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Evaluation of Anti-inflammatory activity and toxicity studies of *Chloroxylon sweitenia* in Rats

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ABSTRACT

The extract of *Chloroxylon sweitenia* (Family: Rutaceae) leaves were investigated for its anti-inflammatory activity at the different doses in the standard animal models. The experimental paradigms used were carrageenan induced rat paw oedema (acute), and cotton pellet induced granuloma (chronic) models in rats for anti-inflammatory activity. In rats the toxicity was also performed for the extract by oral administration. The chloroform extract of *Chloroxylon sweitenia* (CECS) exhibited significant anti-inflammatory effect at the dose 50, 100 and 200 mg/kg. Maximum inhibition (55.32 %) was noted at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas the Diclofenac (standard drug) produced 61.33 % of inhibition. In the chronic model (cotton pellet induced granuloma) the CECS (200 mg/kg) and standard drug showed decreased formation of granuloma tissue by 52.32 % and 56.32 % ($p < 0.001$) respectively. The CECS further evaluated for their toxicity effect at the doses of 100 mg/kg administered for 14 days to orally in rats. At the end of experiments the blood, liver function and kidney metabolism was observed. The effect of CECS was assessed by the change in the body weight, lipid peroxidation and glutathione content (GSH) activities were measured from hepatic tissues. The hematological profile and different biochemical parameters such as SGOT, SGPT, and ALP were also estimated. Thus, the present study revealed that the chloroform extract of *Chloroxylon sweitenia* exhibited significant anti-inflammatory activity in the tested models Toxicity study indicates that the extract is non-toxic at the tested doses.

Key words: *Chloroxylon sweitenia*, carrageenan, cotton pellet induced granuloma, anti-inflammatory activity.

INTRODUCTION

Inflammation is a natural response of the mammalian body to a variety of hostile agents including parasites, pathogenic microorganism, toxic chemical substance and physical damage to tissue. Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. The inflammatory process is invariably characterized by a production of prostaglandins, leukotrienes, histamine,bradykinin, platelet-activating factor (PAF) and by a release of chemicals from tissues and migrating cells [1)]. Carrageenan-induced local inflammation is

commonly used to evaluate non-steroidal anti-inflammatory drugs (NSAID). It appears that the onset of the carrageenan local inflammation has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radical, as well as to the release of other neutrophil-derived mediators[2]. In particular, the initial phase of inflammation (oedema, 0–1 h) which is not inhibited by NSAID such as Diclofenac or aspirin, has been attributed to the release of histamine, 5-hydroxytryptamine and bradykinin, followed by a late phase (1–6 h) mainly sustained by prostaglandin release and more recently has been attributed to the induction of inducible cyclo-oxygenase (COX-2) in the tissue[3].

The continued search for potential effective and safe anti-inflammatory agents cannot be over-emphasized. This is important, in view of the roles played by the inflammatory process and inflammatory mediators in diseases such as asthma, rheumatoid arthritis, cancer and neurodegenerative disorders. *Chloroxylon swietenia* DC (East Indian satin wood) is a moderate size tree common in dry deciduous forests throughout Indian peninsula and in Ceylon[4]. Stem bark pounded and the juice applied for ophthalmic infection and cataract by Malayalis. Various parts of the plant are traditionally used in rheumatism and the leaves are applied to wounds[5]. This species has been extensively investigated and a number of chemical constituents from the leaves, bark and roots of the plant have previously reported in a number of instances which includes alkaloids[6-7], cumarin [8-9], lignans[10]. However, no work has been reported on the anti-inflammatory effects on acute and chronic phases of inflammation, *C. sweitenia*. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory and toxicity studies of chloroform extract of *C. sweitenia* (CECS) in standard animal models.

MATERIALS AND METHODS

Plant Material

The plants *Chloroxylon sweitenia* were collected in the month April 2004 from the Komarapalayam, Namakkal district, Tamilnadu, India. The plant material was taxonomically identified by the Botanical survey of India, Coimbatore, Tamilnadu and the voucher specimen SK-7 was retained in our laboratory for future reference. The leaves of each of the plant material were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no 40 and stored in an airtight container for further use.

Chemicals and Reagents

The chemicals used in the present study were carrageenan (S. D. Fine Chemicals Limited, Bombay), and Diclofenac sodium (Torrent, Bombay).

Preparation of Extract

The dried powdered plant material was extracted with chloroform in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 7.75 %). The extract showed positive test for alkaloids, steroids, and tannins. The extract at the different doses of 50, 100 and 200 mg/kg was suspended in aqueous Tween 80 solution (2 %) and Diclofenac (10 mg/kg) in saline were used for the present study.

Animals

Swiss albino mice of either sex weighing between (18-22 g) or Albino Wistar rats of the either sex (180-200 g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet with water *ad libitum*.

Investigation of Anti-inflammatory effects by Carragenan Induced Paw odema

The rats were divided into 5 groups (n = 6). The extract and the standard used for this study were prepared in the same manner as mentioned earlier. Animals were deprived of food and water for 18 hours before the experiment. On the day of the experiment they were assigned to 5 groups of six animals each. They were marked and numbered for identification. Paw oedema was induced by subplantar injection into the rat right hind paw of 0.1 ml sterile saline containing 1% carrageenan (control group). A group of rats were treated with test compounds and standard drugs were administered orally concomitantly with carrageenan injection. Control group of animals received the same volume of vehicle instead of the tested agents. The volume of the paw was measured by a plethysmometer immediately after the injection as previously described[11-12]. Subsequent readings of the same paw were carried out at one-hour intervals up to 4 h and compared to the initial readings. The increase in paw volume was taken as oedema volume. The percentage of inhibition of inflammation was calculated for comparison.

The ratio of the anti-inflammatory effect of CECS was calculated by the following equation: anti-inflammatory activity (%) = (1-D/C) x 100, where D represents the percentage difference in paw volume after CECS was administered to the rats, and C represents the percentage difference of volume in the control groups.

Cotton pellets-induced granuloma

The rats were divided into five groups (n = 6). The cotton pellet granuloma model investigated the proliferation phase of inflammation[13]. The extract of different doses (50,100 and 200 mg/kg) and Diclofenac at 10 mg/kg body weight was given to the animals orally. After 30 min the animals were anesthetized. After shaving the fur, the rats were anaesthetized and 10 mg of sterile cotton pellets were inserted, one in each axilla. The extracts was administered daily for a period of seven days. The rats were sacrificed after a high dose of anesthesia on the eighth day and the pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37° C for 24 h and dried at 60° C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

Short term toxicity study

The extract administrated at the doses of 50 and 200 mg/kg orally once daily and it was continued upto 14 days to observe any short-term toxicity. Then, at the end of experiments the hematological profile and different biochemical parameters of hepatorenal function were observed.

Blood collected and hematological parameters were determined as described in hematological studies [14].

Liver and other important internal organs were removed, weighed and observed for pathological changes.

The blood was centrifuged at 3000 rpm at 4°C for 10 minutes to separate serum. The activities of serum glutamate oxaloactetate transaminase level (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assayed by method [14] . The alkaline phosphatase activity in the serum was measured according to the procedure of King (1965a) method by spectrophotometrically[15]. Further, liver biochemical parameters were estimated by methods described in estimation of biochemical parameters.

Biochemical assays and hematological studies

Hemoglobin content, leucocytes counts, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by standard methods[16] .Blood urea was determined by the diacetyl monoxime method of Leclerc and Schwarz (1957). Hemoglobin content [18] was estimated from the peripheral blood of normal, control and treated animal groups. Protein content was measured by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as the standard[17].

The liver was excised, rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4), blotted and weighed. The homogenate was processed for estimation of lipid peroxidation and GSH. Assay for microsomal lipid peroxidation was carried out by the measurement of thiobarbituric acid reactive substances (TBARS) in the tissues reported [19] by Ohkawa *et al.*, 1979. The pink chromogen produced by the reaction of malondialdehyde, which is a secondary product of lipid peroxidation reaction with thiobarbituric acid was estimated at 532 nm. Reduced glutathione (GSH) in the tissues was assayed by the method of Ellman [20] (1979). GSH estimation is based on the development of yellow color when 5,5'-dithiobis (2-nitro benzoic acid) di-nitrobisbenzoic acid was added to compounds containing sulphydryl group.

Statistical Analysis

The results are expressed as mean ± SEM. The statistical analysis was performed by ANOVA test.

RESULT

The chloroform extract of leaves part of *Chloroxylon sweitenia* was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in table 1, 2,3 and 4. As shown in table 1, the chloroform extract showed inhibition of 39.07, 44.90, 55.32 % at the doses of 50, 100 and 200 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas the standard drug showed 61.33 **%** of inhibition. As shown in table 3, in the chronic model (cotton pellet induced granuloma), of the different doses of the CECS and standard drug showed decreased formation of granuloma tissue at 28.48, 41.13, 52.32 **%** and 56.32 % ($p < 0.001$), respectively. The heamatological profile and biochemical parameter were shown in table 4.Hematological parameters like hemoglobin remained unaltered at the

dose of 50 and 200 mg/kg. The hematological parameters such as urea, transaminase activities increased at the dose of 200 mg/kg. A comparison of activities of the lipid peroxidation, GSH and the levels of SGOT, SGPT and associated biochemical parameter for the study were compared with corresponding values in a normal, control and standard shown in table 3 and 4.

DISCUSSION

The potential of the CECS for its anti-inflammatory effect and short-term toxicity was investigated. The effect of CECS at the dose of 50,100 and 200 mg/kg showed significant anti-inflammatory activity. Significant anti-inflammatory activity was observed for CECS in carrageenan-induced oedema and also the chronic models.

The present study establishes the anti-inflammatory activity of chloroform extract of *Chloroxylon sweitenia*. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and is commonly used to induce acute inflammation and is believe to be bi-phasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome [21)]. It has been reported that second phase of edema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-edematous effect of natural products[22-23)].

Prostaglandins play a major role in the development of second phase of reaction that is measured at 3 hours time. These mediators take part in the inflammatory response and are able to stimulate nociceptor and thus induce pain [24)]. It is also the prostaglandin amplifies the pain mechanism and enhances vascular permeability whilst the leukotrienes contract smooth muscles blood vessels. And it leads to enhance the vascular permeability and mediate proinflammatory and allergic response[25-26)]. Diclofenac is a potent onhibitor of prostaglandin formation and thereby reduces inflammation and arthritic pain. Furthermore, diclofenac has been reported to reduce chemotaxis oxygen derived free radical generation and neutral production [36)]. Based on these reports, it can be inferred that the inhibitory effect of the extract of *Chloroxylon sweitenia* on carrageenin-induced inflammation in rats may by due to inhibition of the mediators responsible for inflammation.

Chronic inflammation is a reaction arising when the acute response is in sufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophills and exudation. The cotton pellet granuloma widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet of the correlates with the amount of granulumatous tissues[27-28)] Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides[29-30)]. This result indicates the efficacy of the extract, which possesses anti-inflammatory activity.

Serum transaminase elevation has been reported to be associated with a number of inflammatory disorders and hepatocelluar damage. Leakage of large quantities of enzymes into the blood stream is often

associated with massive necrosis of the liver. Serum enzymes elevation has also been reported to be associated with number of inflammatory disorders [31] .It is also reported that the low heamoglobin concentration [32] with increased level of serum alkaline phosphatase [38] and serum creatinine[34] are noted in chronic inflammatory disease such as rheumatoid arthritis, which is usually associated with anorexia and weight loss. Also, methyl prednisolone was reported that the level of serum protein content is lowered in rheumatoid arthritis in human[35]. The present study revealed that CECS is non-toxic at the tested doses and also indicates that for the safety use of the extract.

From the above discussion, the extract exhibited significant anti-inflammatory activity and did not affect the heamatological and biochemical profiles of hepato-renal function. Thus this study substantiates the use of this plant as an anti-inflammation folklore medicine. Further detailed investigation is underway to determine the exact phytoconstituents responsible for the anti-inflammatory activity.

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Table 1. Effect of the *Chloroxylon sweitenia* extract on carrageenan and induced pedal oedema

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Carrageenan control	0	0.732 ± 0.063	-
Diclofenac	12.5	0.283 ± 0.024	61.33
CECS	50	0.446 ± 0.035	39.07
CECS	100	0.403 ± 0.028	44.90
CECS	200	0.327 ± 0.017	55.32

Values are mean ± SEM (n = 6). Experimental groups were compared with control $p < 0.001$.

Table 2. Effect of the *Chloroxylon sweitenia* extract on cotton-pellets Induced granuloma in rats

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	Percentage of inhibition
Control	0	47.4 ± 4.12	-
Diclofenac	12.5	20.7 ± 1.82	56.32
CECS	50	33.9 ± 2.43	28.48
CECS	100	27.9 ± 1.70	41.13
CECS	200	22.6 ± 2.2	52.32

Values are mean ± SEM (n = 6). Experimental groups were compared with control $p < 0.001$.

Table-3. Effect of *Chloroxylon sweitenia* extract on hematological and different biochemical parameters in rats

S.No.	Parameters	Experimental			
		Control (Normal saline 5 ml /kg)	CECS (50 mg/kg)	CECS (200 mg/kg)	Diclofenac (12.5 mg /kg)
1	Hemoglobin (g %)	12.4 ± 1.10	12.5 ± 0.83	12.3 ± 1.04	12.4 ± 1.21
2	Haematocrit %	37.7 ±1.94	37.7 ± 1.43	35.7 ± 1.23 [b]	36.9 ± 1.24
3	MCHC %	33.4 ± 2.83	33.2 ± 2.17	32.4 ± 0.77	33.5 ± 0.71
4	MPV	134.8 ± 7.36	132.4 ± 3.31 [b]	138.6 ± 4.65[a]	141 ± 3.72 [b]
5	Platelets (10^3x mm^3)	1358 ± 41.2	1355.0 ±74.1	1346 ± 57.13 [a]	1257.4 ± 42.2

	Liver and Kidney function test				
6		114.9 ± 8.57	114.8 ± 6.44 [a]	110.5 ± 1.62	98.8 ± 1.34 [a]
7	SGOT (U/L)	20.7± 1.72	20.1 ± 2.21 [b]	19.5 ± 1.74	19.7 ± 1.88
8	SGPT(U/L)	60.2 ± 3.04	59.9 ± 4.8 [b]	67.4 ± 4.65	43.7± 3.95 [b]
9	ALP (U/L)	0.38 ± 0.02	0.39 ± 0.02	0.48 ± 0.03	0.45 ± 0.02 [a]
10	Creatinine (mg/dl)	18.4 ± 0.87	16.5 ± 1.48	21.5 ± 1.74	17.6 ±1.29 [a]
11	BUN (mg/dl)	7.2 ± 0.04	7.23 ± 0.65	6.92 ± 0.53	6.85 ± 0.54

Protein (g/dl)

Values are mean ± SEM (n=6). Groups was compared with normal control group [a] $p< 0.001$, [b] $p< 0.01$

Table.4. Effect of *Chloroxylon sweitenia* extract on Lipid peroxidation and Glutathione status in rats

S.No.	**Parameters**	**Experimental**			
		Control (Normal saline 5 ml /kg)	**CECS (50 mg/kg)**	**CECS (200 mg/kg)**	**Diclofenac (12.5 mg /kg)**
1.	Lipid peroxidation	0.97 ± 0.060	0.98 ± 0.84	1.17 ± 1.04	1.13 ± 1.21
2.	Reduced Glutathione	2.37 ±1.94	2.38 ± 1.43	2.52 ± 1.23 [b]	2.47 ± 1.24

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