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Original Article

IN-VIVO ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC ROOT EXTRACT OF *BAUHINIA VARIEGATA* LINN

P. MEENA PRABHA*[1], V. KAMALAKKANNAN[2], K.S.G.ARUL KUMARAN[3], R.SAMBATHKUMAR[4]

[1]Department of pharmacognosy, J.K.K.Nattraja College of Pharmacy,Kumarapalayam-638183 Namakkal(Dt),Tamilnadu,India, [2]Department of pharmaceutics, J.K.K.Nattraja College of Pharmacy,Kumarapalayam-638183 Namakkal(Dt), Tamilnadu,India, [3]Department of pharmaceutics (H.O.D), K.M.C.H college of Pharmacy, Coimbatore-641048, Tamil nadu,India, [4]Department of pharmaceutics(H.O.D), J.K.K.Nattraja College of Pharmacy,Kumarapalayam-638183 Namakkal(Dt), Tamilnadu, India.
Email: meenaprabha.p@jkkn.org

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ABSTRACT

Objective: The objective of present study was to evaluate the anti-inflammatory activity of ethanolic extract of *Bauhinia variegate* root.

Methods: In the present study BVEE at 200 and 400 mg/kg body weight was studied for anti-inflammatory activities in different animal models.. Anti- inflammatory activity was carried out by using carrageenan induced paw edema model and cotton pellet induced granuloma model in wistar rats.

Results: The results shows that BVEE possess anti-inflammatory activity in acute as well as sub acute models of inflammation in rats. BVEE (200 and 400 mg/kg) showed significant ($p < 0.01$) anti-inflammatory activity by reducing the paw edema volume in carrageenan-induced paw edema in rats in the late phase (2to4h)regulated by prostaglandins and leucotrienes and in cotton pellet induced granuloma model BVEE decreased dry weight of granuloma.

Conclusion: The observed pharmacological activity may be due to presence of phytochemical compounds present in the extract like alkaloids, flavonoids, phenols, saponins and tannins.

Keywords: Anti-inflammatory activities, BVEE- Bauhinia variegata ethanolic extract, Carrageenan induced paw edema, Cotton pellet induced granuloma method.

INTRODUCTION

Pain is sensorial modality representing in many cases the only symptom for diagnosis of several diseases [1].While inflammation is a complex defensive mechanism which consists of highly sequential events provoked by number of stimuli like pathogens, noxious mechanical and chemical agents, and autoimmune responses. The subsequent cascade of events which takes place in inflammation is characterized by various signs and symptoms like redness, swelling, heat, and pain. A regulated response protects against further injury and clears damaged tissue in physiological conditions while in pathological condition inflammation may result in tissue destruction and lead to organ dysfunction [1]. All the steroidal and non steroidal anti-inflammatory drugs (NSAID's) available in market cause undesired and serious side effects during their clinical use, so studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs. The currently used anti-inflammatory drugs may not be useful in all cases so there is increased focus on plant research and their active constituents [2].

Bauhinia variegata (family-Leguminosae) is a medium sized deciduous tree, sparingly grown in India.The plant is found in Throughout India, growing wild and as a garden plant. Widely planted in the tropics and warm regions of the world, including the southern margin of the United States from Florida to California. This plant is used traditionally in scrofula, diarrhea, anticancer, pain, rheumatism, delirium, and depressant[1].These active constituents have been attributed the therapeutic activity of the plant. Therefore, the present study was undertaken to evaluate their anti-inflammatory activities.

MATERIALS AND METHODS

Drugs and chemicals

Diclofenac sodium (gift sample from Microlabs) Carrageenan (Sigma- Aldrich, St. Louis, MO, USA), ethanol (Qualigens, Mumbai, India) petroleum ether (jk chemicals, Hyderabad) all reagents used in the experiment were of analytical grade respectively.

Collection and authentication of plant material

The Plant specimen for the proposed study was collected from Nellore, Andhra pradesh It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai (**PARC/ 2010/ 628**).

Preparation of extracts

The freshly collected Root of *Bauhinia variegate* Linn of this plant was chopped, shad dried and coarsely powdered. The powder was defatted with petroleum ether (60-80^{o}c) then successively extracted with ethanol (90% v/v) using soxhlet extractor. The ethanolic extracts were dried under reduced pressure using a rotary vacuum evaporator. The percentage yield was 3.912 % w/w for ethanol extract.

Animals

Wistar albino rats of either sex (175-200g) were obtained from saastra Bioscience Research Laboratories in Saastra College of pharmaceutical education and research centre, Varigonda village, Totapalligudur Mandal, SPSR Nellore dist. Animals were housed in plastic cages at an ambient temperature (25±2^{o}C) and relative humidity of 45-55%. A 12:12 hr light- dark cycle was maintained during the experiments. They were fed with balanced rodent pellet diet from saastra Bioscience Research Laboratories, Nellore and water *ad libitum* throughout the experimental period.

Animals were acclimatized to their environment for at least one week before experimentation. The animals were randomly divided into different groups. Each animal was housed separately after recording its body weight and had kept separate marks for identifying the dose level, group and individual number.

IAEC approval The Institutional Animal Ethics Committee (IAEC)

approved the protocol of the study on Registration no. 1243/bc/08/ CPCSEA dated 9.02.2011. All animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) under the "Guidelines for Care and Use of Animals in Scientific Research".

Preliminary phytochemical screening

Qualitative preliminary phytochemical screening was carried out for evaluation of tannins, alkaloids, flavonoids, saponins, etc using standard procedures and tests. [3,4]

Acute toxicity study

Acute toxicity study – up and down procedure was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD) 423. In this experiment three groups of albino Swiss mice n=6 were used. **[**5] Animals were fasted overnight with water *ad libitum* and food was withheld for 3-4 hrs after oral administration of the BVEE. First group of animals were treated with starting dose of 1000 mg/kg body weight of BVEE orally.

Second group of animals were treated with a maximum dose of 2000 mg/kg body weight of BVEE. Control group was treated with normal saline. Animals were observed individually after dosing. Observation included mortality and gross behaviors e.g. body positions, locomotion, rearing, tremors were observed. The effect of BVEE on passivity, grip strength, pain response, stereotypy, righting reflex, and mortality were assessed.

Pharmacological evaluation

Carrageenan - induced paw edema method

Group 1 (Control): Receive vehicle,

Group 2 (Test): Ethanolic extract of root of Bauhinia variegata (200mg / kg, p.o),

Group 3 (Test): Ethanolic extract of root of Bauhinia variegata (400mg / kg, p.o),

Group 4 (Standard): Diclofenac sodium (5 mg / kg, p.o)

Paw oedema was induced by injecting 0.1 mlof 1% carrageenan in physiological saline into the subplantar tissues of the left hind paw of each rat. [6, 7, 8, 9] The ethanol extracts of Bauhinia variegata root (200 & 400 mg/kg) and Diclofenac sodium was administered to the groups of group 2, group 3, group 4 orally 30 min prior to carrageenan administration. The paw volumes have to be measured at 60, 120, 240 minutes by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group were compared with the control group and standard.

The Percentage inhibition was calculated by using formula,

% inhibition = C – T/ C x 100

C means difference between right and left paw volume in control.

T means difference between right and left paw volume in test and standard,

Cotton pellet granuloma method

Group 1 (Control): Receive vehicle,

Group 2 (Test): Ethanolic extract of Bauhinia variegata root (200mg / kg, p.o),

Group 3 (Test): Ethanolic extract of Bauhinia variegata root (400mg / kg, p.o),

Group 4 (Standard): Diclofenac sodium (5 mg / kg, p.o),

Cotton pellets weighing 30±1 mg was autoclaved and implanted subcutaneously into both sides of the groin region of each rat. The ethanol extracts of Bauhinia variegata root at concentration 200mg/kg, 400mg/kg have to be administered orally for Group 2 and 3 animals for 7 days. Group IV animals received Diclofenac at a dose of 5 mg/kg orally for same period. On 8thday the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60^{o}C, weighed and compared with control and standard group.

The Percentage inhibition was calculated by using formula.

% inhibition = C – T/ C x 100

C means weight of granuloma in control groups.

T means weight of granuloma in test and standard groups.

Statistical analysis

The results were expressed as Mean ± Standard Error of Mean (SEM). [10, 11] The groups were compared by one way analysis of variance (ANOVA) followed by post hoc "Dunnett's Multiple comparison test" to analyze statistical significance. $P < 0.01$ was considered to be significant.

RESULTS

Phytochemical analysis

Preliminary phytochemical qualitative analysis of BVEE showed the presence of alkaloids, saponins, flavonoids, tannins, phenol compounds in the extract.

Acute toxicity study

No abnormality in the gross behavioral studies also no mortality were noted. Based on these observations two different doses (200 and 400 mg/kg) were selected for the pharmacological studies.

It predicted that BVEE does not show any marked sign of toxicity and mortality up to 1000 mg/kg body weight orally in mice for 24 hrs and was considered as safe for pharmacological activity. According to OECD – 423 guidelines, the LD_{50} dose was fixed as 200 and 400mg/kg.

Table 1: Table showing acute toxicity study

Parameters	Group I	Group II	Group III
Alertness	Normal	Normal	Normal
Passive/acute	Positive	Positive	Positive
Restlessness	Absent	Absent	Absent
Aggressiveness	Absent	Absent	Absent
Touch response	Normal	Normal	Normal
Tremors	Absent	Absent	Absent
Pain response	Normal	Normal	Normal
Convulsion	Absent	Absent	Absent
Righting reflex	Positive	Positive	Positive
Gripping strength	Present	Present	Present
Pinna reflex	Present	Present	Present
Corneal reflex	Normal	Normal	Normal
Writhing	Absent	Absent	Absent
Pupils	Normal	Normal	Normal
Urination	Normal	Normal	Normal
Salivation	Absent	Absent	Absent
Skin colour	Normal	Normal	Normal
Lacrimation	Absent	Absent	Absent
Respiration	Normal	Normal	Normal

Anti inflammatory activity

Carrageenan induced paw edema in rats

Treatment with BVEE at a dose of 200 mg/kg and 400 mg/kg exhibited a significant decrease in paw volume. BVEE at 200 & 400 mg/kg showed significant ($p< 0.01$) decrease in paw volume at 2nd and 4th h. Diclofenac (5 mg/kg) exhibited a significant ($p< 0.01$) reduction in paw volume at 2nd and 4th h as compared to vehicle control. The percentage inhibition of change in paw volume of BVEE at 200 mg/kg and 400 mg/kg was found to be 20.00% and 33.33 % respectively at 2h. However the maximum percentage inhibition was found to be at 4th h 23.80% and 38.00 % for 200 mg/kg and 400 mg/kg of BVEE respectively. The percentage inhibition of Diclofenac (5 mg/kg) was found to be 46.60 % and 47.61 % at 2nd & 4th h respectively when compared with carrageenan control animal.

Table 2: Table showing Carrageenan Induced paw edema Method

S. No.	Treatment	Dose	Mean edema volume (ml)			% Inhibition		
		(mg/kg)	1st hr	2nd hr	4th hr	1st hr	2nd hr	4th hr
1	Group 1	1 ml	0.11 ± 0.0063**	0.15±0.0063	0.21±0.0082*	-	-	-
2	Group 2	200	0.09±5.7490**	0.12±4.2248	0.16±3.0136*	18.18	20.00	23.80
3	Group 3	400	0.08±6.0707**	0.10±4.2184**	0.13±3.0202**	27.27	33.33	38.00
4	Group 4	5	0.06±5.7490**	0.08±4.2153**	0.11±4.6837*	45.45	46.60	47.61

Group 1 – Control, **Group 2** - Bauhinia variegata ethanolic extract 200mg, **Group 3** - Bauhinia variegata ethanolic extract 400mg, **Group 4** - Diclofenac sodium , Values are mean ± SEM of 6 parallel measurement. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6), All the values are significant **P< 0.01 when compared against control., All the values are significant *P< 0.01 when compared against standard

Cotton pellet-induced granuloma in rats

The chronic anti-inflammatory activity of the test extract, Diclofenac sodium was used as standard control. The percentage decrease in the dry weight of granulomatous tissue by the various samples in mice was found out and all the values are compared by using t-test. Significant reduction in the granulomatous tissue.

In cotton pellet granuloma, the BVEE (200 and 400 mg/kg) significantly (p< 0.01) inhibited the granuloma formation when compared to vehicle control group. The degree of inhibition was dose dependent. The BVEE at 200, and 400 mg/kg inhibited the granuloma formation by 38.44%, and 43.91% respectively. Diclofenac (10 mg/kg) significantly (p< 0.01) inhibited the granuloma formation by54.76%.

Table 3: Table showing Cotton pellet induced granuloma method

Treatment	Dose (mg/kg)	Weight of dry cotton pellet granuloma (mg)	% inhibition
Group 1	1 ml	148.14 ±0.1009 **	-
Group 2	200	91.19 ±0.0626*	38.44
Group 3	400	83.09 ±0.1992	43.91
Group 4	5	67.01 ±0.0999**	54.76

Group 1 - Control , Group 2 - Bauhinia variegata ethanolic extract 200mg, Group 3 - Bauhinia variegata ethanolic extract 400mg, Group 4 - Diclofenac sodium , Values are mean ± SEM of 6 parallel measurement., Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6), All the values are significant **P< 0.01 when compared against control., All the values are significant *P< 0.01 when compared against standard

DISCUSSION

The use of traditional medicine is widespread and plants still present a large source of structurally novel compounds that might serve as leads for development of novel drugs [12]. The present investigation was carried out to scientifically evaluate the traditional claim of Bauhinia variegata as anti- inflammatory. On acute oral toxicity the extract was found to be safe up to 2000 mg/kg. Phytochemical screening showed the presence of saponins, phenols, flavonoids, alkaloids and tannins.

Carrageenan induced paw oedema which is a classical model of acute inflammation has been widely used in the study of steroid and non steroid anti-inflammatory drugs [13]. Carrageenan-induced inflammation has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation [14]. Carrageenan is a family of linear sulphated polysaccharides extracted from the red seaweed marine alga *Chondrus crispu*. Lambda carrageenan is used in animal models of inflammation to test anti-inflammatory activity because dilute carrageenan solutions (1-2%) injection causes swelling and pain [15]. The edema produced by subplantar injection of carrageenan in rat hind paw is biphasic over 4 or more hours. The early phase is attributed due to release of serotonin and histamine while later phase is sustained by prostaglandins and leucotrienes [16] and continuity between two phases is provided by kinins [17]. The second phase is sensitive to most clinically effective anti-inflammatory drugs. The BVEE was found to significantly inhibit carrageenan induced rat paw edema in the late phase regulated by prostaglandins and leucotrienes.

Cotton Pellet induced granuloma in rats is a chronic model of inflammation which has been widely used to assess activity of anti-inflammatory drugs on proliferative phase of inflammation [13]. Proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels which are the basic sources of highly vascularized reddish mass is termed as granulation tissue is seen during repair process of inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed [18]. In the present study significant activity of BVEE was seen against cotton pellet induced granuloma in rats indicating ability of BVEE in reducing number of fibroblasts and synthesis of collagen and muco polysaccharide, natural proliferative events of granulation tissue formation. The presence of phenolic compounds in the extracts may be responsible for the anti -inflammatory activities in both the models [19].Therefore anti - inflammatory activity of BVEE can be attributed to its phytochemical compounds present in the extract.

CONCLUSION

BVEE showed anti-inflammatory activity in carrageenan (acute) and cotton pellet induced granuloma model (sub acute). This activity can be contributed to the phytochemicals present in the extract like alkaloids, phenolic compounds, flavanoids and tannins.

CONFLICT OF INTERESTS

Declared None

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