Research Paper

Analgesic Activity of Traditionally used Fruit Aqueous Extracts of *Garcinia indica*

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Pain is becoming an increasingly serious problem in the world. *Garcinia indica* Choisy (Clusiaceae) fruits, commonly known as 'Kokum' are widely used in Ayurveda to relieve pain. This study aimed to evaluate the analgesic activity of *Garcinia indica* fruit rind extract in experimental rats. The aqueous extract of shade-dried fruit rind of *Garcinia indica* was used for the study. The hot plate method was used to evaluate centrally mediated analgesic activity while peripheral analgesic activity was tested by sodium chloride-induced writhing test in rats. To assess the analgesic activity, hot-plate latent pain response, tail clip, tail immersion and sodium chloride-induced writhing tests were used in the experimental rat model at the doses of 250 and 500 mg/kg of *Garcinia indica* fruit rind extract were used. In the hot plate and tail clip, *Garcinia indica* fruit rind extract at doses 250 and 500 mg/kg showed a significant ($p<0.001$) dose-dependent increase in the response latency when compared to the control animals and at 180 min when compared to animals treated with standard drug tramadol. *Garcinia indica* fruit rind extract at 250 and 500 mg/kg showed no significant prolongation of the tail withdrawal time in rats submitted to the tail immersion test. In the sodium chloride-induced writhing response, *Garcinia indica* fruit rind extract at doses of 250 and 500 mg/kg body weight, produced a significant ($p<0.001$) dose-dependent decrease in the number of writhes when compared to the untreated group. *Garcinia indica* fruit rind extract at both doses caused a significant ($p<0.01$) reduction in the number of writhes at 60 min compared to animals treated with the standard drug aspirin. In conclusion, the analgesic effects of *Garcinia indica* fruit rind extract are probably mediated by both cerebral and peripheral inhibitory mechanisms. The findings also support the traditional usage of *Garcinia indica* fruit in pain relief.

Key words: *Garcinia indica*, fruit aqueous extract, analgesic activity

A painful sensory and emotional experience that is linked to or characterized by existing or prospective tissue damage is known as pain[1] which is globally becoming a greater problem[2]. An estimated 20 % of adults worldwide are suffering from pain and each year, 10 % of cases of chronic pain are newly diagnosed[2]. Neuropathic pains can result from damage to the neurons in the peripheral and central nervous systems caused by a variety of disorders[3]. A wide range of naturally occurring compounds found in plants could serve as a basis for the creation of novel pharmaceuticals. Analgesic and anti-inflammatory properties of numerous therapeutic plants have long been utilized by traditional healers[2]. *Garcinia indica* Choisy (Clusiaceae) (*G. indica*) commonly known as 'Kokum', is one of the 200 species in the genus *Garcinia* found in the Afro-Asian countries and about 30 species are found in India[4]. The fruit is widely utilized in Indian tradition to treat pain, rheumatism, edema and cardiac issues[5-8]. The main compound garcinol (camboginol), a tri-isoprenylated chalcone has been reported in *G. indica* and other species for antioxidant activity[9]. Other components of *G. indica*, such as isoliquiritigenin and chalcone, relieve rheumatic and other kinds of pain. The anti-inflammatory actions of garcinol are based on its strong potency in specifically

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reducing Prostaglandin E_2 (PGE_2) synthesis and 5-lipoxygenase product generation. This rationalizes its usage as a therapeutic agent[10]. The aqueous and ethanol extracts of *G. indica* fruit rind has shown anti-inflammatory activity comparable to that of aspirin when evaluated using the carrageen-induced paw edema mode[11]. However, no scientific information addressing the analgesic effects of *G. indica* fruits has been documented. The present study evaluates *G. indica* aqueous fruit rind extract for its role in centrally and peripherally mediated analgesia.

MATERIALS AND METHODS

Collection and identification of plant material:

The fruits of *G. indica* were collected from the local forest of Sinquerim located in Goa, India. The fruits were identified and authenticated at the Department of Ethnobotany and Plant Biotechnology, ICMR-National Institute of Traditional Medicine, Belagavi, where a voucher specimen (RMRC-518) was deposited.

Preparation of extract:

The fruits of *G. indica* were shade dried, rind separated from the seeds and pulverized into a coarse powder. The powder was macerated in water with a ratio of 1:5 (drug:water) using 5 % chloroform as a preservative. The maceration was shaken at regular intervals, for 1 w. The macerated extract was filtered using a muslin cloth and the filtrate was evaporated in a temperature-controlled water bath. The dried aqueous *G. indica* Fruit Rind Extract (GIFRE) was kept in an airtight container and stored at 4° until further use.

Experimental animals:

Healthy adult Wistar rats (180-250 g body weight (b.wt)) were procured from Shri Venkateshwara Enterprises, Bangalore. The animals were housed in groups of six animals each in polypropylene cages at room temperature with a 12 h light/dark cycle. The animals were given standard animal feed (Amrut Enterprises, Sangli, Maharashtra) and clean drinking water *ad libitum*. The animals were acclimatized to normal laboratory conditions for a week before the initiation of the experiment. All the tests were carried out on overnight fasted animals. The experiment was conducted prior as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The present study was duly reviewed and approved by the Institutional Animal Ethics Committee of KLEU's College of Pharmacy, Belagavi (IAEC Reg No. CPCSEA/221).

Drugs and chemicals:

Tramadol hydrochloride (Wockhardt Ltd.) and aspirin were used as the standard drugs. Tramadol was purchased from the local pharmacy and aspirin was obtained as a gift sample from Shreya Life Sciences, Aurangabad. Sodium chloride (Rankem) and sodium CMC (HiMedia Labs) used were of analytical grade.

Selection of dose:

The median Lethal Dose (LD_{50}) value of the aqueous extract has been reported to be >2000 mg/kg[11]. For the assessment of analgesic activity, 250 and 500 mg/kg doses of GIFRE were selected.

Grouping and dosing of animal:

The animals were divided into four groups (six animals each). Group I was assigned as negative control and received the vehicles, normal saline 2 ml/kg post operation (p.o.). Group II was served as positive control and treated with standard drugs; tramadol 10 mg/kg p.o., for the hot plate latent pain response test, Haffner's tail immersion test and tail immersion test; while aspirin at a dose of 10 mg/kg p.o., for sodium chloride-induced writhing response. group III and Group IV were used as test groups and received GIFRE 250 mg/kg p.o., and 500 mg/kg p.o., respectively.

Evaluation of analgesic activities of the fruit extract:

Hot-plate latent pain response test: The hot-plate latent pain response test was performed as per the method described by Vogel *et al*.[12]. Briefly, a temperature-controlled hot plate was used at 55°-56°. The animals were placed individually on the hot plate and the time until either licking or jumping occurred was recorded by a stopwatch. The latency was recorded after 30, 60, 90 and 180 min following administration of the standard and test drugs.

Tail clip test:

The tail clip test was performed as per the method described earlier[12,13]. An artery clip was applied to the root of the tail of the rats and the reaction

time (attempt to bite the tail or remove the clip) was noted after 30, 60, 90 and 180 min following administration of the standard and test drugs. The time between stimulation of onset and response was measured by a stopwatch in 1/10 second increments.

Haffner's tail immersion test:

To distinguish central and peripherally acting analgesics, the tail immersion test was carried out[12]. The animals were placed into individual restraining cages leaving the tail hanging out freely. The tail was marked at a lower 5 cm portion and immersed in a cupful of water of exactly 55°. Within a few seconds, the rat reacted by withdrawing its tail. This reaction time was recorded by a stopwatch after 30, 60, 90 and 180 min following administration of the standard and test drugs.

Sodium chloride-induced writhing response:

Peripheral analgesic activity was evaluated by sodium chloride-induced writhing in rats[9]. The method of Fukawa *et al*.[14] was used with modifications. To induce writhes, 1 ml/kg of 8 % sodium chloride was injected intra-peritoneally in rats and the number of writhes was recorded for each animal, after 30, 60 and 120 min following administration of the standard and test drugs. Writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Statistical analysis:

The data were analyzed using two-way Analysis of Varience (ANOVA) followed by Bonferroni's Multiple Comparison Test (Graph Pad Prism software, version 5.01) and expressed as mean±standard error of mean. The $p \leq 0.005$ was considered statistically significant.

RESULTS AND DISCUSSION

GIFRE at 250 and 500 mg/kg produced a dose-dependent increase in the latency period of pain induced by the heating plate (Table 1). GIFRE at the dose 250 and 500 mg/kg and tramadol 10 mg/kg significantly ($p<0.001$) prolonged the time taken for the pain response at 30, 60, 90, 120 and 180 minutes compared to the control. Both the doses (250 and 500 mg/kg) produced a significant ($p<0.001$) increase in the reaction time at 180 min, compared to tramadol. The reaction time steadily increased with the peak effect seen at 3 h for both doses.

GIFRE at the dose 250 and 500 mg/kg significantly ($p<0.001$) increased the latency period at 90, 120 and 180 min compared with control group while, tramadol (10 mg/kg) significantly ($p<0.001$) increase the latency period 30, 60, 90, 120 and 180 minutes compared to the control. GIFRE at 250 and 500 mg/kg produced a dose-dependent increase in the latency period of pain produced by applying an artery clip to the tail of the rat (Table 2). Treatment at doses of 250 and 500 mg/kg of GIFRE produced a gradual increase in response latency, with a significant ($p<0.001$) increase in the response time at 180 min compared to tramadol. Whereas tramadol administration produced a significant ($p<0.001$) increase in the response latency starting at 30 min and with maximum response latency seen at 120 min.

GIFRE at 250 and 500 mg/kg doses showed no significant prolongation of the tail withdrawal time in rats submitted to the tail immersion test (Table 3). Standard drug tramadol (10 mg/kg), however, showed a significant ($p<0.001$) increase in the tail withdrawal time at 90, 120 and 180 min.

GIFRE at 250 and 500 mg/kg and aspirin at 10 mg/kg significantly ($p<0.001$) suppressed the 8 % sodium chloride-induced writhing response compared to the control group (Table 4). GIFRE at 250 and 500 mg/kg significantly ($p<0.01$) reduced writhing response at 60 min compared to aspirin at a dose of 10 mg/kg treated group. Interestingly, GIFRE at 250 mg/kg produced a greater percentage inhibition of writhing at all the time intervals compared to the 500 mg/kg dose, but this was not statistically significant. Standard drug aspirin produced increasing inhibition of writhing, with maximum inhibition seen at 2 h.

The analgesic activity of GIFRE was evaluated by different experimental models of pain *viz*., narcotic models like a hot plate, tail clip tests and a non-narcotic model like 8 % sodium chloride-induced writhing behavior possess analgesic activity both centrally as well as peripherally. The hot-plate and tail-clip tests are useful in elucidating centrally mediated analgesic responses, focusing mainly on changes above the spinal cord level. The painful experience is short-lasting and it is well accepted that agonists of opioid receptors play a major

role in mediating analgesia in acute pain models. The hot plate test is considered to be selective for opioid-like compounds, which are centrally-acting analgesics in several animal species[15]. Prolongation of the reaction time in the hot plate test indicates the involvement of supraspinal mechanisms[16]. Treatment with GIFRE showed significant analgesic activity in the hot plate and tail clip tests that may in part, be mediated by opioid receptors. These findings indicate that GIFRE may contain opioid-like compounds that are responsible for the analgesic activity.

TABLE 1: EFFECT OF GIFRE TREATMENTS ON THE HOT-PLATE TEST

Group	Response latency					
	0 min	30 min	60 min	90 min	120 min	180 min
Group I (2 ml/kg)	2.16±0.06	2.1±0.07	2.18±0.06	2.180±0.08	2.17±0.07	2.19±0.08
Group II (10 mg/kg)	2.17±0.08	5.23±0.29$^{###}$	10.27±0.16$^{###}$	15.23±0.22$^{###}$	14.48±0.18$^{###}$	12.42±0.16$^{###}$
Group III (250 mg/kg)	2.13±0.09	4.31±0.14^{a}	8.37±0.18^{a}	10.26±0.15^{a}	13.3±0.19^{a}	14.22±0.30^{a},***
Group IV (500 mg/kg)	2.18±0.07	4.91±0.23^{a}	9.63±0.19^{a}	12.16±0.22^{a}	15.07±0.11^{a}	16.1±0.15^{a},***

Note: $^{###}$p<0.001 as compared to group I; ^{a}p<0.001 as compared to group I and ***p<0.001 as compared to group II

TABLE 2: EFFECT OF GIFRE TREATMENTS ON HAFFNER'S TAIL CLIP TEST

Group	Response latency					
	0 min	30 min	60 min	90 min	120 min	180 min
Group I (2 ml/kg)	5.07±0.05	5.02±0.06	5.13±0.05	5.04±0.08	5.12±0.05	5.08±0.06
Group II (10 mg/kg)	5.03±0.06	6.28±0.18$^{###}$	8.08±0.27$^{###}$	11.7±0.2$^{###}$	11.06±0.26$^{###}$	10.15±0.24$^{###}$
Group III (250 mg/kg)	5.06±0.11	5.2±0.07	6.15±0.08	8.05±0.21^{b}	9.98±0.10^{b}	11.00±0.08^{b},**
Group IV (500 mg/kg)	4.97±0.12	5.36±0.13	6.74±0.18	8.26±0.19^{b}	10.87±0.35^{b}	11.63±0.33^{b},***

Note: $^{###}$p<0.001 as compared to group I; ^{b}p<0.001 as compared to group I; **p<0.01 and ***p<0.001 as compared to group II

TABLE 3: EFFECT OF GIFRE TREATMENTS ON THE TAIL IMMERSION TEST

Group	Response latency					
	0 min	30 min	60 min	90 min	120 min	180 min
Group I (2 ml/kg)	1.32±0.10	1.30±0.11	1.32±0.09	1.40±0.14	1.43±0.16	1.40±0.13
Group II (10 mg/kg)	1.3±0.07	1.75±0.11	1.76±0.14	3±0.30$^{###}$	3.52±0.33$^{###}$	2.43±0.21$^{###}$
Group III (250 mg/kg)	1.38±0.12	1.4±0.13	1.58±0.13	2.19±0.29	2.06±0.27	1.8±0.12
Group IV (500 mg/kg)	1.42±0.15	1.51±0.17	1.57±0.18	2.05±0.19	2.07±0.14	1.78±0.07

Note: $^{###}$p<0.001 as compared to group I

TABLE 4: EFFECT OF GIFRE TREATMENTS ON 8 % SODIUM CHLORIDE-INDUCED WRITHING RESPONSE

Group	No. of writhes			% Inhibition of writhing		
	30 min	60 min	120 min	30 min	60 min	120 min
Group I (2 ml/kg)	9.17±0.60	9±0.73	9.17±0.60	-	-	-
Group II (10 mg/kg)	5±0.97$^{###}$	5.33±0.71$^{###}$	1.67±0.21$^{###}$	45.45653	41.82393	81.8152
Group III (250 mg/kg)	3.17±0.40^{c}	2.33±0.33^{c},**	1.33±0.49^{c}	65.45217	74.55002	85.4587
Group IV (500 mg/kg)	3.5±0.76^{c}	2.5±0.56^{c},**	2.67±0.56^{c}	61.81957	72.72826	70.9065

Note: $^{###}$p<0.001 as compared to group I; ^{c}p<0.001 as compared to group I and **p<0.01 as compared to group II

The acetic acid-induced writhing test is known to be a sensitive analgesic method providing reasonable parallelism to clinical potency. However, CNS-depressant and anti-histaminic drugs exhibit a potent anti-writhing action. Such an unspecific property of the writhing test in mice has been reported by many others. In this respect, the sodium chloride-induced writhing test in rats was shown to have a definite advantage over the acetic acid writhing test. By evaluating the analgesic activity of the writhing-in response using mice, it is only possible to inject the irritant like acetic acid once because the response induced by the irritant lasts over 60 min. In the sodium chloride-induced writhing test, the response occurs within 30 s and ceases within 3 min. Further, chronic challenges of sodium chloride do not cause injury to abdominal organs. Accordingly, it is possible to repeatedly challenge an animal with sodium chloride. This test is sensitive and predicts accurately analgesic activity from rat to man. Furthermore, this test is very useful for examining changes in drug activity during chronic administration[14]. Intraperitoneal administration of 8 % sodium chloride produced an immediate writhing response consisting of a wave of contraction and relaxation, passing caudally along the abdominal wall, sometimes accompanied by the twisting of the trunk followed by extension of the hind limbs. It also produced licking, biting and scratching behavior directed to the site of the injection. Treatment with GIFRE at 250 and 500 mg/kg significantly ($p<0.0001$) reduced writhes as compared to the 8 % sodium chloride-induced group. The highest percentage inhibition of writhing was shown by GIFRE at a dose of 250 mg/kg at 120 min as compared to the aspirin-treated group.

It has been suggested that hypertonic sodium chloride may act directly on nociceptors[17]. Also, it is known that chemically induced nociception can be due to acute inflammation in the peritoneal area[18]. Peripheral inflammation increases prostaglandin levels at the site of inflammation, which contributes directly to inflammation and pain. Leukotriene as well as peptide leukotrienes results in an increase in vascular permeability and chemotaxis of polymorphonuclear leucocytes as well as the release of mediators from leucocytes which sensitize nociceptors[15]. It is well documented that peripheral inflammation involves an increase in Cyclooxygenase (COX)-2 mediated prostaglandin synthesis in the CNS including the spinal cord, elevating the PGE_2 levels in the Cerebrospinal Fluid (CSF), which contributes to peripheral pain responses. Fever is also triggered by an elevation of PGE_2 in the brain[19]. This may be an explanation for the hyperthermia observed in the rats injected with 8 % sodium chloride only.

The tail immersion method distinguishes central and peripherally acting analgesics[12]. The GIFRE administered in rats did not show a significant effect when compared to standard drug treatment however showed a significant increase in tail withdrawal time. Studies have revealed that garcinol inhibited the activity of 5-lipoxygenase blocked the microsomal PGE synthase-1 mediated conversion of PGH_2 to PGE_2 and interfered with isolated COX-1 enzyme. The high potency of garcinol in selectively suppressing PGE_2 synthesis and 5-lipoxygenase product formations provides the molecular basis for its anti-inflammatory effects and rationalizes its therapeutic use[10]. Recent findings suggest that both aqueous and alcoholic extracts of *G. indica* fruit rind contain active phytoconstituents that show anti-inflammatory action by inhibiting either synthesis, release, or inflammatory action of mediators such as histamine, serotonin, prostaglandins, bradykinin and lysozymes[11]. These observations provide a possible basis for the peripheral analgesic action of GIFRE.

The extract of GIFRE exhibited significant analgesic activity in central as well as peripheral analgesic models and possibly mediated its effect through diverse mechanisms that may involve both, central and peripheral pathways. Free radicals are now implicated in pain and some plant antioxidants have pain-alleviating properties. This may be attributed to the presence of flavonoids and tannins in the extract[16]. *G. indica* fruits contain tannins and flavonoids and possess antioxidant activity (the principal antioxidant being garcinol) which could play an important role in inducing analgesia.

Based on the current findings, it can be concluded that the analgesic effects of *G. indica* fruit rind aqueous extract are probably mediated by both cerebral and peripheral inhibitory mechanisms. The findings also support the traditional usage of *G. indica* fruit in pain relief. However, the accurate mechanisms through which *G. indica* fruit exerts its analgesic effects are unknown. It is recommended

that further studies be done to ascertain the precise mechanism of action of the anti-pain activity as well as the identification of phytoconstituents responsible for the analgesic effect.

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Conflict of interests:

The authors declare no conflict of interest.

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