ORIGINAL ARTICLE

Preliminary phytochemical, toxicity and anti-inflammatory evaluation of *Commelina benghalensis*

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Background: *Commelina benghalensis* is a widely used ethno medicinal plant for various diseases in India, but only few studies have been conducted in this plant. **Objective:** The present work was performed to screen phytochemical, toxicity and anti-inflammatory activity of hydroethanolic extract of leaves of *C. benghalensis* (Family: Commelinaceae). **Materials and Methods:** Hydroethanolic extract of leaves of *C. benghalensis* (HECB) was prepared and subjected to preliminary phytochemical investigations. Acute and sub-acute toxicity tests were performed in female Wistar rats. The anti-inflammatory activity was studied using carrageenan-induced rat paw edema, cotton pellet granuloma and xylene-induced ear edema models at two different doses (200 mg/kg and 400 mg/kg of body weight). **Results:** HECB did not show any toxic reactions in female rats, and a dose of 400 mg/kg exhibited significant anti-inflammatory activity in all three models as compared to the control group. Indomethacin 10 mg/kg also showed significant anti-inflammatory activity in all three models. **Conclusion:** These experimental results have established a pharmacological evidence for the folkloric use of the *C. benghalensis* as an anti-inflammatory agent. Determination of the median lethal dose (LD_{50}) revealed that the *Commelina* extracts was safe.

Key words: Anti-inflammatory, Carrageenan, *Commelina benghalensis*, Cotton pellet, Xylene, sub-acute toxicity

INTRODUCTION

Inflammation is the response of living tissues to injury, and it involves complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair.[1] Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoke serious adverse reactions.[2]

Traditional medicines play an important role in health services around the globe. About three-quarters of the world population relies on plants and plant extracts for healthcare.[3] *Commelina benghalensis* (family Commelinaceae), locally known as Dholpata, is a perennial herb native to tropical Asia and Africa. It is used in the Indian subcontinent as a folk medicine for the treatment of variety of ailments. The plant is used for mouth thrush,[4] inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children,[5] insanity and exophthalmia.[6] In China, *C. benghalensis* is used medicinally as a diuretic, febrifuge and anti-inflammatory agent.[7] A study reported that leaves of *C. benghalensis* contains antioxidants-like lutein and β-carotene in the range of 84-187% and 50-115%.[8] The above-stated folkloric uses formed the basis for the present study, which aimed at exploring the preliminary phytochemical, toxicity and anti-inflammatory evaluation of hydro-ethanolic extract of leaves of *C. benghalensis*.

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MATERIALS AND METHODS

Drugs and Chemicals

Indomethacin, carrageenan and carboxy methyl cellulose were purchased from Sigma Aldrich, Germany. Xylene was purchased from Merck Industries, India, and all other chemicals used were of analytical grade. SGPT, SGOT, BUN and serum glucose strips were obtained from Idex.

Collection and Preparation of Plant Material

C. benghalensis was collected from Sagar Districts of Madhya Pradesh, India. It was identified, collected

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and authenticated. A voucher specimen (voucher specimen number –4653-54) was deposited in the herbarium of the Department of Botany, Gauhati University, Guwahati, Assam. The leaves were washed with water and shade dried in the open air, then pulverized to dry powder using electric grinder.

Extraction of Plant Material

The powdered crude drug (800 g) was subjected to extraction process by cold maceration with 70% ethyl alcohol in room temperature for 7 days. The extract was filtered, concentrated using rotary vacuum evaporator (Buchi) and heated on a water bath at 45 ± 5°C to obtain HECB. The yield obtained was approximately 15% w/w.

Animals

Wistar albino rats (150-200 g) and Swiss albino mice (25-30 g) of either sex were used for the present investigation and were obtained from Central Animal Facility, NIPER, Guwahati. Animals were housed under standard environmental conditions of temperature (25 ± 2°C) and light and dark cycle (12:12 h). Rats were fed with standard pellet diet and water *ad libitum*. All experimental studies were conducted with permission from the Institutional Animal Ethics Committee, Gauhati Medical College, Guwahati (IAEC No.O9/NIPER/CPCSEA/351).

Preliminary Phytochemical Screening

Preliminary phytochemical screening of *C. benghalensis* extract was performed using specific reagents through standard procedures.[9]

Acute Dose Toxicity Study

The acute toxicity study was conducted as per the OECD guidelines 425. Initially, HECB was administered orally at a limit dose of 2000 mg/kg to a single female rat. The rat was observed closely for the first 4 h and then periodically up to 24 h for any toxic symptoms and mortality. After 24 h, the same dose was administered to four more female rats.[10]

Sub-acute Dose Toxicity Study

Sub-acute dose toxicity study was performed according to the standard method, as described previously.[11] Briefly, three groups containing 6 female rats (130-150 g) each were used. Group I was kept as control and groups II and III received 200 mg/kg and 400 mg/kg of the extract, respectively. Single daily dosing was given continuously for 14 days. Initial and final body weights, water and food intake, state of faecal matter, body temperatures and mortality rate were monitored. The animals were sacrificed on the 15th day. Haematological and serum biochemical parameters were determined using Automated Countess Cell Counter (Invitrogen) and Automated Vet Analyzer (Idexx), respectively.

Anti-inflammatory Study

Experimental design

Animals were randomly divided in to 4 groups containing 6 rats/mice each; Group 1: vehicle control (1% CMC), Group 2: HECB 200 mg/kg, Group 3: HECB 400 mg/kg, Group 4: indomethacin 10 mg/kg of body weight.

Carrageenan-induced paw edema in rats

Carrageenan-induced paw edema model was performed as described previously.[12,13] Briefly, the rats were fasted for 12 h before the experiment, but water was given *ad libitum*. Animals were pre-treated with vehicle and drugs orally 1 h before the experiment. The edema was induced by subcutaneous injection of freshly prepared carrageenan solution (0.05 ml of 1% solution in 0.9% sodium chloride solution) in the sub-plantar region of the left hind paw using a 27-gauge needle. The paw volume was measured just before the injection of carrageenan and after 1, 2, 4 and 6 h of carrageenan injection using water displacement plathysmometer (UGO Basile, Italy).

$$\% \text{ inhibition of edema} = (1 - Vt/Vc) \times 100$$

Where, Vt = Mean paw edema volume of the treated group.
Vc = Mean paw edema volume of the control group.

Cotton pellet granuloma

This is a well-known animal model for screening of chronic anti-inflammatory activity of drugs. Rats were fasted for 12 h before the start of the experiment, but water was given *ad libitum*. After 1 h of administration of either control, graded dose of HECB or indomethacin, the rats were anaesthetized by ketamine (30 mg/kg, i.p). Back of the rats were shaved and disinfected by 70% ethanol. Incisions were made on lumbar region using surgical blade. By a blunted forceps, a subcutaneous tunnel was formed and a sterilized cotton pellet (15 ± 1 mg) was placed on both the sides of the scapular region. The rats were given respective drugs for 7 days, as described in the experimental design. On 8th day, the animals were sacrificed using high dose of ether and the cotton pellets were removed. The cotton pellets were dried at 60 ± 5°C until a uniform weight was obtained and weighed.[14-16]

$$\text{Granuloma inhibition (\%)} = (1 - Wt/Wc) \times 100$$

Where, Wc = Mean weight of dried cotton of the control group.
Wt = Mean weight of dried cotton of the treated group.

Xylene-induced ear edema in mice

Adult Swiss Albino mice were divided in to 4 groups ($n = 6$), fasted overnight and allowed water *ad libitum*. All treatments were given as described in the experimental design section. After 1 h, the mice were treated with 30 µl of xylene in the anterior and posterior surfaces of the right ear lobe. The left

ear was considered as control. Two hours after induction of inflammation, the mice were killed by overdose of ether anaesthesia and both the ears were removed. Circular sections (7-mm diameter) of both the ears were punched out using a cork borer and weighed. Edema was quantified as the weight difference between the two ear plugs.[14]

Percentage edema inhibition = 100 × (1− (Rt − Lt)/(Rc − Lc)

Where,
Rt = mean weight of right ear plug of the treated group.
Lt = mean weight of left ear plug of the treated group.
Rc = mean weight of right ear plug of the control group.
Lc = mean weight of left ear plug of the control group.

Statistical Analysis

Values were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA (Graph pad prism version 6), followed by Dunnets *post hoc* test; $P < 0.05$ were considered statistically significant.[17]

RESULTS

Preliminary Phytochemical Screening

The preliminary phytochemical screening of HECB revealed the presence of alkaloids, flavonoids, tannins, carbohydrates and saponins [Table 1].

Table 1: Preliminary phytochemical screening of hydro ethanolic extract of *Commelina benghalensis*

Phytochemical	Observation
Alkaloids	++
Carbohydrates	+
Flavonoids	++
Saponins	+
Tannins	+
Steroids	–
Terpenoids	–

Symbol (+) indicates presence and (−) indicates absence of phytochemicals

Acute Toxicity Study

HECB did not show any toxic reactions and mortality up to a dose of 2000 mg/kg. Therefore, LD_{50} of HECB should be >2000 mg/kg. For the current research work, 200 mg/kg and 400 mg/kg were taken as the treatment dose.

Sub-acute Dose Toxicity Study

In sub-acute dose toxicity studies, the extract treatment (200 and 400 mg/kg p.o. for 14 days) did not cause any mortality. There were no considerable changes in body weight, behavioural parameters and locomotion in treated animals as compared to the control group. The haematological and serum biochemical parameters of the extract treated rats showed no significant ($P \geq 0.05$) change as compared to the control rats [Table 2].

Anti-inflammatory Activity

Carrageenan-induced paw edema

HECB 400 mg/kg and indomethacin 10 mg/kg significantly inhibited the carrageenan-induced paw edema at the 6th h ($P < 0.05$). HECB produced a dose-dependent inhibition of carrageenan edema, which was comparable with known anti-inflammatory drugs. Percentage inhibition of edema shown by HECB 400 mg/kg was 60.29% and indomethacin 10 mg/kg was 57.35% respectively at the 6th h [Table 3].

Cotton pellet granuloma

Both doses of HECB and 10 mg/kg indomethacin significantly inhibited the granuloma formation in cotton pellet method ($P < 0.001$). Indomethacin showed more granuloma inhibition (54.44%) as compared to HECB 400 mg/kg (48%) [Table 4].

Xylene-induced ear edema

Topical application of *Commelina* extract reduced the edema development in xylene model of acute inflammation. HECB at 200 mg/kg and 400 mg/kg significantly reduced

Table 2: Effect of HECB in haematological and serum biochemical parameters in sub-acute dose toxicity study

Test treatment	S. Glucose mg/dl	Protein g/dl	Urea mg/dl	SGPT IU/L	SGOT IU/L	WBC count (x10^3 cells/mm^3)
Vehicle control	88.93±4.32	5.76±0.48	18.02±0.61	22.68±1.38	53.52±2.34	6.35±0.27
HECB 200 mg/kg	87.30±4.01	5.42±0.56	18.27±0.43	23.71±1.49	53.38±2.23	6.63±0.41
HECB 400 mg/kg	89.25±5.27	6.12±0.43	18.96±0.51	22.30±1.54	53.61±1.72	6.39±0.60

Values are expressed as mean±SEM (n=6). Not significant (P≥0.5) as compared to the vehicle control group. HECB – Hydroethanolic extract of leaves of *C. benghalensis*; SGPT – Serum glutamic pyruvate transaminase; SGOT – Serum glutamic oxaloacetic transaminase; WBC – White blood cells

Table 3: Effect of HECB in carrageenan-induced paw edema

Dose	Edema volume (ml)			
	1 h	2 h	4 h	6 h
Control CMC (1%)	0.42±0.06	0.51±0.03	0.62±0.04	0.68±0.06
HECB 200 mg/kg	0.32±0.12 (23.8)	0.36±0.14 (29.41)	0.39±0.08 (37.09)	0.34±0.12 (50)
HECB 400 mg/kg	0.26±0.14 (38.09)	0.31±0.07 (39.21)	0.32±0.06 (48.38)	0.27±0.11*(60.29)
Indomethacin 10 mg/kg	0.23±0.06 (45.23)	0.28±0.05 (45.09)	0.3±0.11 (51.61)	0.29±0.1*(57.35)

Values of edema are mean±S.E.M, n=6, *P<0.05 as compared to the control group, Percentage inhibition of edema are represented in parenthesis. HECB – Hydroethanolic extract of leaves of *C. benghalensis*. CMC – carboxy methyl cellulose

Table 4: Effect of HECB in cotton pellet granuloma method in rats

Group	Dose	Dry weight of cotton pellet in mg	Granuloma inhibition (%)
Control	CMC (1%)	66.35±0.05	-
HECB	200 mg/kg	38.99±0.49*	41.32
HECB	400 mg/kg	34.48±0.032*	48
Indomethacin	10 mg/kg	30.25±0.05**	54.44

Values of edema are mean±S.E.M, n=6, ***P<0.001 as compared to the control group. HECB – Hydroethanolic extract of leaves of *C. benghalensis*; CMC – Carboxy methyl cellulose

Table 5: Effect of HECB on xylene-induced ear edema in mice

Group	Dose	Edema (mg)	Inhibition (%)
Control	CMC (1%)	0.503±0.06	-
Test 1	HECB 200 mg/kg	0.2±0.04*	60
Test 2	HECB 400 mg/kg	0.17±0.01*	66
Standard	Indomethacin 10 mg/kg	0.14±0.12**	72

Values of edema are mean±S.E.M, n=6, *P<0.05, **P<0.01 as compared to the control group. HECB – Hydroethanolic extract of leaves of *C. benghalensis*; CMC – Carboxy methyl cellulose

the inflammation ($P < 0.05$), and the percentage of edema inhibition was 60% and 66% respectively [Table 5].

DISCUSSION

Preliminary phytochemical evaluation revealed the presence of alkaloids, flavonoids, tannins, carbohydrates and saponins in the *Commelina* extract. It have been reported that flavonoids, tannins and saponins shows promising anti-inflammatory activity.[18-20] Therefore, these phytochemicals may be the reason for anti-inflammatory activity of HECB. Both acute and sub-acute toxicity studies failed to reveal any toxic effect of *Commelina* extract and LD50 should be >2000 mg/kg of body weight.

Carrageenan-induced rat paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs.[21] The carrageenan-induced edema has been described as a biphasic event including a rapid early phase (0-2 h), triggered by the concerted release of histamine, 5-hydroxytryptamine and cyclooxygenase products, and a more sustained late phase (2-6 h), regulated by neutrophil infiltration, sustained production of arachidonic metabolites and nitric oxide produced by inducible nitric oxide synthase (iNOS).[22,23]

In carrageenan-induced model, HECB 400 mg/kg showed significant reduction of paw edema at the 6th h with 60.29% of edema inhibition. These results indicated that *Commelina* extracts were effective in preventing sustained late phase (2-6 h) edema and that this may be due to cyclooxygenase inhibition or iNOS inhibition.

Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms. An acute, transient phase, characterized by local vasodilatation and increased capillary permeability; a sub-acute phase, characterized by infiltration of leukocytes and phagocytic cells; and a chronic proliferative phase, in which tissue degeneration and fibrosis occur.[24] During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small vessels, which are the basic sources of forming a highly vascularised reddish mass termed granular tissue. The cotton pellet granuloma formation is a widely used method for assessing anti-inflammatory activity of compounds against chronic inflammation.[25] In the present study, both the doses of HECB significantly inhibited the granuloma formation, and it may be due to the inhibition of proliferation of macrophages, neutrophils, fibroblasts and multiplication of small vessels.

Xylene causes instant irritation of the mouse ear, which leads to fluid accumulation and edema that are characteristic of the acute inflammatory response. Suppression of this response is likely an indication of anti-phlogistic effect of the test drug.[26] It have been reported that in xylene-induced ear edema model, the application of xylene produces neurogenous edema with partial association of 'substance P', which is a neuropeptide released during neurogenous inflammation.[24,27] In our study, HECB at 200 mg/kg and 400 mg/kg significantly inhibited the xylene-induced ear edema. This indicates that *Commelina* extracts have anti-phlogistic and substance P inhibition activity.

CONCLUSION

From the above results, it can be concluded that *C. benghalensis* is a safe drug for acute and long-term use. Furthermore, the anti-inflammatory activity exhibited by the HECB is a result of the synergistic action of the bioactive compounds present in the leaves. These experimental results established the pharmacological evidence for the traditional uses of *C. benghalensis* in inflammatory conditions. However, it needs isolation, structural elucidation and screening of any of the above-mentioned active principle (s) to establish the clinical utility.

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