Evaluation of Anti-inflammatory Activity of Chloroform Extract of *Bryonia laciniosa* in Experimental Animal Models

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The anti-inflammatory effect of the leaves of *Bryonia laciniosa* was evaluated using carrageenan, dextran, histamine, serotonin induced rat paw oedema and cotton pellet induced granuloma (chronic) models in rats. In mice, carrageenan peritonitis test was performed for the extract by oral administration. The chloroform extract of *Bryonia laciniosa* (CEBL) exhibited significant anti-inflammatory effect at the dose 50, 100 and 200 mg/kg. Maximum inhibition (52.4%) 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 exhibited significant anti-inflammatory activity in dextran induced paw oedema in a dose dependent manner. 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 CEBL (200 mg/kg) and standard drug showed decreased formation of granuloma tissue by 50.1 and 57.3% ($p<0.001$) respectively. The extract also inhibited peritoneal leukocyte migration in mice. Thus, the present study revealed that the chloroform extract of *Bryonia laciniosa* exhibited significant anti-inflammatory activity in the tested models.

Key words *Bryonia laciniosa*; carrageenan; cotton pellet induced granuloma; anti-inflammatory activity

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus the present investigation to carried out to evaluate the anti-inflammatory potential of *Bryonia laciniosa*. The plant *Bryonia laciniosa* LINN (Family: Cucurbitaceae) is a shrub found wildly in India, Philippines and some parts of Africa. It has considerable reputation as a potent adjunct in the treatment of various ailments such as jaundice, inflammation and fever.[1] The leaf extract of the plant is being used as a cathartic and hot aqueous extract of the roots and seeds has an effect on conception in barren women.[1] Goniothalamin, punicic acid and lipids were previously isolated from the whole plant of *Bryonia laciniosa*.[2,3] 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 *Bryonia laciniosa* (CEBL) for anti-inflammatory activity in acute and chronic models in rats. Leukocyte infiltration using carrageenan peritonitis test was also studied in extract treated mice. 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 *Bryonia laciniosa* (Family: Cucurbitaceae) was collected in the month of April 2001 from the Kolli Hills, Tamilnadu, India. The plant material was taxonomically identified by the Botanical survey of India, Shibpur, Howrah and the voucher specimen GMS-25 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, U.S.A.), 5-hydroxy tryptamine hydrochloride (serotonin) (Sigma, U.S.A.), dextran (Sigma, U.S.A.), and indomethacin (IPCA, 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 14.25%). The extract showed positive test for steroids, triterpenoids and lipids. The extract at the different doses of 50, 100 and 200 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*.

Toxicity Study An acute toxicity study relating to the determination of LD_{50} was performed.[4]

Carrageenan-Induced Rat Paw Oedema 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. The different groups were treated with CEBL (50, 100, 200 mg/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.*) and control vehicle were administered orally. The paw volume was measured at 0 and 3 h after carrageenan injection using plethysmometer.[5] The animals were pretreated with the extract 1 h before the administration of carrageenan. The extract and the standard used for this study were prepared in the same manner as mentioned earlier. The ratio of the anti-inflammatory effect of CEBL was calculated by the following equation: anti-inflammatory activity (%)$=(1-D/C)\times100$, where D represents the percentage difference in paw volume after CEBL was administered to the rats, and C represents the percentage difference of volume in the control groups.[8]

Dextran Induced Paw Oedema The animals were treated as in case of carrageenan induced paw oedema mod-

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els, except that in place of carrageenan, dextran (0.1 ml, 1% w/v in normal saline) were used.[5)]

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.[6)]

Cotton Pellets-Induced Granuloma The rats were divided into four 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 CEBL (50, 100, 200 mg/kg, *p.o.*) and 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 measure of granuloma formation.[6)]

Mouse Carrageenan Peritonitis The mice were divided into 5 groups ($n=6$). Inflammation was induced by modification of the technique as previously described.[7)] The extract was administered orally at doses of 50, 100 and 200 mg/kg and indomethacin at a doses of 10 mg/kg *p.o.* and carrageenan (0.25 ml, 0.75% in saline) was injected intraperitoneally 1 h later and after 4 h the animals were sacrificed by cervical dislocation for further investigation. Ca^{2+} and Mg^{2+} free phosphate buffered saline was used during the collection of peritoneal fluids. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined by microscopic counting.[8,9)] The percentage of the leukocyte inhibition was calculated by the following equation: $\%=(1-T/C)\times100$, where T represents the treated groups leukocyte counts and C represents the control groups leukocyte counts.

Neutrophils changes was calculated by the following equation: neutrophils changes=neutrophils counts of treated groups/neutrophils counts of control groups×100.

Statistical Analysis The results are expressed as mean±S.E.M. The statistical analysis was performed by analysis of variance (ANOVA) test.

RESULTS

The chloroform extract of *Bryonia laciniosa* was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are tabulated in Tables 1, 2 and 3. The chloroform extract exhibited significant anti-inflammatory activity at the tested doses of 50, 100, and 200 mg/kg in a dose dependant manner.

As shown in Table 1, the chloroform extract showed maximum inhibition of 52.4% at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas the standard drug showed 62.1% of inhibition. In dextran induced paw oedema the chloroform extract showed significant inhibition (34.4, 43.2, 52.1%) in a dose dependent manner as compared with control. As shown in Table 2, in case of histamine and serotonin induced paw oedema, the chloroform extract showed 54.9 and 52.3% of inhibition at the dose of 200 mg/kg whereas indomethacin showed 59.8 and 59.5% of inhibition respectively. As shown in Table 3, in the chronic model (cotton pellet induced granuloma), the CEBL (200 mg/kg) and standard drug showed decreased formation of granuloma tissue at 50.1 and 57.3% ($p<0.001$), respectively.

The CEBL also inhibited peritoneal leukocyte migration (36.4, 56.9, 77.4% at the doses of 50, 100, 200 mg/kg respectively) whereas the inhibition produced by indomethacin (10 mg/kg) 36.1% was found to be in carrageenan induced peritonitis model as shown in Table 4.

Table 1. Effect of the *Bryonia laciniosa* Extract on Carrageenan and Dextran Induced Pedal Oedema

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Carrageenan control	0	0.736±0.070	—
Indomethacin	10	0.320±0.030	62.1
CEBL	50	0.452±0.040	38.6
CEBL	100	0.408±0.030	43.0
CEBL	200	0.350±0.030	52.4
Dextran control	0	0.625±0.050	—
Indomethacin	10	0.242±0.020	61.3
CEBL	50	0.420±0.040	34.4
CEBL	100	0.355±0.040	43.2
CEBL	200	0.300±0.030	52.1

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

Table 2. Effect of *Bryonia laciniosa* Extract on (Histamine and 5-HT) Induced Pedal Oedema in Rats

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Histamine control	0	0.542±0.050	—
Indomethacin	10	0.218±0.020	59.8
CEBL	50	0.344±0.040	36.5
CEBL	100	0.295±0.030	45.0
CEBL	200	0.244±0.030	54.9
Serotonin control	0	0.625±0.050	—
Indomethacin	10	0.253±0.020	59.5
CEBL	50	0.403±0.040	32.3
CEBL	100	0.366±0.040	41.4
CEBL	200	0.298±0.030	52.3

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

Table 3. Effect of the *Bryonia laciniosa* Extract on Cotton-Pellets Induced Granuloma in Rats

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	Percentage of inhibition
Control	0	47.1±1.9	—
Indomethacin	10	20.4±0.7	57.3
CEBL	50	34.4±1.1	26.8
CEBL	100	28.5±0.9	39.5
CEBL	200	23.5±0.6	50.1

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

Table 4. Effect of the Chloroform Extract of *Bryonia laciniosa* on Leukocytes Migration in Peritoneal Exudation by Induced Carrageenan Mice

	Treatment	Parameters			
		Leukocytes (10^5 ml^{-1})	Leukocytes inhibition	Neutrophils (10^5 ml^{-1})	Neutrophils changes
1	Control	4.29±0.44	—	2.55±0.41	—
2	CEBL 50 mg/kg	2.73±0.12	36.4	0.98±0.05	38.4
3	CEBL 100 mg/kg	1.85±0.16	56.9	0.33±0.07	12.9
4	CEBL 200 mg/kg	0.97±0.09	77.4	0.71±0.08	6.6
5	Indomethacin 10 mg/kg	2.74±0.16	36.1	0.91±0.47	35.7

Values are mean±S.E.M. (n=6). Experimental groups were compared with control $p<0.01$.

DISCUSSION

The CEBL was evaluated for its anti-inflammatory activity in acute and chronic models. Significant anti-inflammatory activity was observed for CEBL in both carrageenan and dextran induced oedema models. The chloroform extract showed maximum inhibition of 52.4% at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema. Carrageenan induced oedema is commonly used as an experimental animal model for acute inflammation and 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.[10,11] The CEBL also exhibited significant anti-inflammatory property in dextran induced paw oedema model. Dextran induced paw oedema 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.[12] The extract effectively suppressed the inflammation produced by both carrageenan and dextran.

The extract also effectively suppressed the inflammation produced by mediators *viz.* histamine and serotonin. The CEBL 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 prostaglandins might be involved in inflammation.

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation.[13,14] Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The CEBL 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.[15]

Leukocyte aggregation at the site of inflammation is a fundamental event in the inflammatory process. Cell migration occurs as a result of much different process including adhesion and cell mobility.[16] In the present investigation we compared the effect of the extract at the doses of 50, 100 and 200 mg/kg with indomethacin on the cell migration. The CEBL also inhibited the carrageenan induced leukocyte migration in peritonitis model in mice. The CEBL was found to inhibit leukocyte migration more potent than indomethacin. The extract (in peritonitis model) drastically reduced the migration of neutrophils.

From the above discussion, the chloroform extract from the leaves of *Bryonia laciniosa* exhibited significant anti-inflammatory activity in both acute and chronic inflammatory models. Further detailed investigation is underway to determine the exact phytoconstituents those are responsible for the anti-inflammatory activity.

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