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Analgesic and Anti-inflammatory activity of fruit extract phytocompounds of *Chloroxylon swietenia* DC

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Abstract
Chloroxylon swietenia is a popular medicinal plant that is used traditionally in indigenous medicinal practices in India and Sri Lanka to treat wounds, relieve pain and inflammation. However, there are no reports found on the scientific evaluation of *C. swietenia* fruits for their analgesic and anti-inflammatory activities. The present study was aimed to check the analgesic and anti-inflammatory activities of fruit ethanol extract of *C. swietenia* in various experimental models in rats. Analgesic activity was determined using the tail immersion, hot plate methods, and writhing test in acetic acid-induced rats, whereas anti-inflammatory studies were performed in carrageenan-induced inflammation in rats. The results of increased fruit ethanol extract concentration i.e. 500 mg/kg body weight dose level have shown promising effects on all selected experimental models, in the tail immersion test it showed withdrawal time of 7.59 ±0.07 sec. while standard Diclofenac treated group showed 9.49 ±0.17 sec, similarly for hot plate method it showed 10.43 ±0.05 sec. which is near to standard Diclofenac treatment group 11.34 sec, and in acetic acid-induced writhing 42.33±1.86 writhes was observed in 500mg/kg b. w. treated group whereas 31.67±1.45 writhes were recorded in standard Diclofenac treated group. Anti-inflammatory activity of carrageenan-induced rats, paw edema, and swelling was under control and effectively reduced in the fruit ethanol extract-treated group noticeably in comparison with the control group. This study concludes that *Chloroxylon swietenia* fruit extract possesses analgesic and anti-inflammatory potential which is an indication of the traditional claim of this plant in the Indigenous system of medicine which might be due to the presence of a therapeutic group of phytochemicals i.e. phenolic, alkaloids, tannins, and steroids, etc.

Keywords: analgesic, anti-inflammatory, *Chloroxylon swietenia*, writhing test

Introduction
Pain is an intolerable effect that signals as a caution of injury or hazard in the physiological system and one of the principal causes for patients give attention to medical care [1]. Pain is usually transitory, lasting only until the noxious stimulus is removed or the underlying damage or pathology has healed, but some painful conditions, such as rheumatoid arthritis, peripheral neuropathy, cancer, and idiopathic pain, may persist for years [2]. Due to adverse side effects of synthetic non-steroidal anti-inflammatory drugs liver damage, cardiovascular problems, renal failure, erectile dysfunction, manic depression, hypertension, cramps and dizziness, the appearance of dormant diabetes, skin atrophy, decreased bone density, gastrointestinal tract ulcers, dependence, constipation and respiratory problems caused by NSAIDs [3] and addiction caused by opiates. Therefore, the dependence on these drugs for analgesic treatment has not remained effective in all the cases [4]. Therefore, analgesic and anti-inflammatory drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates.
During this process, the research on the safety and effectiveness of plant-based drugs used in traditional medicine have been rewarded great attention because they are inexpensive and have few side effects, and according to WHO nearly 80% of the world population depends mainly on plant-based drugs [5]. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. In the field of pain management, constant research based on natural pharmacophores and their interaction with analgesic targets has led to the search for many potential therapeutic agents [6].
Chloroxylon swietenia is a medium-sized deciduous medicinal plant of the Rutaceae family having extensive medicinal uses in traditional remedies. Tribal people living in the tropical forest areas of Tamil Nadu and South India anciently use *Chloroxylon swietenia* plants to treat several diseases [7]. Leaf paste of this plant is used to treat wounds, cuts, burns, and skin diseases also for wounds infected with animal worms, fungal skin infections, and rheumatism. Stembark is used as an astringent, effective in treating ophthalmic infectivity, common colds, and coughs. The root paste is used as a balm in the treatment of headache [8]. According to ethnopharmacology reports, the plant leaves and stem bark have been pharmacologically evaluated for antimicrobial [9, 10], antidiabetic [11], hepatoprotective [12], insecticidal [13], and antioxidant properties [14]. There are many phytochemicals identified and isolated from leaves and stembark, such as terpenes (Geraniol, limonene, and linalool), flavonoids (isoquercetrin) from leaves, alkaloids (Skimmianine, alfa-fagarine, and furanoquinoline), lignans (hinokinin, savinin, collinusin, and syringaresinol) from bark, phenolics (2, 4-Dihydroxy 5-prenyl cinnamic acid), as well as coumarins (nodakenetin, isopimpinellin, and bergaptan) from the heartwood of the plant *Chloroxylon swietenia* DC [7, 15, 16].
Although the plant has traditional uses there are no scientific reports to-date on fruit and its isolates. As a part of our continuing screening of medicinal plants with analgesic and

anti-inflammatory activity, the current study was undertaken to assess the analgesic and anti-inflammatory efficacy of fruits of *Chloroxylon swietenia* in experimental rat models.

Material and Methods

Plant material

Chloroxylon swietenia DC plant fruits were collected from Shimoga district, Karnataka, India during May and June month. The plant has been identified and confirmed by an in-house taxonomist Dr. V Krishna, Professor, Post Graduate Studies and Research in Biotechnology, Kuvempu University, Shimoga, Karnataka. Fruits were shade dried and then pulverized with a powered mixer to obtain a bristly powder and successive extraction of phytochemicals was carried out with ethanol using a soxhlet apparatus. Later solvent from the extract was fully recovered by a rotary vacuum evaporator. The extract was well-maintained in a vacuum desiccator until use.

Experimental animals

Wistar albino rats (150-200gm body weight) of either sex were selected. The animals were caged in an animal housed under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 2°C) with a relative humidity of 75±5% and had free access to standard diet and water. The rats were left to acclimatize to the laboratory conditions for 7 days before the experimental session. All the experiments were done in agreement with the guidelines, as adopted by the Institutional Animal Care Committee, CPCSEA, India. The Institutional Animal Ethical Committee permitted the studies under the certification (NCP/IAEC/CL/213/01/2012-13).

Analgesic activity

Standard methods viz., tail immersion, hot plate, and acetic acid-induced writhing response methods were employed to determine the analgesic activity.

For each test, healthy albino rats of either sex (150-200g) were distributed into 4 groups of 6 animals each.

The animals were fasted overnight before the start of the experiment, with free access to water. All drugs/vehicle were administered orally. Group I received the vehicle (1% CMC suspension) and served as the control group. The group II, rats were administrated with the standard drug Diclofenac sodium (25mg/kg b.w.). Group III & IV were treated with ethanol extract (200 and 500 mg/kg b.w. dose based on acute toxicity test) respectively [17].

Tail immersion method

After administration of the test sample, all the experimental rats were kept into separate restraining cages leaving the tail hanging out freely. The animals were placed in cages 30 min before testing allowing them to adapt. The 5cm tail tip is marked. This portion of the tail is dipped in a beaker of freshly filled water of exactly 55±0.5˚C. Within few seconds the rat responds by withdrawing the tail. The response time is noted in 0.5-sec units by a stopwatch. After each determination, the tail was carefully dried. The reaction time is determined before and periodically after oral administration of the test drug, e.g., after 0.5, 1, 2, 3, and 4 hours. The cut-off time of the immersion is 15 seconds. The withdrawal time of untreated animals is between 1 and 5.5 seconds. A withdrawal time of more than 6 seconds is regarded as a positive response [18].

Eddy's Hot plate method

Experimental rats were kept on a hot plate (55±0.5˚C), the time for forepaw lifting or jumping was considered as the response time. Rats showing response time between 3-5 sec. were selected. Animals not responding in this period were discarded. The response time was noted at 30, 60, 120, 180 & 240 minutes after administration of the test compounds or the standard drug to determine the onset and duration of action. One hour after the administration of samples, rats were individually placed on the hot plate of the analgesiometer maintained at 55˚c. Analgesic activity was determined by comparing with the control group [19].

Acetic Acid-Induced Writhing Response

After thirty minutes of test sample administration, a 0.7% acetic acid (10 ml/kg) solution was injected intraperitoneally to all the animals in different groups. The number of writhes (abdominal constrictions) occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptive response [20].

Results were expressed as mean percent inhibition of writhing (PIW):

$$PIW = \frac{\text{No. of Writhes (control) - No. of Writhes (sample)}}{\text{No. of Writhes (control)}} x\ 100$$

Anti-Inflammatory Activity

Carrageenan induced paw edema

The rats were divided into 4 groups of 6 rats each. In the control group, I received 1% CMC suspension. The positive control group II was treated orally with the standard drug, indomethacin (10 mg/kg b.w.). FEE was administered to groups III and IV in doses of 200 and 500 mg/kg b.w. All the suspensions were administered 30 min before the induction of edema by administering 0.1 ml of 1% w/v carrageenan in saline. The degree of paw edema (mm) of all the groups was measured immediately and at 30, 60, 120, and 240 min. interval after the carrageenan injection, by using Vernier caliper [21].

Statistical Analysis

Data are presented as mean ± standard error of the mean. Data were analyzed with ANOVA and GraphPad prism 5. Where the control group was compared with the remaining groups. Significance was set at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

Results

In the analgesic tail-immersion test (pain induced by hot water), the FEE-treated group considerably increased pain tolerance time 5.43 ±0.14 and 7.59 ±0.07 seconds at 200 mg and 500mg/kg doses respectively, which proved a notable dose-dependent analgesic response against thermal discomfort. An increased pain tolerance time was witnessed in the case of 500mg/kg FEE (Fruit ethanol extract). This effect was comparable to that of Diclofenac sodium treated group tolerance time of 9.49 ±0.17 seconds suggesting the central analgesic effect of FEE as shown in figure-1(a).

The results of the hot plate test are presented in figure-1 (b). The fruit ethanol extract showed a dose-dependent increase in the withstand period to thermally induced analgesic

activity, FEE exhibited a maximum latency time of 8.30 ±0.09 and 10.43 ±0.05 sec. for 200 and 500 mg/kg b.w doses respectively, while Diclofenac sodium exhibited the maximum reaction time of 11.34 sec. at the dose of 10mg/kg. The results showed that the ethanol extract significantly elevated the pain threshold as compared to control and the activity was constant during the complete observation time of 240 min.

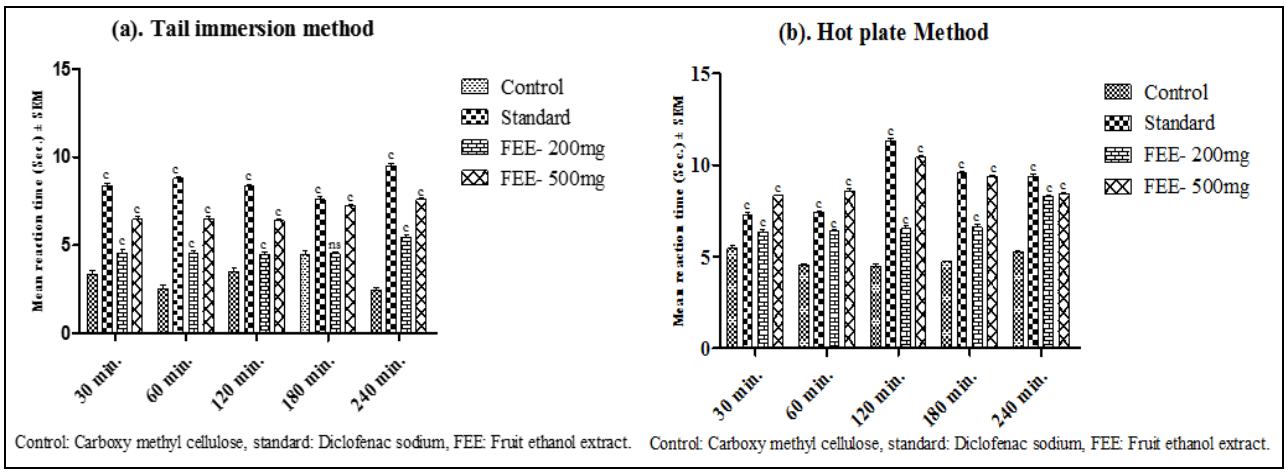

Fig 1: Analgesic activity of *Chloroxylon swietenia* fruit ethanol extract in rats.

Bars represent mean±S.E. Statistical significance symbols represent $^{a}p\leq0.05$, $^{b}p\leq0.01$, $^{c}p\leq0.001$, and ns= non-significant. P-value indicates the significant difference from the comparison of the control group with standard, FEE-200 mg and FEE-500mg treated groups.

Table 1: Effect of fruit ethanol extract on acetic acid-induced writhes in rats.

Groups	No. Of writhes between 5 to 20 mins. Mean ± S.E.M	Percent inhibition of writhing (PIW)
I- (Control) 1 % CMC	74.33±0.88	-
II- (Standard) 25 mg/kg	31.67±1.45^{c}	57.39
III- (FEE) 200 mg/kg	54.67±1.20^{c}	26.44
IV- (FEE) 500 mg/kg	42.33±1.86^{c}	43.05

Control: Carboxymethylcellulose, standard: Diclofenac sodium, FEE: Fruit ethanol extract. Statistical significance symbols represent $^{a}p\leq0.05$, $^{b}p\leq0.01$, $^{c}p\leq0.001$, and ns= non-significant. P-value indicates the significant levels in comparison of G -1 with G-2, G-3, and G-4.

Oral administration of FEE (200 and 500 mg/kg b.w) produced a dose-dependent analgesic response, the results of the acetic acid-induced writhing test in experimental animals indicate a favorable increase in the stimulation time required to produce writhing movements when treated with fruit ethanol extract. The obtained results were analogous to that of the reference drug (diclofenac sodium 10 mg/kg) treated group (31.67±1.45), the no. of writhing movements were also beneficially decreased to 54.67±1.20 and 42.33±1.86 in 200 mg/kg and 500 mg/kg FEE treated rats respectively, as compared to the Carboxymethylcellulose treated animals (74.33±0.88), suggesting a peripheral antinociceptive analgesic effect (Table -1).

After Carrageenan injection the rat paw edema volume was measured every hour (up to 4h) in all experimental rats. In the beginning volume of paw edema was increasing immediately after 1% w/v carrageenan injection in all experimental groups every hour. The observed volume started to decrease in the fruit ethanol extract-treated group in a dose-dependent manner which is significant in comparison with Carboxymethylcellulose treated group and competitive to standard Indomethacin drug-treated group while recording edema up to 240min(4hours) (Figure- 2).

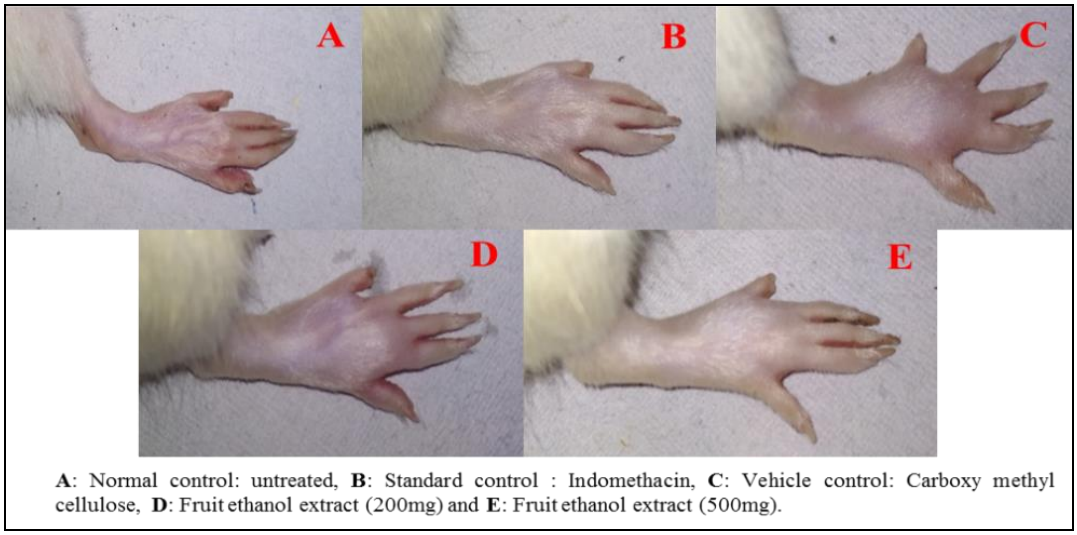

Fig 2: Carrageenan induced paw edema

Table 2: Effect of fruit ethanol extracts on Carrageenan induced paw edema

4Groups	Inflammation (mm)				
	30 min.	60 min.	120 min.	180 min.	240 min.
I- (Control) 1 % CMC	2.73±0.06	2.56±0.08	2.30±0.03	2.04±0.04	1.84±0.04
II (Standard) 10 mg/kg	2.58±0.07 ns	2.27±0.08 b	1.88±0.06 c	1.63±0.04 c	1.27±0.03 c
III- (FEE) 200mg	2.63±0.08 ns	2.27±0.04 b	2.10±0.07 ns	1.80±0.06 ns	1.57±0.03 b
IV-(FEE) 500mg	2.80±0.07 ns	2.41±0.09 ns	1.90±0.08 c	1.53±0.07 c	1.17±0.04 c

Control: Carboxymethylcellulose, standard: Indomethacin, FEE: Fruit ethanol extract. Statistical significance symbols represent $^{a}p\leq0.05$, $^{b}p\leq0.01$, $^{c}p\leq0.001$, and ns= non-significant. P-value indicates the significant levels in comparison of G -1 with G-2, G-3, and G-4.

Anti-inflammatory activity in carrageenan-induced rat paw edema test, the Carboxymethylcellulose treated control rats showed an average of 1.84±0.04mm edema development at the end of the observation period. Oral administration of FEE to Carrageenan-induced rats inhibited the inflammatory response effectively in a dose-dependent manner at 240 min (4h). It showed a significant reduction in the paw edema with 1.57 ±0.03mm and 1.17±0.04 mm i.e. for 200 mg/kg and 500 mg/kg respectively, which is most significant in a competitive way to the standard Indomethacin treated group with 1.27±0.03mm on average, which is presented in the table- 2.

Discussion

In the present study, the fruit ethanol extract of the *Chloroxylon swietenia* plant showed significant analgesic and anti-inflammatory properties. The fruit ethanol extract was administered orally at the doses of 200 and 500 mg /kg b.w. out of which 500mg/kg b.w. showed better activity than 200mg/kg b.w. The methods selected were thermal nociception tail immersion and hot plate tests which are the two generally used techniques for evaluating central analgesic activity [22, 23]. The chemical nociception in the acetic acid-induced writhing model is for peripherally mediated effects which are standard pharmacological models for the evaluation of analgesia by natural products [24] of *Chloroxylon swietenia* fruit ethanol extract. The Carrageenan-induced edema method was selected to study anti-inflammatory potential [25].

The tail immersion and hot plate test procedures highlight the modifications at the spinal cord level. These are effective methods for the prediction of centrally facilitated anti-nociceptive responses [26]. The procedures are considered for their high resemblance to opioid-derived analgesics [27]. FEE presented prominent anti-nociceptive activity in both experimental models in a dose-dependent way. The tail immersion nociception involves μ2, κ1, and δ2 opioid receptors via spinal reflexes while hot plate nociception involving μ1, κ3, δ1, σ2 opioid receptors via supra-spinal reflex [28, 29, 30]. Inhibition of nociceptive activity indicates a possible inhibition or modification of pain induction through this pathway. Results from both the tests are indicative of spinal and supra-spinal receptors mediated anti-nociceptive activities of *Chloroxylon swietenia* fruit ethanol extract.

Acetic acid-induced release of prostaglandins, serotonin, bradykinin, histamine, TNF (some common endogenous mediators) in the peripheral tissue fluid is associated with the development of pain and writhing [31]. The profound antinociceptive effect of fruit ethanol extract at 500 mg/kg dose in the acetic acid-induced writhing test, therefore, implies that fruit ethanol extract may be involved in the inhibition of inflammatory mediators like cyclooxygenase, lipoxygenase, and others which results in the interruption of signal transduction in primary afferent nociceptors [32]. Therefore, the FEE must have both central and peripheral mechanisms of pain, while NSAIDs inhibit only peripheral pain [33, 34]. The plant extracts of *Chloroxylon swietenia* showed both kinds of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanisms [35].

Carrageenan-induced paw edema is said to be the biphasic latest method for screening anti-inflammatory agents [36]. The first phase (1-2 h) is due to the release of histamine or serotonin and the second phase of edema is due to the release of prostaglandin [37]. The results of this study indicate that the FEE extensively reduced carrageenan-induced paw edema in rats. As a result, the mechanism of action may be by inhibition of histamine, serotonin, or prostaglandin synthesis [38]. These results indicate a significant analgesic and anti-inflammatory activity at both dose levels studied.

The analgesic and inflammatory activity were showed by *Chloroxylon swietenia* fruit extract in various models indicate that the plant extract might possess centrally and peripherally mediated analgesic and anti-inflammatory properties, and this is maybe due to the presence of therapeutic phytochemicals like alkaloids, steroids, tannins, and other polyphenolic compounds [39,40]. The majority of phytoconstituents present in *C. swietenia* fruit are phenolic, alkaloids, tannins, and steroids, etc. [41]. The results of the present study support the traditional use of *Chloroxylon swietenia* in some painful conditions. However, further research with isolated compounds is required to better understand the principal lead compounds that contribute to the effect and the mechanisms involved in the process.

Conclusions

The present study revealed that the analgesic and anti-inflammatory activities of *Chloroxylon swietenia* fruit ethanol extract on selected rat models strongly support its use in traditional medicine. The present research findings demonstrated the scientific validation for the traditional uses of this plant as an analgesic and inflammatory medicine. Further studies are necessary on the active phytoconstituents of *Chloroxylon swietenia* fruit that are responsible for their analgesic and inflammatory properties.

Ethical Clearance

The study protocol was approved by the National College Pharmacy, Shimoga.

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Conflict of interest
The authors declare no conflict of interest regarding the publication of this article.

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