

Evaluation of Anti-inflammatory activity and toxicity related studies of *Chloroxylon sweitenia* in standard animal models

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Summary

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, histamine, serotonin 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 methanol extract of *Chloroxylon sweitenia* (MECS) exhibited significant anti-inflammatory effect at the dose 50, 100 and 200 mg/kg. Maximum inhibition (51.70 %) was noted at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas the Indomethacin (standard drug) produced 62.1 % of inhibition. The extract also exhibited significant inhibition on the hind paw oedema in rats caused by histamine and serotonin respectively. In the chronic model (cotton pellet induced granuloma) the MECS (200 mg/kg) and standard drug showed decreased formation of granuloma tissue by 50.53 % and 57.0 % ($p < 0.001$) respectively. The MECS 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 MECS was assessed by the change in the body weight, lipid peroxidation, glutathione content (GSH), superoxide dismutase (SOD) and catalase (CAT) 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 methanol 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: carrageenan, cotton pellet induced granuloma, anti-inflammatory activity.

Introduction

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 cells¹⁾. 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⁾

Chloroxylon swietenia DC (East Indian satin wood) is a moderate size tree common in dry deciduous forests throughout Indian peninsula and in Ceylon³⁻⁴⁾. 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 wounds⁵⁾. The bark is used as an astringent. 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 alkaloids⁶⁻⁷⁾, coumarin⁸⁻⁹⁾, lignans¹⁰⁾. However, no work has been reported on the anti-inflammatory effects on acute and chronic phases of inflammation, *C. swietenia*. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory and toxicity studies of methanol extract of *C. swietenia* (MECS) in standard animal models.

Result

The methanol extract of leaves part of *Chloroxylon swietenia* was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in table 1, 2 and 3. The methanol extract exhibited significant anti-inflammatory activity at the tested doses of 100, 200 and 400 mg/kg in a dose dependant manner. As shown in table 1, the methanol extract showed maximum inhibition of 51.70 % at the dose of 400 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas the standard drug showed 56.50 % of inhibition.

As shown in table 2, in case of histamine induced paw oedema, the ethanol extract showed 53.87 % of inhibition at the dose of 400 mg/kg whereas Indomethacin showed 59.80 % of inhibition respectively. As shown in table 3, in the chronic model (cotton pellet induced granuloma), the MECS (400 mg/kg) and standard drug showed decreased formation of granuloma tissue at 50.53 % and 57.0 % ($p < 0.001$), respectively. When the rats were observed for the behavioral changes after orally administration of a single dose of the extract, none of the rats exhibited any abnormal behavioral responses at doses of 100 and 400 mg/kg. These include inactiveness, loss of appetite, slow movement, and dizziness, erection of hairs and hypothermia. Administration of repeated daily doses of 50 and 400 mg/kg for 14 days did not influence the body weight of the mice. The weights of liver, kidney, brain and spleen were also not altered by the treatment. The hematological profile and biochemical parameter were shown in table 4- 5.

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	-
Indomethacin	10	0.283 ± 0.024	61.33
MECS	100	0.446 ± 0.035	39.07
MECS	200	0.403 ± 0.028	44.90
MECS	400	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 *Chloroxylon sweitenia* extract on mediators like histamine and 5-HT induced pedal oedema in rats

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Histamine control	0	0.544 ± 0.045	-
Indomethacin	10	0.207 ± 0.022	61.94
MECS	100	0.328 ± 0.030	36.70
MECS	200	0.298 ± 0.024	45.22
MECS	400	0.251 ± 0.021	53.86
Serotonin control	0	0.623 ± 0.045	-
Indomethacin	10	0.256 ± 0.017	59.73
MECS	100	0.407 ± 0.035	34.67
MECS	200	0.361 ± 0.033	43.01
MECS	400	0.295 ± 0.020	52.64

Values are mean ± SEM (n = 6).

Experimental groups were compared with control p < 0.001.

Table 3. 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 ± 3.2	-
Indomethacin	10	20.7 ± 1.5	56.32
MECS	100	33.9 ± 1.7	28.48
MECS	200	27.9 ± 1.8	41.13
MECS	400	22.6 ± 2.1	52.32

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

Table 4. Effect of *Chloroxylon sweitenia* extract on hematological and different biochemical parameters in rats

S.No	Parameters	Experimental			
		Control (Normal saline 5 ml /kg)	MECS (100 mg/kg)	MECS (300 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 ³ x mm ³)	1358 ± 41.2	1355.0 ± 74.1	1346 ± 57.13 ^a	1257.4 ± 42.2
Liver and Kidney function test					
6	SGOT (U/L)	114.9 ± 8.57	114.8 ± 6.44 ^a	110.5 ± 1.62	98.8 ± 1.34 ^a
7	SGPT(U/L)	20.7 ± 1.72	20.1 ± 2.21 ^b	19.5 ± 1.74	19.7 ± 1.88
8	ALP (U/L)	60.2 ± 3.04	59.9 ± 4.8 ^b	67.4 ± 4.65	43.7 ± 3.95 ^b
9	Creatinine (mg/dl)	0.38 ± 0.02	0.39 ± 0.02	0.48 ± 0.03	0.45 ± 0.02 ^a
10	BUN (mg/dl)	18.4 ± 0.87	16.5 ± 1.48	21.5 ± 1.74	17.6 ± 1.29 ^a
11	Protein (g/dl)	7.2 ± 0.04	7.23 ± 0.65	6.92 ± 0.53	6.85 ± 0.54

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

Table 5. Effect of different doses of methanol extract of the *Chloroxylon sweitenia* on different biochemical parameters

Parameters	Normal Saline (0.9% NaCl 0.5ml/kg)	MECS 100 mg/kg	MECS 300 mg/kg	Standard 12.5 mg/kg
Lipid peroxidation (n moles MDA/ g of tissue)	0.97 ± 0.03	0.98± 0.04	0.97 ± 0.02	0.99 ± 0.02
GSH (mg/g of tissue)	2.36 ± 0.03	2.39 ± 0.12*	2.46 ± 0.17*	2.54 ± 0.21
SOD (Units / mg Protein)	4.38 ± 0.43	4.42 ± 0.27	4.49 ± 0.22	4.56 ± 0.33*
Catalase (Units / mg tissues)	25.9 ± 1.91	26.3 ± 0.11	26.8 ± 0.11	27.4 ± 1.17

Values are mean ±SEM (n=7) comparisons group 1 and 2. The experimental groups compared with normal groups. Statistical significance* p < 0.01

DISCUSSION

The potential of the MECS for its anti-inflammatory effect and short-term toxicity was investigated. The effect of MECS at the dose of 100, 200 and 400 mg/kg showed significant anti-inflammatory activity. The methanol extract of *Chloroxylon sweitenia* was evaluated for its anti-inflammatory activity in acute and chronic models. Significant anti-inflammatory activity was observed for MECS in carrageenan, mediators induced oedema and also the chronic models.

The present study establishes the anti-inflammatory activity of methanol extract of. 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 lysosome¹⁶⁾. It has been reported that

second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to assess the anti-oedematous effect of natural products¹⁷⁻¹⁸⁾. Prostaglandin's 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 pain¹⁹⁾. 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 be due to inhibition of the mediators responsible for inflammation.

Histamine is the one the important inflammation mediators and it is potent vasodilators substance and increase the vascular permeability²⁰⁻²¹⁾. The study showed that the dose MECS effectively suppressed the odema produced by the histamine, which is indicates that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandin's might be involved in the inflammation. So, it me suggested that its anti-inflammatory activity is possible backed by its anti-histaminic activity.

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 granulomatous tissues²²⁻²³⁾. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-sterodal 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 mucopolysaccharides²⁴⁻²⁵⁾. The MECS showed significant anti-inflammatory activity in cotton-pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

Serum enzymes elevation has also been reported to be associated with number of inflammatory disorders²⁶⁾. It is also reported that the low heamoglobin concentration²⁷⁾ with increased level of serum alkaline phosphatase²⁸⁾ and serum creatinine²⁹⁾ are noted in chronic inflammatory disease such as rheumatoid arthritis, which is usually associated with

anorexia and weight loss. Methyl prednisolone therapy in rheumatoid arthritis produced elevation of serum ALP and no changes in SGOT, alkaline phosphatase, creatinine and urea nitrogen. Also, methyl prednisolone was reported that the level of serum protein content is lowered in rheumatoid arthritis in human³⁰⁾. The present study revealed that MECS is non-toxic at the tested doses. Oral administration of *C. sweitenia* leaf extract (200 mg/kg body weight) for 14 days resulted in a significant reduction in thiobarbituric acid reactive substances while increased glutathione in liver which clearly shows the antioxidant property of MECS 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 hematological 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. However, in view of the antioxidant activity observed in oral administration experiment using rats with extract, seem to be effective for reducing oxidative stress and is expected to contribute to the prevention of free radical –related disease such as diabetes mellitus, cancer, and chronic inflammation.

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), histamine (Sigma, USA), 5-hydroxy tryptamine hydrochloride (serotonin) (Sigma, USA), and Indomethacin (IPCA, Bombay).

Preparation of Extract

The dried powdered plant material was extracted with methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 12.25 %). The extract showed positive test for alkaloids, steroids, and tannins. The extract at the different doses of 100, 200 and 400 mg/kg was suspended in aqueous Tween 80 solution (2 %) and Indomethacin (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 odema¹¹⁻¹³

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 described¹¹⁻¹²⁾. 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 MECS was calculated by the following equation: anti-inflammatory activity (%) = $(1-D/C) \times 100$, where D represents the percentage difference in paw volume after MECS was administered to the rats, and C represents the percentage difference of volume in the control groups.

Mediator induced inflammation

The anti-inflammatory activity of the extract was measured with phlogistic agents (*viz.* histamine, 5-HT) which act as mediator of inflammation. The paw oedema was induced in rats by sub plantar injection of freshly prepared histamine (1 mg/kg) and serotonin (1 mg/kg) solutions respectively and the paw oedema was measured as mentioned earlier.¹¹⁾

Cotton pellets-induced granuloma

The rats were divided into five groups (n = 6). The cotton pellet granuloma model investigated the proliferation phase of inflammation⁷⁾. The extract of different doses (50,100 and 200 mg/kg) and Indomethacin 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 weight¹²⁾. 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 100 and 400 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 studies¹⁴⁾. 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 oxaloacetate transaminase level (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assayed by method 15⁸⁾ (Bergmeyer and Bernt 1974). The alkaline phosphatase activity in the serum was measured according to the procedure of King (1965a) method by spectrophotometrically 16⁹⁾. Further, liver biochemical parameters were estimated by methods described in estimation of biochemical parameters.

Biochemical assays and hematological studies

Blood was obtained from the tail vein, 24 h after last dose. For the total count blood was drawn into RBC or WBC pipettes, diluted and counted in a Neubauer counting chamber. The hemoglobin concentration was determined by Sahli's Hemoglobinometer method. Differential count of leukocytes was done on a freshly drawn blood film using Leishman's stain. Hemoglobin content¹⁷⁾ RBC and WBC count¹⁸⁾ and differential leukocyte count¹⁹⁾ was estimated from the peripheral blood of normal, control and treated animal groups.

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 20¹³⁾ 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 Ellman¹⁴⁾. GSH estimation is based on the development of yellow color when 5,5'-dithiobis (2-nitro benzoic acid) di-nitrobenzoic acid was added to compounds containing sulphhydryl group. Proteins were estimated by the method of Lowry *et al.*, (1951) using bovine serum albumin as the standard¹⁵⁾.

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