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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF Alternanthera brasiliana LEAVES

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ABSTRACT

To study the anti-inflammatory activity of a hydro-ethanolic extract of *Alternathera brasiliana* leaves (HEAB).The study was carried out using Wistar albino rats (150-180 gm) and Swiss albino mice (20-25 gm) of either sex. HEAB was prepared by cold maceration. The effect of HEAB was investigated for anti-inflammatory activity using carrageenan induced paw edema (acute), cotton pellet granuloma (sub-acute) and formaldehyde induced arthritis (chronic) method. The analgesic activity of HEAB was studied using formalin induced paw licking. Statistical analysis was done by using Oneway analysis of variance (ANOVA) followed by post hoc Dunette's test. p<0.05 was considered statistically significant.The anti-inflammatory activity of HEAB (100, 200 & 400 mg/kg, p.o.) showed significant (p<0.05) decrease in carrageenan induced paw edema. In sub-acute model HEAB (200 & 400 mg/kg, p.o.) significantly decrease the granuloma formation. In chronic model, HEAB (100, 200 & 400 mg/kg, p.o.) the arthritis was significantly lowered. HEAB did not show any significant effect in early phase of formalin paw licking while it significantly decrease the paw licking in late phase at given doses.HEAB showed significant anti-inflammatory activity.

KEYWORDS: Inflammation, Hydro-ethanolic extraction, Cold maceration, OECD.

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INTRODUCTION

Inflammation is a complex defense mechanism which involves various responses with the objective of eliminating noxious stimulus. These responses could be localized or generalized. Inflammation occurs in three different phases. which involves different physiological and immunological mediators. The three phases are - acute phase which involves transient local vasodilatation and increased capillary permeability; subacute phase which involves infiltration of leucocytes and other phagocytic cells and chronic phase which involves degeneration of the affected tissue and fibrosis.¹ Chronic inflammation is associated with certain severe disease like rheumatoid arthritis, Crohn's disease, type II diabetes, Alzheimer's disease,etc.² Already available anti-inflammatory drugs, non steroidal (NSAIDs) anti-inflammatory drugs and corticosteroids only provide symptomatic relief and suffer from many drawbacks. NSAIDs produce gastric ulcers while corticosteroids are toxic and relapse of the disease occur on discontinuation of therapy.³ Hence, the search for safer anti-inflammatory drug is still on. According to World Health Organization (WHO) estimation, about 80% of the population in countries relies developina on herbal medicines at least for their primary health care.⁴ Traditional Indian medical systems such as Ayurveda and Unani rely heavily on plant products.⁵ North-east region of India is an abode of various medicinal plants which are utilized by the local tribes for the treatment of their ailments. The folklore claim of the inhabitants should be scientifically verified with regard to better utility of the medicinal plants from this regions.⁶ Alternanthera brasiliana (Amaranthaceae) is a herbaceous plant, also known as "penicilina" or "terramicina", and is widely used by rural communities as a agent.7,8 medicinal Α. brasiliana mainly contains flavonoids as 3-O-robinobioside derivatives of kaempferol and guercetin. It is against inflammation, cough and used diarrhoea in popular Brazilian medicines.⁹ This plant exhibits antiviral properties.⁷ The hydroalcoholic extract from the whole plant had been proved to possess promising analgesic effects in mice but in other study this plant was found ineffective as anti-microbial. Methanolic

extract of the leaves of the plant was found to have wound healing effect.¹⁰ Aqueous or ethanolic extract of A. brasiliana leaves were proved able to block human mitogen induced lymphocyte proliferation, without any toxic effect.⁹ The ethanomedicinal uses and inhibitory effects on human mitogen induced proliferation on leuckocytes suggested that the leaves might possess anti-inflammatory activity. This stimulated our interest to study the effect of the leaf extract of this plant on experimentally induced inflammation.

MATERIALS AND METHODS

i) Collection and preparation of plant material

Leaves of *A. brasiliana* were collected in the month of December from the local botanical garden. The plant material was authenticated by Regional Research Laboratory, Jorhat, Assam (India), where a voucher specimen no. AU/CVSc/PHT/02 was deposited. The leaves were cleaned and shade dried in open air for 8-10 days and then powdered for extraction.

ii) Extraction of plant material

About 600 gm of the dried leaf powder was extracted with two and half liter (about four times) of mixture of water & ethanol (50:50) by cold maceration in the dark for seven days with occasional stirring (twice daily).⁹ The extract was concentrated in rotary evaporator at reduced pressure. The concentrated extract was reduced to a semisolid mass by drying on a water bath at $40\pm5^{\circ}$ C to obtain hydroethanolic extract of leaf of *A. brasiliana* (HEAB), (yield approx. seven percent w/w). The HEAB was subjected to phytochemical screening for the verification of the presence of phytoconstituents.

iii) Animals

Adult Wistar albino rats (150-180 g) and Swiss albino mice (20-25 g) of either sex were used. The animals were housed under conditions of $25\pm2^{\circ}$ C, 45-55% relative humidity and standard light and dark cycle (07:00 am to07:00 pm) and had free access to water and standard diet (fortified with minerals and vitamins).The animals were allowed to acclimatize to the laboratory conditions and kept on overnight fasting prior to the experiments. All the experiments were performed as per Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines and the study was approved by the Institutional Animal Ethics Committee (IAEC).

iv) Chemicals

Indomethacin, carrageenan and formalin were purchased from Sigma Aldarich, Germany. All other chemicals were of analytical grade purchased locally.

v) Acute toxicity study

Toxicological studies were done according to the guidelines of Organization for Economic Cooperation & Development (OECD-425). After taking reference of the work done by Barua et al, direct limit test was done.¹⁰ The limit test was done with HEAB (2000 mg/kg, p.o.), suspended in 0.4% carboxy methyl cellulose (CMC) solution, on five female Wistar albino rats (150-180 g).

vi) Anti-inflammatory studies

a) Acute anti-inflammatory activity

The rats were randomly divided into five groups (n = six per group) and the first group served as vehicle control and received 0.4% CMC solution (0.1 ml/100 g, p.o.). Group II, III and IV received HEAB (100, 200 and 400 mg/kg, p.o, respectively). The fifth group was administered indomethacin (5 mg/kg, p.o.) as the standard drug. After 45 min, acute inflammation was produced by the method described by Winter, Risley and Nuss.¹¹ Briefly, 0.1 ml of one percent carrageenan solution (prepared with normal saline) was injected into the plantar aponeurosis of right hind paw of the rats of all five groups. The paw volume was measured by а plethysmometer (UGO BASILE). Measurements were made immediately before and 60, 120, 180 and 240 minutes after carrageenan injection. Inhibitory activity was calculated using the formula:

Percentage inhibition of paw edema =
$$\frac{(C_t-C_o) \text{ control } - (C_t-C_o) \text{ treated}}{(C_t-C_o) \text{ control}}$$
 X100

Where, C_t is paw volume after 180 minutes of carrageenan injection and C_o is paw volume before carrageenan injection.

b) Sub acute anti-inflammatory activity

Cotton pellet granuloma (sub acute inflammation) was produced in rats by method of Winter and Poter with slight modification.¹² The rats were randomly divided into five groups (n = six per group): group I (vehicle control); group II, III and IV (HEAB at 100, 200 and 400 mg/kg, p.o., respectively); group V (indomethacin 1mg/kg, p.o.). On day one, rats received treatments and 45 minutes later, two autoclaved cotton pellets 20±0.5 mg were aseptically implanted under the previously depilated back of rats anaesthetized with diethyl ether. The treatment was administered once daily for the next seven days. On the eighth day, animals were euthanized by overdose of ether. The pellets were dissected out, freed of tissue attachments and dried in the oven overnight at 60°C. The dried pellets were weighed and the weight of the granuloma tissue formed around each pellet was determined. The level of inhibition of granuloma tissue formation was calculated using the relation:

Percentage inhibition =
$$\frac{(T_c - T_t)}{T_c}$$
 X100

Where, T_c was the weight of granuloma tissue of vehical control group and T_t was the weight of granuloma tissue of treated group.

c) Chronic anti-inflammatory activity

The formaldehyde induced arthritis method of Seyle was used.¹³ On first day, The rats were randomly divided into five groups (n = six per group): group I (vehicle control); group II, III and IV (HEAB at 100, 200 and 400 mg/kg, p.o., respectively); group V (indomethacin 1mg/kg, p.o.). 45 minutes later, arthritis was induced by subplantar injection of 0.1 ml of 2.5% formaldehyde solution and the injection was repeated on third day. Arthritis was assessed by measuring the volume of distilled water

displaced by the paw before the induction of arthritis and once every day for ten days, starting from day one, after induction of arthritis. The treatment was continued once daily for the next ten days. The global edematous response was quantified as the area under the curve (AUC) of the time-course of the arthritic event. The AUC was calculated using the trapezoidal rule.¹³ The level of inhibition of arthritis was calculated using the relation:

AUC_c - AUCt X 100 AUC_c

Where, AUC_c was the AUC of the vehicle control group and AUC_t was the AUC of the treated group.

vii)Formalin test

Mice were placed in a glass bell jar for 30 minutes to accommodate to their surroundings, and then removed. The mice were randomly divided into five groups (n= six per group): group I (vehicle control); group II, III and IV (HEAB at 100, 200 and 400 mg/kg, p.o., respectively); group V (aspirin 20 mg/kg, p.o). After 45 minutes, formalin test was performed as described by Hunskaar and Hole with slight modification.¹⁴ Briefly, 0.05 ml of 2.5% solution of formalin (0.92% formaldehyde) made up in phosphate buffer (pH 7.3) was administered subcutaneously into sub-plantar region of right hind paw. The mice were placed under a glass bell jar and the amount of time spent in each one of the four behavioural categories was recorded. The categories were scored as: 'Zero'- if the injected paw was not favoured; 'One'- if the injected paw had little or no weight on it; 'Two'- if injected pas was elevated and was not in contact with any of the surface and 'Three'- if the injected paw was licked, bitten or shaken. Responses were measured for early phase (up to five minutes) and late phase (ten to twenty five minutes) after formalin injection.¹⁵ An average pain intensity score was calculated, according to the weighed score technique as described by Dubuisson and Dennis, by multiplying the amount of time spent in each category by the category score and adding and then finally dividing by the total

time of observation i.e., 300 seconds and 900 seconds for early phase and late phase, respectively.

viii) Statistical analysis

Results were expressed as mean±S.D. (n=six). Statistical analysis were performed with oneway analysis of variance (ANOVA) followed by post-hoc Dunnett's tests. P-value less than 0.05 were considered statistically significant.

RESULTS

i) Phytochemical screening

HEAB obtained was greenish brown in color. Phytochemical screening revealed the presence of flavonoids, phenolics, alkaloids, resins, tannins, triterpenes and sponins.

ii) Acute toxicity study

In the acute toxicity study, no death was observed at dose 2000 mg/kg, p. o. of HEAB in any of the five rats.

iii) Acute anti-inflammatory activity

The effect of HEAB on acute inflammation is shown in figure 1. The results in table 1 revealed that the paw edema was significantly reduced by HEAB at dose 100 mg/kg (20.13% inhibition) and above. Indomethacin significantly inhibited the paw edema (43.01% inhibition).





Effect of HEAB on time course of acute inflammation induced by carrageeenan. Results are given as mean \pm S. D. of six animals in each group. Significance at [#]p<0.05, *p<0.01 and ** p<0.001 when compared to vehicle control using one way ANOVA followed by Dunnett's test.

Table 1Effect of HEAB on carrageenan induced paw edema in rats after 180 minutes.

Group	Treatment	Increase in paw volume after 180 minutes of carrageenan injection (ml)	Percentage inhibition of paw edema (%)
1	Vehicle control	0.25±0.07	-
II	HEAB 100 mg/kg	0.19±0.01 [#]	20.13
III	HEAB 200 mg/kg	0.15±0.02**	33.93
IV	HEAB 400 mg/kg	0.16±0.02**	30.58
V	Indomethacin mg/kg	5 0.13±0.03**	43.01

Results are given as mean±S. D. of six animals in each group. Vehicle control group is compared with rest of the treated groups. Significance at [#]p<0.05, *p<0.01 and ** p<0.001 when compared to vehicle control using one way ANOVA followed by Dunnett's test.

iv) Sub acute anti-inflammatory activity

A dose dependent reduction in the weight of granuloma tissue formed around the cotton pellets was observed with HEAB compared to the vehicle treated rats. However, the degree of reduction was less than the effect caused by indomethacin (Table 2).

Table 2
Effect of HEAB on sub acute inflammation (cotton pellet granuloma) in rats.

Group	Treatment	Weight of granuloma tissue (mg)	Percentage inhibition in granuloma formation (%)
1	Vehicle control	24.65±2.24	-
11	HEAB 100 mg/kg	22.32±2.56	9.48
III	HEAB 200 mg/kg	20.32±1.56**	17.56
IV	HEAB 400 mg/kg	17.28±3.36**	29.90
V	Indomethacin 1 mg/kg	13.87±1.56**	43.73

Results are given as mean±S. D. of six animals (twelve cotton pellets) in each group. Vehicle control group is compared with rest of the treated groups. Significance at ** p<0.001 when compared to vehicle control using one way ANOVA followed by Dunnett's test.

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Figure 2 Effect of HEAB on time course of chronic inflammation induced by formaldehyde in rats. Results are given as mean±S. D. of six animals in each group.

Table 3Effect of HEAB on chronic inflammation (formaldehyde induced arthritis) in rats.

Group	Treatment	AUC (unit ²)	Percentage inhibition in formaldehyde induced arthritis (%)
1	Vehicle control	4.65±0.49	-
11	HEAB 100 mg/kg	3.82±0.16**	17.85
III	HEAB 200 mg/kg	3.17±0.54**	31.83
IV	HEAB 400 mg/kg	3.49±0.43**	24.95
V	Indomethacin 1 mg/kg	2.57±0.31**	40.86

Results are given as mean±S. D. of six animals in each group. Vehicle control group is compared with rest of the treated groups. Significance at ** p<0.001 when compared to vehicle control using one way ANOVA followed by Dunnett's test.

v) Chronic anti-inflammatory activity

Figure 2 represents the effect of HEAB and indomethacin in chronic inflammation produced by formaldehyde. The chronic inflammation was significantly reduced by HEAB while the degree of reduction in case of indomethacin was higher (Table 3).

vi) Formalin Test

Subplantar injection of formalin evoked a characteristic biphasic response. No significant difference was seen in the average pain intensity score of early phase between control group and treatment groups (data not shown). While, in late phase, HEAB produced significantly low average pain intensity score when compared with vehicle control. Aspirin produced the lowest average pain intensity score in the treatment groups (Table 4).

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Table 4Effect of HEAB on formalin induced paw liking in mine (late phase).

Group	Treatment	Average pain intensity score
1	Vehicle control	1.21±0.33
11	HEAB 100 mg/kg	1.09±0.20*
III	HEAB 200 mg/kg	0.84±0.34**
IV	HEAB 400 mg/kg	0.63±0.23**
V	Aspirin 20 mg/kg	0.57±0.11**

Results are given as mean±S. D. of six animals in each group. Vehicle control group is compared with rest of the treated groups. Significance at *p<0.01 and ** p<0.001 when compared to vehicle control using one way ANOVA followed by Dunnett's test.

DISCUSSION

The purpose of the study was to evaluate the anti-inflammatory effect of A. brasiliana in invivo models of inflammation. The preliminary phytochemical screening of HEAB shows the presence of alkaloids, resins, flavonoids, triterpenes, starch and tannins. Kaempferol and guercetin derivatives are the main flavonoids found in the plant A. brasiliana.⁹ Kaempferol and guercetin has shown antiinflammatory properties in in-vitro models of inflammation.^{16,17} Thus the anti-inflammatory activity of HEAB could be due to flavonoids present in the extract. The HEAB did not produce any toxicological and behavioral changes in the rats at a dose of 2000mg/kg and doses of 100, 200 and 400 mg/kg were selected for the present study. Carrageenan induced paw edema was taken as a prototype of acute inflammation because it is most prominent experimental model in search and evaluation of new anti-inflammatory drug. Also, carrageenan is devoid of any systemic effect and results are highly reproducible. Earlier studies have shown that carrageenan-induced paw edema is usually biphasic in nature. The initial phase (0-60 minutes) is mediated by histamine and serotonin. The second phase is known to be influenced by the lipid derived eicosanoids (prostaglandins, leukotrienes, etc) with peak at 180 minutes.^{18,19} Our studies with the time course of carrageenan edema formation revealed that on administration of HEAB, there was significant reduction of the paw edema (in second phases). This suggested that HEAB possesses peripheral action, probably related to arachidonic acid and cyclooxygenase cascade.

Sub-acute and chronic inflammations are the reactions when the acute response is insufficient to eliminate the pro-inflammatory agents. These reactions include proliferation of

infiltration of fibroblast. neutrophils and exudation of fluid. It occurs via development of proliferative cells which can either spread or form granuloma. The ability to inhibit the increase in the number of fibroblasts during granular tissue formation and arthritis formation is an excellent indicator of anti-inflammatory activity.¹⁹ Granuloma is normally observed with inflammatory changes accompanying the cotton pellet implantation in rat models. Cotton pellet granuloma is also known as foreign body granuloma and is model for non-immunological type of inflammation mediated mostly by kinin.¹⁸ kinin is considered to be the main mediator of granuloma, as it causes vasodilation and increase vascular permeability in early stages of inflammation.²⁰ In our present investigation, HEAB significantly decreased the formation of granuloma (in a dose dependent manner), thereby suggesting anti-kinin activity in the proliferative phase of inflammation. Further studies, with chronic inflammatory models of inflammation, indicate that the HEAB (at the doses employed), significantly inhibited formaldehyde mediated arthritis in rats. Thereby, suggesting the possible anti-arthritic activity of the same. These results support the previous results in which leaves of A. brasiliana inhibit the lymphocyte proliferation.⁹ Our study also revealed that HEAB has potent antinociceptive action in the formalin test, which is a model of inflammatory pain. The formalin test has two distinctive phases - early phase and late phase. The early and late phases of formalin test have obvious different properties.²¹ The early (acute) phase is due to direct stimulation of nociceptors (also called neurogenic phase) and the late phase is mainly inflammatory in origin (also called inflammatory phase). The early phase reflects centrally

mediated pain while the pain in the late phase

is due to release of inflammatory mediators.²² HEAB had no effect on the early phase response, but it attenuated nociceptive

response in the late phase. The antiinflammatory potential may account for its peripheral analgesic activity.

CONCLUSION

Present study provides evidence for the anti-inflammatory activity of the hydro-ethanolic extract of *A. brasiliana* leaves in acute and certain aspects of chronic inflammation. The anti-inflammatory activity may derive from a combination of inhibition of pro-inflammatory mediator release, vascular permeability and neutrophil migration. The flavonoids contained in the leaves may be responsible for the anti-inflammatory activity. Further studies are needed to evaluate the ulcerogenic potential and possible mechanism of action of anti-inflammatory activity of *A. brasiliana*.

CONFLICT OF INTEREST

Conflict of interest declared none.

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