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Anti-pyretic and anti-inflammatory activity of chloroform extract of *Croton roxburghii* in standard animal models

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SUMMARY

The chloroform extract of *Croton roxburghii* (Family: Euphorbiaceae) was evaluated for its antipyretic effects in Brewer's yeast induced hyperthermia in rats. The anti-inflammatory effect of the *Croton roxburghii* was also evaluated by using carrageenan, dextran, histamine, serotonin induced rat paw oedema and cotton pellet induced granuloma (chronic) models in rats. The chloroform extract of *Croton roxburghii* (CECR) exhibited significant anti-pyretic and 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 Indomethacin (standard drug) produced 61.33% of inhibition. The extract exhibited significant anti-inflammatory activity in dextran induced paw edema in a dose dependent manner. In the chronic model (cotton pellet induced granuloma) the CECR (200 mg/kg) and Indomethacin (10 mg/kg) showed decreased formation of granuloma tissue by 52.32% and 56.32% respectively. The extract also exhibited a significant antipyretic response in Brewer's yeast induced pyrexia in rats. Thus, the present study revealed that the CECR exhibited significant antipyretic and anti-inflammatory activity in the tested animal models.

Key words: *Croton roxburghii*; Carrageenan induced paw edema; Cotton pellet induced granuloma

INTRODUCTION

Medicinal plants have been used since ancient times as medicines for the treatment of diseases and still play a key role in world health. The chemical diversity of plants has made them one of the main sources for the isolation of bioactive organic compounds (Basso *et al*., 2005). However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use. Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary. The genus croton is a large one with 750 species of trees and shrubs distributed in tropical and subtropical regions of the both hemispheres. The croton family is rich in active alkaloids and several species of croton are well known as medicinal plants. *Croton roxburghii* (Family: Euphorbiaceae) is a medium sized tree. Different part of the plant was used by the local tribal (Andhra Predesh) people of India for the treatment

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of various disorders such as antifertility, snake venom, fever and wounds (Rama and Henry, 1997). Active constituents of the genus croton include proanthocyanidin, tannins, terpenes, alkaloids, flavones, and phenolic compounds (Cai *et al*., 1991; Hermandez and Delgado, 1992; Porras-Reyes *et al*., 1993). However, fewer reports are available with respect to the pharmacological properties of the plant. Hence, the present study was undertaken to evaluate the effect of the chloroform extract of *Croton roxburghii* (CECR) for its anti-pyretic activity by yeast-induced hyperpyrexia in rats. The extract also evaluate for the anti-inflammatory activity in acute and chronic models in rats. The effect of the extract was also compared with that of the standard drug, Indomethacin, a well-known anti-inflammatory agent.

MATERIALS AND METHODS

Plant material

The plant *Croton roxburghii* (Family: Euphorbiaceae) was collected in the month of May 2006 from Nagarjunna Sagar Hills, Andhra Pradesh, India. The plant material was taxonomically identified by the Botanical survey of India, Coimbatore, Tamilnadu, India and the voucher specimen TSK-3 was retained in our laboratory for future reference.

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), dextran (Sigma, USA), paracetamol (Torrent, Bombay) and Indomethacin (IPCA, Bombay).

Preparation of extract

The dried powdered plant (bark) material was extracted with 80% methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained. The semi solid mass was further partitioned with water and successively extracted with chloroform, ethyl acetate, and methanol. The solvent was completely removed under reduced pressure. The yield of chloroform, ethyl acetate, and methanol extract were 5.2, 3.4 and 6.2% w/w respectively. The chloroform extract, at the different doses of 50, 100 and 200 mg/kg was suspended in 0.25% carboxy methyl cellulose solution and Indomethacin (10 mg/kg) in saline was used for the present study.

Phytochemical study profile

The active part the chloroform extract subjected to chemical analysis to determine the classes of compounds present in it. The powdered bark of C*roton roxburghii* treated with ammonium hydroxide the resulting brownish red viscous mass was extracted with chloroform. The chloroform extract was concentrated under reduced pressured *in vacuo* and refrigerated. The resulting mass obtained from chloroform extract was chromotogramed by different solvent system. From the silica gel G thin layer chromatography using the solvent system n-butanol -acetic acid -water (4: 1: 1) was selected for the study and a single sport observed on TLC plate and spraying with dragendroff's reagent gave positive test for alkaloid. The R_f value of the resulting compound was noted (R_f = 0.65). The material was crystallized from hot methanol. The crystalline white compound, it is also shown a single spot on TLC, after spraying with Dragendroff's reagent gave positive test of alkaloid. The mass spectral studies on the compound indicates that the molecular ion peak (m/z 369: 58 (100). The isolated compound IR ($CHCl_3$) 3011, 2943, 2829, 2786, 1600, 1135, 1090 cm^{-1} data suggested to a taspine already reported in the literature and the structure was identified and comparison with the published data (Vaisberg *et al*., 1989). The present study carried out the extract; it is due to the paucity of the sample of the compound.

Animals

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*.

Toxicity study

An acute toxicity study relating to the determination of LD_{50} was performed (Litchfield and Wilcoxon, 1949).

Antipyretic activity

Study on normal body temperature

Rats of either sex were divided into four groups, comprising six in each group for these experiments. The rectal temperature of each rat was measured initially and at every one hour interval for five hours after administration of both 0.25% carboxy methyl cellulose solution (control) and chloroform extract of the *Croton roxburghii* at the dose of 50, 100, and 200 mg/kg respectively.

Yeast induced hyperpyrexia in rats

The antipyretic effect of CECR was evaluated in Wistar albino rats by the method of Loux *et al*., 1972). The rats were divided into five groups containing six in each and trained to remain quite in a restraint cage. A thermister probe was inserted 3 to 4 cm into the rectum and fastened to the tail adhesive tape. After measuring the basal rectal temperature, the animals were given subcutaneous injections of 10 ml/kg of 15% w/v yeast suspended in a 0.5% w/v methylcellulose solution. When the temperature was at peak (18 h after yeast injection) the rectal temperature recording were repeated. Those animals that showed a rise in rectal temperature of more than 1.2 °C were used for the present study. The extract (CECR) was administrated interaperitoneally (i.p.) at 50, 100, and 200 mg/kg to three groups of rats respectively. Similar volumes (5 ml/kg) of normal saline were injected to the control groups. The fifth group of rats received the antipyretic agent, paracetamol, at a dose of 150 mg/kg i.p. Rectal temperatures were recorded immediately before CECR, Paracetamol or vehicle administration and again at 20, 21, 22, and 23 h after yeast injection.

Anti-inflammatory activity

Carrageenan - induced rat paw edema

The rats were divided into 5 groups (n = 6). Acute inflammation was produced by the subplantar administration of 0.1 ml of 1% carrageenan in normal saline in the right paw of the rats. Different groups were treated with CECR (50, 100 and 200 mg/kg), Indomethacin (10 mg/kg) and control vehicle were administered orally. The paw volume was measured at 0 h and 3 h after carrageenan injection using plethysmometer (Winter *et al*., 1957). The animals were pretreated with the extract 1 h before the administration of carrageenan. The extract and the standard drug used for this study were prepared in the same manner as mentioned earlier. The ratio of the anti-inflammatory effect of CECR was calculated by the following equation: anti-inflammatory activity (%) = $(P_1 - P_2/ P_1) \times 100$, where P_1 represents the paw volume of control rats, and P_2 represents the paw volume of the treated groups.

Dextran induced paw edema

The animals were treated as in case of carrageenan induced paw edema models, except that in place of carrageenan, dextran (0.1 ml, 1% w/v in normal saline) was used (Parmar and Ghosh, 1978).

Mediator induced inflammation

The anti-inflammatory activity of the extract was measured with phlogistic agents (*viz*. histamine, 5-hydroxy tryptamine hydrochloride) which are act as mediator of inflammation. The paw edema was induced in rats by sub plantar injection of freshly prepared histamine (1 mg/kg) and serotonin (1 mg/kg) solutions respectively. The paw edema was measured as mentioned earlier (Parmer and Ghosh, 1978).

Cotton pellets-induced granuloma

The inflammation was produced by cotton pellets induced granuloma (Winter *et al*., 1962). The rats were divided into five groups (n = 6). After shaving the fur, the rats were anaesthetized and 10 mg of sterile cotton pellets were inserted, one in each axilla. The CECR (50, 100 and 200 mg/kg, p.o.), Indomethacin (10 mg/kg, p.o.), and control vehicle were administered orally for 7 consecutive days from the day of cotton pellet implantation. The animals were anaesthetized on the eighth day and cotton 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 a measure of granuloma formation.

Statistical analysis

The results are expressed as mean ± S.E.M. The statistical analysis was performed by ANOVA test.

RESULTS

The chloroform extract of *Croton roxburghii* was subjected to chromotogramed by using different solvent system. It was determined by identification reactions based on the chemical group to be determined or thin layer chromatography. From the thin layer chromatography the chloroform extract of *Croton* the spot from the chromatogram med to be alkaloid by using spraying reagent dragendroff's gave positive test for alkaloid. Due to diminutive quantity of the isolated compound, hence, we undertaken the crude part of the chloroform extract *Croton roxburghii* was carried out the present study.

The CECR was evaluated for antipyretic and anti-inflammatory activity in standard experimental animal models and the results are summarized in Table 1, 2, 3, 4, and 5. The chloroform extract exhibited significant anti-pyretic anti-inflammatory activity at the tested doses of 50, 100 and 200 mg/kg in a dose dependant manner. Effect of the CECR on the

Table 1. Effect of chloroform extract of *Croton roxburghii* on normal body temperature in rats

		Rectal temperature in °C before and after the medication					
Treatment	Dose mg/kg (i.p.)	0 h	1 h	2 h	3 h	4 h	5 h
Normal Saline (0.9% NaCl w/v)	5 ml/kg	37.2 ± 0.1	37.2 ± 0.2	37.3 ± 0.2	37.2 ± 0.3	37.4 ± 0.3	37.2 ± 0.3
CECR	50	37.2 ± 0.3	37.2 ± 0.2	36.6 ± 0.2	36.8 ± 0.4	37.2 ± 0.5	37.4 ± 0.17
CECR	100	37.1 ± 0.2	37.2 ± 0.3	36.4 ± 0.3	36.3 ± 0.2	36.2 ± 0.6	36.1 ± 0.2
CECR	200	37.2 ± 0.2	36.5 ± 0.5	35.8 ± 0.4	36.2 ± 0.4	35.9 ± 0.2	35.7 ± 0.2

Each value represents mean ± S.E.M. of six rats. Statistical significance was compared with normal.

Table 2. Effect of chloroform extract of *Croton roxburghii* on yeast-induced pyrexia in rats

	Rectal temperature in °C before and after the medication					
Treatment	0 h	19 h	20 h	21 h	22 h	23 h
Vehicle 5 ml/kg	37.2 ± 0.2	39.3 ± 0.2	39.4 ± 0.2	39.2 ± 0.2	39.3 ± 0.3	39.3 ± 0.2
CECR 50 (mg/kg)	37.1 ± 0.2	39.3 ± 0.1	38.5 ± 0.3	38.1 ± 0.4	37.7 ± 0.5	37.5 ± 0.1
CECR 100 (mg/kg)	37.3 ± 0.2	39.4 ± 0.2	37.9 ± 0.2	37.2 ± 0.2	36.5 ± 0.6	36.2 ± 0.2[a]
CECR 200 (mg/kg)	37.2 ± 0.2	39.2 ± 0.3	37.3 ± 0.3	36.4 ± 0.4[a]	36.2 ± 0.2[a]	36.7 ± 0.3[a]
Paracetamol 150 (mg/kg)	37.1 ± 0.3	39.6 ± 0.3	37.2 ± 0.3	36.4 ± 0.3[a]	36.1 ± 0.3[a]	37.3 ± 0.3

Each value represents mean ± S.E.M. of six rats. [a] $P < 0.01$ Statistically different from the rectal temperature with 19 h yeast injection.

Table 3. Effect of the *Croton roxburghii* extract on carrageenan and dextran induced pedal oedema

Treatment	Dose (mg/kg)	Paw volume (ml)	Inhibition (%)
Carrageenan control	0	0.732 ± 0.063	-
Indomethacin	10	0.283 ± 0.024	61.33
CECR	50	0.446 ± 0.035	39.07
CECR	100	0.403 ± 0.028	44.90
CECR	200	0.327 ± 0.017	55.32
Dextran control	0	0.629 ± 0.047	-
Indomethacin	10	0.247 ± 0.015	60.73
CECR	50	0.426 ± 0.041	32.27
CECR	100	0.360 ± 0.030	42.76
CECR	200	0.295 ± 0.027	53.10

Values are mean ± S.E.M. (n = 6). Experimental groups were compared with control $P < 0.001$.

Table 4. Effect of *Croton roxburghii* extract on mediators like histamine and 5-HT induced pedal oedema in rats

Treatment	Dose (mg/kg)	Paw volume (ml)	Inhibition (%)
Histamine control	0	0.544 ± 0.045	-
Indomethacin	10	0.207 ± 0.022	61.94
CECR	50	0.328 ± 0.030	36.70
CECR	100	0.298 ± 0.024	45.22
CECR	200	0.251 ± 0.021	53.86
Serotonin control	0	0.623 ± 0.045	-
Indomethacin	10	0.256 ± 0.017	59.73
CECR	50	0.407 ± 0.035	34.67
CECR	100	0.361 ± 0.033	43.01
CECR	200	0.295 ± 0.020	52.64

Values are mean ± S.E.M. (n = 6). Experimental groups were compared with control $P < 0.001$.

Table 5. Effect of the *Croton roxburghii* extract on cotton-pellets induced granuloma in rats

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	Inhibition (%)
Control	0	47.4 ± 3.2	-
Indomethacin	10	20.7 ± 1.5	56.32
CECR	50	33.9 ± 1.7	28.48
CECR	100	27.9 ± 1.8	41.13
CECR	200	22.6 ± 2.1	52.32

Values are mean ± S.E.M. (n = 6). Experimental groups were compared with control $P < 0.001$.

normal body temperature in rats is presented in Table 1. This effect was maximal at the doses of 100 and 200 mg/kg in a dose dependent manner and it caused significant lowering of body temperature up to 5 h after its administration.

As displayed in Table 2, the extract of *Croton roxburghii* at the dose 100 and 200 mg/kg caused a significant lowering in rectal temperature of hyperthermic rats. The decrease in rectal temperature still existed when assessment was made 3 h after test drug administration and efficacy was comparable to that of standard drug paracetamol at the dose of 150 mg/kg.

As shown in Table 3 - 5, the chloroform extract showed maximum inhibition of 55.32% at the dose of 200 mg/kg after 3 h of drug treatment in

carrageenan induced paw edema, whereas the standard drug showed 61.33% of inhibition. In dextran induced paw edema the chloroform extract showed significant inhibition (32.27%, 42.76%, and 53.1%) in a dose dependent manner as compared with control. As shown in Table 4, in case of histamine and serotonin induced paw edema, the chloroform extract showed 53.86% and 52.64% of inhibition at the dose of 200 mg/kg whereas indomethacin showed 61.94% and 59.73% of inhibition, respectively. As shown in Table 5, in the chronic model (cotton pellet induced granuloma), the CECR (200 mg/kg) and standard drug showed decreased formation of granuloma tissue at 52.32% and 56.32% ($P < 0.001$), respectively.

DISCUSSION

The potential of the CECR for its antipyretic and anti-inflammatory effect was investigated. Since antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis (Vane, 1987), the yeast-induced hyperthermia in rat model was employed to investigate the antipyretic activity of the chloroform extract of *Croton roxburghii*. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulate the set point at which body temperature is maintained. In fever this set point elevated and the drug like paracetamol do not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature (Gilman, 1990). The present investigation indicated that the chloroform extract of *Croton roxburghii* shown significant anti-pyretic effect. The result seems to support the view that the chloroform extract has some influence on prostaglandin-biosynthesis because prostaglandin is believed to be a regulator of body temperature (Milton, 1982).

The CECR was also evaluated for its anti-inflammatory activity in acute and chronic models. Significant anti-inflammatory activity was observed for CECR in both carrageenan and dextran induced edema models. The chloroform extract showed maximum inhibition of 55.32% at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw edema. The carrageenan-induced edema is the preliminary screening models for searching for potential anti-inflammatory compounds. Edema formation is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the later phase (Castro *et al*., 1968; Alcarz and Jimenez, 1988). The CECR also exhibited significant anti-inflammatory property in dextran induced paw oedema model (Parmer and Ghosh, 1978). Dextran induced paw edema is known to be mediated both by histamine and serotonin. Dextran induces fluid accumulation, which contains little protein and few neutrophils, whereas carrageenan induces protein rich exudation containing large number of neutrophils. The extract effectively suppressed the inflammation produced by both carrageenan and dextran.

Mast cell mediators include arachidonic acid products, biogenic amines, chemo attractants, cytokines, and growth factors, neuropeptides, proteoglycans, and proteolytic enzymes. (Schwartz, 1987; Serafin and Austen, 1987; Kobayashi *et al*., 2000). Mast cells are increasingly recognized as key cells in the development of a number of inflammatory diseases (Theoharides, 1996; Marone *et al*., 2002) including the skin, (Maurer *et al*., 2003) joints, (Wasserman, 1984; Tetlow and Woolley, 1995) and urinary bladder, (Theoharides, 2002) that worsen by stress. The extract also effectively suppressed the inflammation produced by mediator's viz. histamine and serotonin (5-HT). The CECR exhibited a significant inhibition against histamine and 5-HT induced hind paw edema, which indicates that the extracts exhibits its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators *viz*.

histamine, serotonin and prostagandins might be involved in inflammation. So, it may be suggested that its anti-inflammatory activity is possible backed by its antihistaminic 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 granulumatous tissues (Dunne, 1990). Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The CECR 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 (Arrigoni-Maratellie, 1988). From these observations it can be indeed inferred that the endogenous chemical substances liberated during pain, inflammation and fever like histamine, serotonin and arachidonic cascade metabolites are inhibited to produce various activities highlighted in this study.

The present screening permits to support, in part, the attributed properties of the evaluated species, often reported to treat ailments in which inflammatory processes could be involved. Taspine alkaloid was also isolated from the same species like C*roton lechleri* (Vaisberg *et al*., 1989). The biological activity of taspine was also reported with respect of anti-inflammatory activity (Perdue *et al*., 1979), a reverse transcriptase inhibitory activity and wound healing activities (Vaisberg *et al*., 1989). From the studies the presence of alkaloid taspine in C*roton roxburghii* was may be responsible for the antipyretic and anti-inflammatory activity.

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