on Auto-analyzer. Reitman's and Frankel method.(1957).[7-9]

Statistical analysis

Values are expressed as Mean ± SE. The data were analyzed by one way Analysis of variance (ANOVA) followed by Dennett's test. The results were consider statistically significant when $P<0.001$.

RESULTS

Aqueous and ethanolic extracts of GIFR were investigated for their possible anti inflammatory activity in acute experimental animal models. The results are summarized in (**table 1&2**).

Both aqueous and ethanolic extracts of GIFR at dose of 200mg & 400mg/kg showed dose dependent anti inflammatory activity on carrageenan induced paw edema in rats. The potential anti inflammatory activity of both extracts was comparatively same as that of aspirin as standard drug (200mg/kg).

The results showed that GIFR aqueous extract at 200 and 400mg/kg significantly ($p<0.001$) reduced the paw edema volume when compared with control group, similarly GIFR ethanolic extract at 200 & 400mg/kg showed significant ($p<0.001$) reduction in carrageenan induced paw odema volume after 1, 2, 3, 4, & 5th hours. Aspirin at dose of 200mg/kg showed significant ($p<0.001$) reduction as expected.

DISCUSSION

Inflammation is a universal host defense response involving complex network of cell-cell, cell-mediators and tissue interactions. Inflammation is regulated by many pro and anti-inflammatory chemical mediators viz. Histamine, Prostaglandins, leukotriens(LTB_4) Serotonin, Bradykinin, Cytokines (IL-1, IL-6,IL-8,IL-11 & TNFα), reactive oxygen species(ROS), growth factors and lysosomal content of neutrophils.[10,11]Many anti-inflammatory agents acting through by inhibiting one or other steps involved in the synthesis of inflammogen e.g NSAIDS inhibits cycloxygenase enzymes play vital role in the synthesis of prostaglandins that causes inflammation, Corticosteroids by inhibiting synthesis of leukotrines and prostaglandins.[12,13.]

Carrageenan induced paw edema in acute inflammation attribute biphasic episodes incorporate of different inflammatory chemical mediators. The formation of edema is due to the release of histamine, serotonin and bradykinin after between first & second hours of carrageenan injection followed by synthesis of prostaglandin, protease and lysosomes up to sixth hour around the damage tissues.[14,15]

The significant inhibitory activity of GIRF on carrageenan induced paw edema at first hour indicates that the GIRF involve the inhibition of histamine and serotonin release. Since GIRF showed significant inhibitory effect up to fifth hour, this suggests that it may inhibit the synthesis or release of prostaglandins, protease and lysozymes.. The finding suggest that both aqueous and alcoholic extracts of GIFR contains active phytoconstituents which showed anti-inflammatory action by inhibiting either synthesis, release or inflammatory action of mediators such as histamine, serotonin, prostaglandins, bradykinin and lysozyme.

Lysosomal enzymes like non prostatic ACP and ALP are known to be increased in inflammation and serve as markers of inflammation. Levels of these enzymes change with the type of inflammation. Treatment with both the extracts showed significant decreased in the levels.[16]

The presence of flavonoids, tannins, phenolic compounds and citric acid may be responsible for anti-inflammatory activity. Recent findings revealed that Garcinol inhibited the activity of 5-lipoxygenase and blocked the mPGES-1-mediated conversion of PGH2 to PGE2 and interfered with isolated COX-1 is one of the possible key mechanism of its anti-inflammatory activity. It appears that different phytoconstituents of GIFR contribute towards its anti-inflammatory effect.[3, 17-19]

CONCLUSION

In conclusion, result of the present study indicates that both aqueous and ethanolic extracts of GIFR are effective when compared with Aspirin in reducing the inflammation. It is difficult to establish the mechanism of action from the present findings. However further study is required to evaluate its anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors are thankful to the Principal Dr. F.V. Manvi and Vice Principal Prof. A. D. Taranalli, K L E College of pharmacy Belgaum India, for the support and constant encouragement.

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Table 1: Effect of Various Treatments on ACP & ALP Levels

Sr. No.	Groups	ACP (IU/L)	ALP (IU/L)
1	Control (Normal Saline)	3.50±0.0365	810±1.95
2	Aspirin (200mg/kg)	2.82±0.0307[a*]	638±2.38[a*]
3	GIFR aqeeous (200mg/kg)	2.65±0.0224[b*]	613±3.65[b*]
4	GIFR aqueous (400mg/kg)	2.77±0.0211[b*]	475±2.67[b*]
5	GIFR ethanolic (200mg/kg)	2.52±0.0307[b*]	427±3.10[b*]
6	GIFR ethanolic (400mg/kg)	2.62±0.0307[b*]	326±2.49[b*]

Each value is the mean ± SEM of n=6. [a] standard compared with control. [b] All G.I. AqE and AlcE groups compared with control. [*] indicates P<0.001

Table 2: Effect of Various treatments on Carrageenan Induced Rat Paw Edema

Groups & Dose of drug	Edema volume in ml						
	0 hr	30 min	1hr	2hr	3hr	4hr	5hr
Control (2ml/kg Normal Saline)	0.100 ±0.0258	0.483 ±0.101	2.55 ±0.198	2.97 ±0.150	2.65 ±0.138	2.08 ±0.147	1.72 ±0.125
Aspirin (200mg/k)	0.107 ±0.0558	0.583 ±0.0872	1.90 ±0.0516[a*] (25.49%)	1.43 ±0.113[a*] (51.8%)	1.15 ±0.120[a*] (56.60 %)	1.12 ±0.133[a*] (46.15%)	0.767 ±0.133[a*] (55.40%)
GIFR aqueous extract (200mg/k)	0.120 ±0.0224	0.600 ±0.0683	1.63 ±0.0615[b*] (36.07%)	1.92 ±0.0543[b*] (35.35%)	1.45 ±0.175[b*] (45.28%)	0.883 ±0.0703[b*] (57.54%)	0.880 ±0.0671[b*] (48.83%)
GIFR aquous extract (400mg/k)	0.117 ±0.0211	1.70 ±0.0856	1.53 ±0.0715[b*] (40.00%)	0.92 ±0.105[b*] (69.02%)	0.85 ±0.0577[b*] (67.92%)	0.683 ±0.0654[b*] (67.16%)	0.617 ±0.0543[b*] (64.12%)
GIFR ethanolic extract (200mg/k)	0.1120 ±0.0	0.783 ±0.0401	1.78 ±0.122[b*] (30.19%)	1.12 ±0.117[b*] (62.28%)	1.19 ±0.0922[b*] (55.09%)	1.02 ±0.0749[b*] (50.96%)	0.713 ±0.0619[b*] (58.56%)
GIFR ethanolic extract (400mg/kg)	0.106±0.0	0.817 ±0.0477	1.25 ±0.115[b*] (50.98%)	0.98 ±0.115[b*] (67.00%)	1.10 ±0.109[b*] (58.49%)	0.989 ±0.0601[b*] (52.45%)	0.997 ±0.0516[b*] (42.03%)

Each value is the mean ± SEM of n=6 , [a]- standard compared with control. , [b]- All GIFR AqE and ethanolic groups compared with control. [*] indicates p<0.001

Source of support: Nil, Conflict of interest: None Declared