ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF Trichosanthes *bracteata* FRUITS IN ANIMAL MODEL

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Summary

The aqueous extract of the fruits of *Trichosanthes bracteata* was investigated for its anti-inflammatory and analgesic activities in animal models. *Tricosanthes bracteata* is widely used in traditional medicine against cathartic asthma and carminative, purgative with ginger oil used for the relief of long standing and recurrent attack of headache. The extract 100, 200 and 300 mg/kg body weight reduced significantly, the formation of oedema induced by carrageenan and histamine. In the acetic acid induced writhing model, the extract showed a good analgesic effect characterized by reduction in the number of writhes when compared with the control. The extract causes dose dependent decrease of licking time and licking frequency in albino rat injected with 2.5% formalin, signifying its analgesic effect. These results were also comparable to those of indomethacin and cyproheptadine, the reference drug used in this study. Acute toxicity test showed that the plant caused 75% mortality in rats at the dose of 450 mg/kg and above, hence it is a toxic plant, therefore caution should be exercised in its use for medicinal purpose.

Keyword: *Tricosanthes bracteata*, Cucurbitaceae, Analgesic and Anti-inflammatory activity, Indomethacin, Toxicity.

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Introduction

Despite the progress made in medical research for the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases remain one of the world's problem[1-3]. Inflammation is the response of living tissue to injury. It involves a complex array of enzyme activation mediator release, extravasations of fluid, cell migration, tissue breakdown and repair[4,5]. Inflammation has become the focus of global scientific research area because of its implication in virtually all human and animal diseases. Modern system of anti-inflammatory drugs (NSAID) have various side effects like tolerance and dependence induced by opiates the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases[6,7]. Therefore, new anti-inflammatory and analgesic drugs lacking these side effect are being researched as alternative to NSAID and opiates[6,8]. Attention is being focused on the investigation of the safety and efficacy of plant based drugs used in traditional because they are economically have less side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies[3,6,8]. *Tricosanthes bracteata* (Cucurbitaceae) is native to topical and southern India as well as the North India. The genus Tricosanthel consists of 11 species most of which are indigenous and well distributed in India[9-11]. *Tricosanthes bracteata* is widely used in traditional medicine against relief of headache, bath oil and cathartics. *Trichosanthes bracteata* is considered to be medicinally important in several traditional system of ayurvedic medicine; the fruit is used in the treatment of asthma and eczema. In the Unani system of medicine, the fruits are used as a carminative, purgative and an abortifacient to lessen inflammation, cure migraines and reduce heat of the brain, as a treatment for leprosy and rheumatism as well as others[12-14]. The roots of plant are used to treat lungs diseases in cattle and for the treatment of diabetics and headaches[13]. It has also reported the use of this plant in curing bronchitis and the application of seed paste for hoof and mouth disease in cattle's. Phytochemical screening showed that from the pulp of the fruit has been isolated a bitter principle resembling to colcynthin to some extent and named Tricosanthin[15]. Nevertheless the anti-inflammatory and analgesic activities and role of these component i.e. action of the plant are not yet clearly defined. Therefore, *Trichosanthes bracteata* fruits were investigated for their analgesic and ant-inflammatory properties in order to identify the main compound in this pharmacological activity.

Materials And Methods

Plant Material

The fruit of *Trichosanthes bracteata* was collected in June 2009 in the East Bihar province of India. The plant were identified by flower characteristics and later validated by Department of Botany, SDAN College, Jehanabad, Bihar (Voucher no. 231/09) and specimen was deposited in the herbarium of the college. The fruits were dried at room temperature and later ground to powder and preserved in dark glass containers until their extraction.

Extraction of Plant Material

The ground plant material (200g) was shaken in distilled water for 48h on an orbital shaker at room temperature. The extraction was filtered using a Buckner funnel and whatman no. 1 filter paper. The filtrate was concentrated under reduced pressure at 40^{o}C and later lyophilized using freeze drying system for biological investigations. The extract yielded 13.25g of the extract from the aqueous solution and used for the further experiments.

Animals

The animals used in this study were male albino rats weighing between 100 to 250g. They were maintained at the experimental animal house of the SDAN College, Jehanabad, Bihar, India. They were kept in rat cages and feed on commercial rabbit cubes (SAMS Feeds, Bihar Sharif) and allowed free access to fresh water in bottles *ad libitum*. All experimental protocols were in compliance with Magadh University, animal ethical committee as well as international accepted principle for laboratory animal use and care.

Chemicals and Drugs

Carrangeenam, acetic acid and Tween 80 all from Sigma-Aldrich, Denmark were the chemicals used. The standard drugs used were indomethacin and histamine supplied by the Blessings Pharmaceuticals India, Nagpur, Maharastra. All the chemicals and drugs used were analytical grade.

Acute Toxicity Test

The acute toxicity of *Trichosanthes bracteata* aqueous extract was determined in rats according to the method of Hilay *et al* [16]. Rats fasted for 16h were randomly divided in to groups of six rats per group. Graded dose of the extract (200, 400, 800, 1600 and 3200 mg/kg) were separately administered to the rats in each of the group by means of standard needles. All the animals were then allowed free access to food and water and observed over a period of 48h for signs of acute toxicity. The number of deaths with in this period was recorded.

Anti-inflammatory Activities

Carrageenan induced rat Paw Oedema

Twenty rats were used in this study and they were divided into five groups per group. Each group one of the following treat with plant extract (100, 200, 300 mg/kg body weight) Indomethacin (10 mg/kg body weight) as vehicle control (0.9% normal saline in 3% Tween 80 (2 ml/kg), which were administered orally. Acute inflammation was produced by the sub-planar administration of 0.1ml of carrageenan in normal saline that contains Tween 80 in the right paw of rats. The paw diameter was measured at 0, 60, 120 and 180 min after carrageenan injection using a micrometer screw gauge. Increase in the linear diameter of the right hind paws were

taken as indication of paw oedema. Oedema was assessed in terms of the difference in the zero time liner diameter of the injected hind paw and its linear diameter at time (*i.e.* 60, 120, 180min) following carrageenan administration. The anti-inflammatory effect of the extract was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1\text{-}D/C)\ 100$$

Where D denotes the percentage difference in paw diameter after the extract was administered to the rats and denoted the percentage difference of diameter in the control groups. The percentage inhibition of the inflammation was calculated from the formula:

$$\text{\% inhibition} = DO\text{-}DT/DO \times 100$$

Where DO was the average inflammation (hind paw oedema) of the control groups of rats at a given time and DT was the average inflammation of the drug treated (extract or reference indomethacin) rats at the same time [17].

Histamine induced rat paw oedema

Using the method of Perianayagam *et al*, the paw oedema was produced by sub-planer administration of 0.1% freshly prepared solution of histamine into the right hind paw of rats. Twenty rats were divided into groups of five rats per group and each group received one of the following treatment plant extract (100, 200, 300 mg/kg body weight) indomethacin (10 mg/kg body weight) or vehicle control (0.9% normal saline in 3% Tween 80 (2 ml/kg) which were administered orally. The paw diameter were recorded immediately before administrating the histamine injection (0 min) and every 60 min for 180 hours after the histamine injection. The drug and extracts were similarly administered 60 min before eliciting paw oedema. The anti-inflammatory effect of the extract was calculated using the formula given above.

Analgesic Activity

Formalin Test

Formalin test was conducted as described by Dharmasiri *et al*. Male rats were treated respectively with 100, 200 and 300 mg/kg of *Trichosanthes bracteata* extract with 10 mg/kg of indomethacin and 2 ml/kg of normal saline. Thirty minutes later, the rats were injected with 0.5ml of 2.5% formalin into the right hand foot pad and were immediately placed in a transparent plastic cage separately. The licking time and frequency of the injected paw were recorded for 30 min[14].

Statistical Analysis

The observations were expressed in mean ±S.D. The difference in response to test drug was determined by one way analysis of variance followed by Doncan's test $P<0.05$ was considered significant.

Results

Acute Toxicity

Oral administration of graded doses (450, 900, 1800 and 3600 mg/kg) of the aqueous extract of *Trichosanthes bracteata* to rats produce significant change in behavior with resulting deal of all the animals in this group. No mortality was however recorded for 300 mg/kg dose after 72 h of administering the extract to the animals.

Carrageenan induced edema in rats

The effect of indomethacin (10 mg/kg) on carrageenan induced paw oedema was mast pronounced 180 min after carrageenan injection while the 100, 200, and 300 mg/kg dose of the extract showed highest activity at 120 min. The anti-inflammatory effect of the extract were most potent with its lowest dose (Table 1).

Table 1. Anti-inflammatory activities of aqueous extract of *Trichosanthes bracteata* fruits and indomethacin on carrageenan induced oedema in the right hind limb of rats.

Time (min)	Control	Extract (mg/kg)			Indomethacin 10 mg/kg
		100	200	300	
60	11.9±1.9	12.0±3.1	11.8±2.7	8.9±2.6	7.9±3.1
120	12.8±2.3	10.1±2.8	11.9±3.1	7.7±0.9	6.5±3.2
130	14.3±2.8	8.9±2.4	7.3±0.9	5.4±0.7	0.9±0.2

Mean±SD n=4

Histamine induced paw edema

The effect of the extract 200, 300 mg/kg and the reference drug on histamine induced paw oedema was most significant at 180 min after histamine injection while 100 mg/kg dose of the extract showed its highest activity at 120 min indicating that the extract may be more potent as compared with the reference drug used. The anti-inflammatory activity of extract also exhibited a dose dependant trend (Table 2).

Table 2. Anti-inflammatory activities of aqueous extract of *Trichosanthes bracteata* fruits and cyproheptadine on histamine induced oedema in the right hind limb of rats.

Time (min)	Control	Extract (mg/kg)			Cyproheptadine
		100	200	300	
60	20.9±1.9	21.1±0.9	18.8±1.6	16.7±1.2	13.8±1.3
120	29.8±1.8	20.9±1.2	15.6±1.4	8.9±1.8	10.8±0.8
180	31.2±1.5	18.9±0.8	9.1±3.1	2.8±1.3	0.9±0.3

Mean ±S.D n=4

Formalin induced paw licking test

The treatment with the extract at 100, 200, 300 and indomethacin at 10 mg/kg caused significant decreases in licking time and frequency of licking of the formalin injected paw rats (Table 3). The 100 mg/kg dose showed the highest activity.

Table 3. Analgesic effect of aqueous extract of *Trichosanthes bracteata* fruits and indomethacin on rats using formalin

Control		Extract (mg/kg)			Indomethacin (10 mg/kg)
		100	200	300	
Duration (Sec)	13.1±1.9	8.2±0.9	7.9±0.8	6.9±0.8	4.9±0.7
Frequency (30 min)	21.4±2.8	7.9±0.4	12.9±1.4	13.9±1.2	13.9±1.7

Control $P<0.05$

Discussion

Carrageenan induced oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic the first phase (60 min) involves the release of serotonin and histamine while the second phase (over 60 min) is mediated by prostaglandins, the cyclo-oxygenase products, and the continuing between the two phase is provided by kinins[5]. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation[18]. This study has shown that the aqueous extract of the fruits of the *Trichosanthes bracteata* possessed a significant anti-oedematoganic effect paw oedema induced by carrageenan. Since carrageenan induced anti-inflammation model is a significant test for anti-inflammatory agent acting by the mediators of acute inflammation[9]. The results of this study showed that *Trichosanthes bracteata* can be effective in acute inflammatory disorder.

The extract also caused pronounced reduction in the oedema produced by histamine. This results tends to suggest that anti-inflammatory activity of extract is possibly supported by its anti-histamine property. The antihistamic effect of the extract increased with increase in the dose of the extract hence the effect is dose dependent. The antihisamic effect of the 300 mg/kg dose of extract is comparable to cyproheptadine an antihistamic and antiserotonergic agent. Histamine is an important inflammation mediator, potent vasodilatation substance and also increases the vascular permeability. Since the extract effectively suppressed the oedema produced by histamine.

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibers by formalin while the pain in the late phase was due to inflammatory mediators like histamine[6]. This test is believed to be a mere valid analgesic model. In this study the extract caused a dose dependent decrease in licking time and licking frequency by the rats injected with formalin signifying the analgesic effect of the extract. Phytochemical screening showed that a bitter principle resembling colcynthin to some extant and named Trichosanthin glycosides were isolated from the fruits of *Trichosanthes bracteata*[12]. NSAID such as indomethacin used in this study are known to inhibit cyclo-oxygenase enzyme I and II which are implicated in the production of inflammation and analgesic activities exhibited by this extract was similar to that of indomethacin which suggest that the plants activity may be mediated by cyclo-oxygenase I and II inhibition.

Conclusion

The results of the study have demonstrated that aqueous extract of *Trichosanthes bracteata* fruits showed strong anti-inflammatory and analgesic activities on the animal models investigated. Acute toxicity test showed that the plant caused 75% mortality in rats at the dose ranges of 450 to 3200 mg/kg. Though the study has provided some justification for the folkloric use of the plant in several communities for condition such as headache, cathartics and bath oil but caution should be exercised in its used for medicinal purpose. The preliminary results of present

investigations appear to indicate that *Trichosanthes bracteata* is a medicinal plant and have higher anti-inflammatory and analgesic property.

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