International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 20-06-2022, Accepted: 06-07-2022, Published: 22-07-2022
Volume 7, Issue 3, 2022, Page No. 16-25

Pharmacological evaluation of *Bauhinia variegate* root for analgesic and anti-inflammatory activity

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Abstract
The research was designed to evaluate the analgesic and anti-inflammatory activity of aqueous extract of *Bauhinia variegate* roots using different animal models.
Analgesic activity was assessed by using various two animal models namely, Writing Test using acetic acid in mice and Hot Plate Method. The degree of analgesic activity was determined by the number of writhing and was recorded for each animal by Writing Test using acetic acid in mice model and the delay in reaction time for of each animal when place in hot plate maintained at 55 ± 0.1^0c is recorded in Hot Plate Method. Anti-inflammatory activity was evaluated using Formalin induced paw edema model, Croton oil ear edema in rats, turpentine oil granuloma model.
Aqueous extract of *Bauhinia variegate* root produced significant analgesic activity in both Hot plate and acetic acid induced writhing models in mice. In hot plate method percentage increase in reaction time was evaluated where as in acetic acid induced writhing model percentage decrease in writhing was determined. Evaluation of anti-inflammatory activity was done by Formalin induced paw edema model, Croton oil ear edema model and turpentine oil induced granuloma model respectively. In Formalin induced paw edema model mean change in paw volume and percentage protection were calculated. In croton oil ear edema model the difference between untreated ear and treated ear were determined which indicated degree of inflammatory edema. In turpentine oil granuloma model the change in paw volume and percentage inhibition of inflammation were calculated.
The study revealed that the *Bauhinia variegate* roots possess a significant analgesic and anti-inflammatory activity.

Keywords: *Bauhinia variegate*, analgesic, anti-inflammatory, Writing Test, Hot Plate Method, Formalin induced paw edema model, Croton oil ear edema

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. It is a subjective experience which cannot be objectively defined or quantified satisfactorily. Pain acts as a warning signal against disturbances either in the body or in the external environment of an individual and thus has a protective function. As a symptom, pain demands instant relief and in practice its dramatic relief highly impresses a layman. Pain receptors are distributed throughout the body.
Clinically, pain can be considered as:

- Superficial or cutaneous pain.
- Deep non-visceral pain from muscles, joints, ligaments and bones.
- Visceral pain.
- Referred pain.
- Psychogenic or functional pain.

Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be one of the most widely used groups of therapeutic agents. These drugs are widely used in the treatment of pain, fever and inflammation. The anti-inflammatory action of NSAID's is considered to be inhibition of prostaglandin synthesis at the site of injury. The anti-inflammatory potency of different compounds roughly corresponds with their potency to inhibit COX. Prostaglandins are active mediators of inflammatory responses and also provide cyto- protection in the stomach and intestine. The key enzyme of their biosynthesis is prostaglandin H_2 synthase (PGHS or cyclooxygenase [COX]) which is a bifunctional enzyme exhibiting both cyclooxygenase and peroxidase activities. The cyclooxygenase component converts arachidonic acid to a hydro per-oxy endoperoxide (PGG_2) and the peroxidase component reduces the endoperoxide to the corresponding alcohol (PGH_2), the precursor of Prostaglandins, thromboxane's and prostacyclin's. It is found that three distinct COX isoforms exist. COX-1 is expressed in all tissues and is involved in the regulation of physiological functions maintaining platelet aggregation and homeostasis of the GI tract and the kidney. COX-2 is rapidly induced in inflammatory cells in

response to cytokines such as tumor necrosis factor-α (TNF-α), interleukins, growth factors and so on [6]. These drugs have side effects especially on the gastro intestinal tract.

In India numerous invaluable plants are used in ethnomedical practices as well as in Ayurveda and Siddha. The pharmacological properties of many such plants are not sufficiently evaluated in the light of modern science. The active principles of plant-derived products have been isolated and characterized, and their mechanisms of action are understood. It is also a function of the traditionally-held belief that the synergistic combination of several active principles in some herbal preparations is responsible for their beneficial effects [4, 5].

The plant *Bauhinia variegate* Linn. Is from Caesalpiniaceae family. It is a medium- sized, deciduous tree, found throughout India. It is traditionally used in bronchitis, leprosy and tumors. The stem bark is used as astringent, tonic and anthelmintic. The plant are reported to contain flavone glycosides, flavonoids (2S)-5,7- dimethoxy-3′,4′- methylene di-oxy flavanone, serine proteinase inhibitor 6-butyl-3-hydroxy flavanone and a new di-hydro di-benzoxepin, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2- methyldibenz [b, f] oxepin3-8. These active constituents have been attributed the therapeutic activity of the plant. Therefore, the present study was undertaken to evaluate their anti-inflammatory and analgesic activities of the plant *Bauhinia variegate* extract [11, 12].

Methodology

Material selection

Animal selection: Swiss albino mice weighing 18-30 gm, and albino rats of Wistar strain weighing 200- 250g were used for the study. The mice and rats were inbred in the central animal house, under suitable conditions of housing, temperature, ventilation and nutrition were used for antidepressant activity. They were kept in clean dry cages week before the beginning of the experiment to acclimatize with the experimental conditions. The animals were fed with standard pelleted diet (Lipton India Ltd., Mumbai) and distilled water *ad libitum* was maintained at 21^0C-23^0C under a constant 12hrs light and dark cycle. The animal care and experimental protocols were in accordance with CPCSEA /IAEC.

Housing: Mice as well as albino rats were housed in groups of six in each clean cage. The bedding material of the cages was removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. Bedding materials used in sufficient amount to keep animals dry between cage changes without coming into contact with watering tubes. Drinking tubes were examined routinely to ensure their proper function.

Chemicals used

Table 1: Chemicals used in the study

Sl no	Chemicals	Supplier
1.	Distilled water	HiMedia Laboratories Pvt. Ltd. Nashik
2.	Tween 80	HiMedia Laboratories Pvt. Ltd. Nashik
3.	phenyl benzoquinone	Central Drug House Ltd, New Delhi
4.	DMSO	Central Drug House Ltd, New Delhi
5.	Ibuprofen	Central Drug House Ltd, New Delhi
6.	Aspirin	Nice chemical Pvt. Ltd, Cochin
7.	Tramadol	Nice chemical Pvt. Ltd, Cochin
8.	Paracetamol	Nice chemical Pvt. Ltd, Cochin

Plant material: The *Bauhinia variegate roots* belonging to the family Fabaceae were collected from Kesavan's plantations (Dealers in Ayurvedic pharmaceuticals), Wayanad, Kerala and the annexure of the same is enclosed. It is preserved in the departmental library for future reference.

Extraction and preparation of test sample: Naturally available roots of *Bauhinia variegate* will be collected and shade dried and will be authenticated. Coarse powder of the air-dried root was subjected to successive solvent extraction method using distilled water chloroform mixture in a Soxhlet extraction unit till exhaustion to get aqueous extract. Each aqueous extract was carefully evaporated in a rotary evaporator under controlled temperature and reduced pressure to get the extract and the yield and percentage yield of various extracts is calculated. The extract will be suspended in 1% tween 80 and will be administered orally [19].

Phytochemical screening [19, 20]

Each extract was subjected to phytochemical screening and the preliminary chemical examination of ethanol extract revealed the presence of steroids, flavonoids, tannins, coumarins, carbohydrates and reducing sugars. Flavonoids exhibit varied biological activities that include analgesic, anti-inflammatory, antioxidant, hepatoprotective and anti-ulcer activities. Tannins are protectants. Based on this, it was contemplated to carry out the screening of aqueous extract for analgesic, anti-inflammatory activities.

Dose Fixation: A dose of 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg body weight were chosen as per the previous work.

Analgesic Activity [32, 33]

Preparation of animals: The animals were selected in such a way that they were free from illness, injury, disease and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Only those animals which are healthy having weights 18-30 g were selected and maintained at standard laboratory conditions.

Preparation and administration of doses: All the doses were prepared in distilled water using 1% Tween 80 solution as suspending agent and administered orally.
The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 h.

Observations: Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 h. additional observations like changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and Somatic motor activity and behavioral pattern were also done. Attention was also given to observations of tremors and convulsions.

Analgesic activity determination

Hot plate test using mice: Albino mice were randomly grouped into six groups of six animals each. Group I served as control. *Bauhinia variegate* aqueous extract (BVAE) 50, 100 and 200mg/kg body weight, p.o., and the standard tramadol at 5 mg/kg body weight i. p., was administered to the animals of group II to group VI respectively. The delay in response time (Jumping and hind paw licking response) of animals when placed on the hot plate which was maintained at 55 ± 1ºC was recorded at 0,30,45,60,90,120 and 180 min. the percentage increase in reaction time was calculated.
Percentage protection against thermal pain was calculated by applying the formula:

% protection against thermal pain = $(Ta - T_b) \times 100 / T_b$

Where,
Ta – Mean reaction time of test and
Tb – Mean reaction time of control.

Abdominal writhing test using acetic acid in mice: Albino mice were used for the study and were divided into six groups of six animals in each. Group I served as control. The II, III, IV and V group animals received *Bauhinia variegate* aqueous extract 50,100, 150 and 200mg/kg body weight respectively by oral route. Group VI animals received the standard drug Aspirin 100 mg/kg body weight by oral route.
Writhing were induced 30 min later by i. p. injection of 0.1 ml of 0.6 % acetic acid to all animals of the various groups. The numbers of writhes were counted for 20 min, starting immediately after acetic acid injection. Percentage protection was calculated for all the groups by applying the formula:

$$\text{Percentage protection} = \frac{\text{Writhing in control} - \text{Writhing in test}}{\text{Writhing in control}}$$

Statistical Analysis: The data were expressed as mean ± SD. Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's and Tukey's test. P- value <0.05 was regarded as statistically significant.

ANOVA (Analysis of variance): In statistics, analysis of variance is a collection of statistical models and their associated procedures, in which the observed variance is partitioned into components due to different explanatory variables. In its simplest form ANOVA gives a statistical test of whether the means of several groups are all equal and therefore generalize Dunnett's multiple comparison tests to more than two groups.

Anti-inflammatory activity [42, 43, 44]

Formalin induced paw edema model: Animal Used was Albino Rats of Wistar strain. Chemical agent used to induce inflammation: Formalin (0.1ml injected intra peritoneally to sub-plantar region of left hind paw) by Oral route
The method was used for this study. Animals were divided into five groups denoted as Control group, Positive control group (Standard-Ibuprofen 100mg/kg) group), Test group I (BVAE 50), Test group II (BVAE 100), Test group III (BVAE150) and Test group VI (BVAE 200). Each group consisting of 6 albino Wister rats.
Control group received orally 0.1ml of 1% suspension in sodium CMC at the dose of 10 ml/kg body weight and Positive control group received orally at the dose of 100mg/kg body weight. Test group I, II, III and IV were treated with test Sample orally at the dose of 50, 100, 150 and 200mg/kg body weight. 0.2 ml of 3 % formalin was injected into the dorsal surface of the left hind paw of rats 1 h after oral administration of the extracts. The time spent by each animal in licking the injected paw was observed for 5 min. (from 0- 5min post formalin injection) and 10 min (from 20-30 min post formalin injection). The mean of the licking time was determined and compared with the mean for the control group.

Croton oil ear edema

Albino rats were divided into six groups of six animals each. Animals were treated orally with the extract (BVAE 50, BVAE 100, BVAE 150 and BVAE 200mg/kg), Ibuprofen (100 mg/kg) and distilled water (3 ml/kg). Thirty minutes later, edema was induced in each rats group by applying a drop of croton oil to the inner surface of the right ear. After 15 min, the animals were sacrificed under ether anesthesia and both ears cut off, sized and weighed. The anti-inflammatory activity was expressed as the difference in (weight of untreated ear – weight treated ear) of edema in the treated mice in comparison with the control rats.

Turpentine oil-induced granuloma pouch bioassay

Subcutaneous dorsal granuloma pouch was made in ether anaesthetized rats by injecting 2 ml of air, followed by injection of 0.5 ml of turpentine oil into it15,16. All drugs were administered orally one hour prior to turpentine oil injection and continued for seven consecutive days. On day 7, the pouch was opened under anesthesia, the amount of exudate was taken out with a syringe, the volume was measured and compared with those of the control and standard group.

Statistical Analysis

The results were expressed as mean + SEM, Statistical significance was determined by one-way ANOVA (Analysis of Variance) followed by t test by using the Graph pad Instant version and compared with control.

Results

Photochemical screening

Table 2: Photochemical screening

sl. no.	Name of the test	Observation BVAE	Conclusion
I.	**Tests for Steroids** Salkowski reaction Liebermann Burchard Liberman's reaction	 + + +	Steroids were present in aqueous extract.
II.	**Tests for Saponins** Foam test Haemolytic test	 + +	Saponins were present in aqueous extract.
III.	**Tests for Tannins and Phenolic Compounds** Lead acetate test 5% Fe Cl_3 test Bromine water test	 + + +	Tannins were present in aqueous extract.
V.	**Tests for Flavonoids** Shinoda test Lead acetate test Alkaline solution	 + + +	Flavanoids were present in aqueous extract.
VI.	**Tests for Reducing Sugars** Fehling's test Benedict's test	 - -	Reducing sugars were absent in ethanolic extract.

Thus we can conclude from above observations that aqueous extract contain steroids, saponins, tannins, phenolic compounds and flavonoids as active constituents (Table No.2).

Analgesic Activity of Bauhinia Variegate Root [32, 33]

The aqueous extracts of leaves of *Bauhinia variegate* were evaluated for analgesic activity by hot plate and acetic acid induced writhing models, the results obtained are as follows;

Hot plate method: The aqueous extracts significantly and dose dependently protected the mice against thermally induced pain stimulus. All the extracts at various time intervals at which they were tested produced increase in reaction time.

The comparison of analgesic activity with the standard drug Tramadol at various time intervals is as follows. At 30 min, only *Bauhinia variegate* aqueous extract (BVAE) 200mg/kg body produced analgesic activity comparable (P<0.05) to that of standard. The percentage protection against thermally induced pain stimulus by BVAE 200 and the standard drug, tramadol was 85.33±5.20 and 69.83±6.73 respectively. At 45 min BVAE 200

produced analgesic activity comparable (P<0.05) to that of tramadol, the percentage protection was 75.92±7.21 and 81.42±5.30 respectively.
At 60 min *Bauhinia variegate* aqueous extract (BVAE) 150mg/kg body weight and *Bauhinia variegate* aqueous extract (BVAE) 200mg/kg body weight produced analgesic activity comparable (P<0.05) to that of tramadol. At 90, 120 and 180 min, all extracts at all doses produced analgesic activity better (P<0.01) than /tramadol. (Table No:3). Maximum analgesic activity was observed in *Bauhinia variegate* aqueous extract (BVAE) 200mg/kg body weight and therefore which is selected as optimum dose for the analgesic activity

Table 3: Analgesic effect of *Bauhinia variegate* aqueous extract (BVAE) and tramadol in mice by hot plate method

Treatment	Dose (mg/kg)	Percentage increase in reaction time					
		30 min	45 min	60 min	90 min	120 min	180 min
Standard (STD) (Tramadol)	5	69.83 ±6.74	81.42 ±5.30	78.74 ±6.40	71.77 ±7.00	66.45 ±7.47	31.69 ±8.07
BVAE 50	50	41.75 ±11.26	64.44 ±8.73	81.49 ±16.81	83.44 ±7.00**	80.30 ±6.67**	66.87 ±12.79**
BVAE 100	100	46.97 ±15.05	70.30 ±19.31	83.08 ±6.06*	80.87 ±6.67**	80.87 ±8.38**	66.67 ±14.91**
BVAE 150	150	64.25 ±15.05	73.24 ±10.05	80.25 ±11.05	82.36 ±7.05	83.89 ±15.15	64.25 ±8.25
BVAE 200	200	85.33 ±5.20*	79.92 ±7.21	78.64 ±5.66*	85.35 ±5.21**	86.29 ±5.19**	47.12 ±18.48**

n=6, values represent mean ±SD

Where, BVAE 50, BVAE 100, BVAE 150 and BVAE 200 indicates *Bauhinia variegate* aqueous extracts at doses 100, 200 and 400 mg/kg body weight respectively.
*Symbols represent statistical significance. * * P < 0.01., *P < 0.05. as compared to tramadol.

Acetic acid induced writhing test: Results of acetic acid induced writhing response in mice indicates that *Bauhinia variegate* aqueous extract produced analgesic activity in a dose dependent manner. BVAE 50, BVAE 100, BVAE 150, BVAE 200 and Aspirin produced significant (P<0.01) decrease in writhing's induced by acetic acid when compared to control. BVAE 200 produced maximum (P<0.01) decrease in the number of writhes when compared with all other groups.
The percentage decrease in writhing by various extracts was compared to that of the standard drug aspirin. BVAE 200 produced maximum percentage decrease in writhing which was better (P<0.01) than that of standard. The percentage decrease in writhing ± SEM by BVAE 200 and aspirin were found to be 81.28±2.04 and 76.60±1.53 respectively**.** The aqueous extract at lower dose did not produce significant decrease in writhing when compared to standard (Figure No:1).

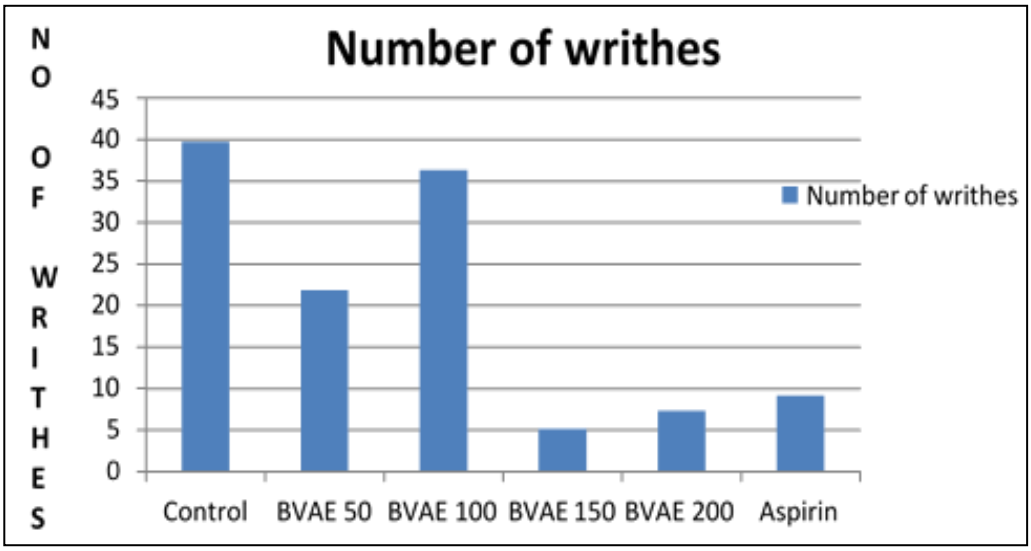

Fig 1: analgesic activities of bauhinia variegate aqueous extracts on acetic acid induced writhes. where n=6, values are mean ± SEM

Where, BVAE 50, BVAE 100, BVAE 150 and BVAE 200 indicates *Bauhinia variegate* aqueous extracts at doses 100, 200 and 400 mg/kg body weight respectively.

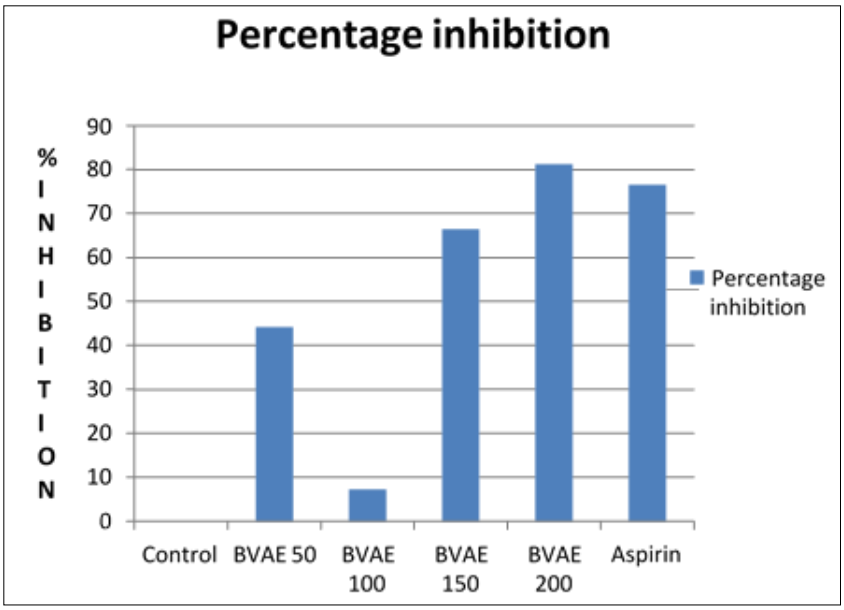

Fig 2: Analgesic activity of *Bauhinia variegate* aqueous extracts on acetic acid induced writhes.

Where, n=6, values are mean ±SEM**,** BVAE 50, BVAE 100, BVAE 150 and BVAE 200 indicates *Bauhinia variegate* aqueous extracts at doses 100, 200 and 400 mg/kg body weight respectively.

Anti-Inflammatory Activity of Bauhinia Variegate Aqueous Extracts (BVAE) [42, 43, 44]
Formalin Induced Paw Edema Model

Table 4: acute anti-inflammatory activity of the *bauhinia variegate* aqueous extracts and ibuprofen (reference drug) on formalin induced paw edema in wistar rats.

Group	N	Inflammatory activity					
		30 min	60 min	120 min	180 min	240 min	300 min
Control	6	1.25±0.014	1.30±0.01	1.34±0.03	1.32±0.056	1.26±0.084	1.24±0.070
BVAE 50	6	1.09±0.013	1.15±0.049	1.20±0.021	1.14±0.049	1.12*±0.070	1.06*±0.021
BVAE 100	6	1.08±0.056	1.17±0.014	1.25±0.042	1.23±0.035	1.10*±0.014	1.00*±0.028
BVAE 150	6	1.095±0.038	1.185±0.046	1.26±0.055	1.235±0.026	1.105±0.035	1.01±0.016
BVAE 200	6	1.10±0.056	1.20±0.014	1.27±0.042	1.24±0.035	1.11*±0.014	1.00*±0.028
Ibuprofen (100mg/kg)	6	1.25±0.007	1.31±0.028	1.33±0.028	1.20±0.007	1.02±0.014	0.93*±0.035

Data are the mean ± SEM values for six rats in each group.
*p < 0.05, **p < 0.01 as compared to the control. At 200mg/kg dose (1.00±0.028), the activity of the extract showed almost similar activity compare to standard drugs.

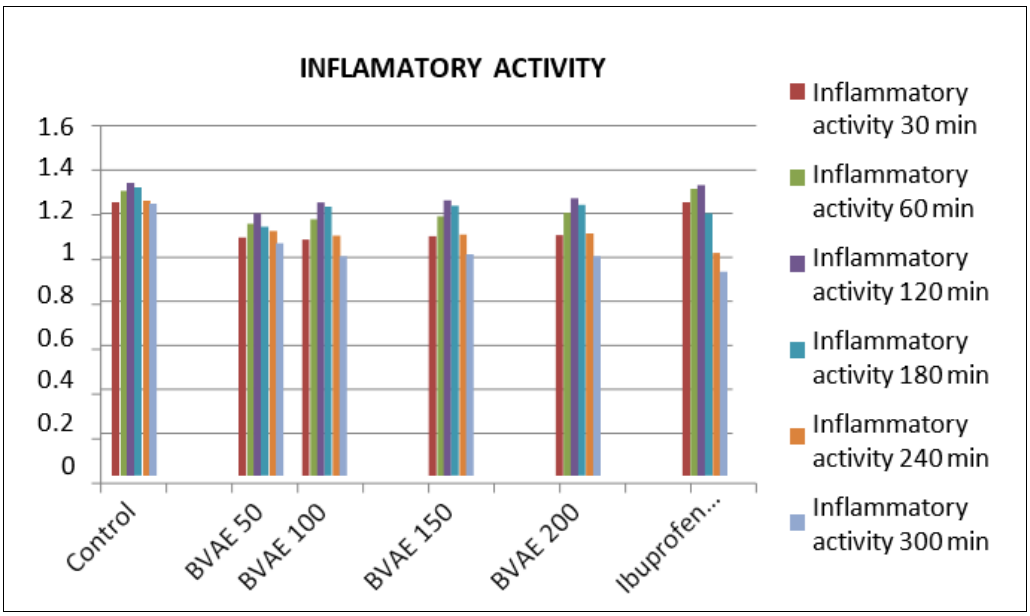

Fig 3: acute anti-inflammatory activity of BVAE and Ibuprofen formalin induced Paw Edema in Wistar rats

Croton Oil Ear Edema Model

Table 5: Effect of BVAE on croton oil ear edema in rats

Group	Dose (mg/kg)	N	Weight of Untreated ear (Right ear) (mg)	Weight of treated ear (Left ear) (mg)	Difference
Control	-	6	37.53 ±1.08	25.02 ±1.17	13.17 ±1.24
BVAE 50	50mg/kg	6	37.49 ±0.37	28.14 ±0.28	9.35±0.09
BVAE 100	100mg/kg	6	37.02 ±0.51	29.04 ±1.20	7.98* ±0.85
BVAE 150	150mg/kg	6	36.95±0.26	30.15±0.84	6.54*±0.54
BVAE 200	200mg/kg	6	36.82 ±0.44	30.66 ±0.63	6.16**±0.69
Ibuprofen	100mg/kg	6	37.43 ±0.64	32.47 ±0.57	4.95* ±0.11

Data are the mean ± SEM values for six rats in each group. *P < 0.05, **P< 0.01 as compared to the control.

Turpentine Oil-Induced Granuloma Pouch Bioassay

The effect of standard Ibuprofen at dose of 100 mg/kg and test drug BVAE at four different concentrations 50, 100, 150 and 200 mg/kg b. w. on turpentine oil induced
granuloma pouch bioassay and is tabulated in Fig:4.

A dose dependent reduction in volume of exudate in ml was observed by BVAE extracts and the potency of anti-inflammatory activity was evaluated using percentage inhibition of inflammation brought about by BVAE. It was found to be:

BVAE 200 >BVAE 150 > BVAE 100> BVAE 50. So it can be concluded BVAE exhibits anti-inflammatory activity in a dose dependent manner.

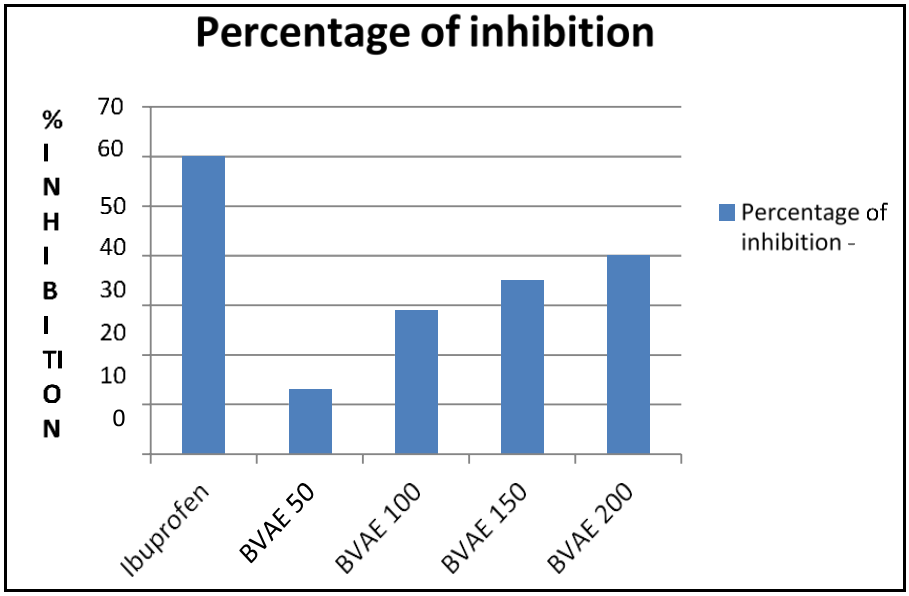

Fig 4: Effect of turpentine oil induced granuloma in rats

The result revealed that the aqueous extract of *Bauhinia variegate* have anti-inflammatory activity. The maximum anti-inflammatory activity was observed in BAAE 200mg/kg body weight.

Discussion

Analgesic activity [33, 35]

Antinociceptive or analgesic activity of *Bauhinia variegate* was evaluated using both chemical and thermal models of nociception in mice. These models are used to detect central and peripheral analgesics respectively. Acetic acid induced writhing test is used for detecting both central and peripheral analgesics, where as hot plate model is more sensitive to centrally active analgesics.

Acetic acid induced writhing test: Acetic acid induced writhing test is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test. However the test is not specific as it does not indicate whether activity is central and/or peripheral. The intraperitoneal administration of acetic acid produces abdominal writhing response due to sensitization of chemo sensitive nociceptors by prostaglandins[58]. Acetic acid releases PGE_2 and $PGF_2\alpha$ as well as lipoxygenase product into the peritoneal fluid.

BVAE produced decrease in number of writhes at doses BVAE 200, BVAE 150and BVAE

The percentage decrease in writhing ± SEM by BVAE 200 and aspirin were found to be 81.28±2.04, and 76.60±1.53 respectively**.** The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptors to prostaglandins. It is therefore possible that the extracts exert their analgesic effect probably by inhibiting the synthesis or action of prostaglandins.
The analgesic effect of the extracts may therefore be due to either its action on visceral receptors sensitive to acetic acid, or due to the inhibition of the production of algo genic substances or the inhibition at the central level of the transmission of painful impulses.

Hot plate test: Thermal induced nociception indicates narcotic involvement. The ability of the extracts to prolong the reaction latency to thermally induced pain (Hot plate test) in mice further suggests central analgesic activity. Thermal nociceptive tests are sensitive to opioid μ receptors. The ethanolic extracts significantly and dose dependently increased the reaction time at the various time intervals at which they were tested. At higher doses the extracts showed activity which was comparable to that of Aspirin (30, 45 and 60 min) and was better than aspirin at 90, 120 and 180 min. This indicates that the extracts exhibit analgesic effect by central action.
From the results obtained by both the models it can be concluded that the extracts may be showing analgesic activity both by peripheral and central mechanisms. Flavonoids, alkaloids and saponins are reported to have analgesic effect.
The analgesic effect of the extract may be due to the presence of flavonoids, tannins, alkaloids and saponins either singly or in combinations which were found to be present in the extracts during phytochemical tests.

Anti-inflammatory activity [42, 43, 44]

Formalin induced paw edema model

In the case of Formalin induced paw *Bauhinia variegate* aqueous extract (BVAE) (PLEE 200 - 1.27±0.042) exhibited almost similar anti-inflammatory activity compared to standard ibuprofen (1.33 ±0.028). Anti-inflammatory activity exhibited by *Bauhinia variegate* aqueous extract was found to be in a dose dependent manner, that is at lower dose less activity and higher dose more activity. So the order of exhibition of activity was observed as: BVAE 200> BVAE 150 > BVAE 100 > BVAE 50. Decline in anti-inflammatory activity was observed after 120 minutes.

Croton oil ear edema model

In the case of Croton oil ear edema model *Bauhinia variegate* aqueous extract (BVAE 200) (6.16±0.69) exhibited almost similar anti-inflammatory activity compared to standard ibuprofen at (4.95±0.11). Anti-inflammatory activity exhibited by *Bauhinia variegate* aqueous extr**act** was found to be in a dose dependent manner, that is at lower dose less activity and higher dose more activity. So the order of exhibition of activity was observed as: BVAE 200> BVAE 150 > BVAE 100 > BVAE 50. Decline in anti-inflammatory activity was observed after 120 minutes.

Turpentine oil-induced granuloma pouch bioassay

The effect of standard Ibuprofen at dose of 100 mg/kg and test drug BVAE at four different concentrations 50, 100, 150 and 200 mg/kg body weigh on turpentine oil induced granuloma pouch bioassay and is tabulated.
A dose dependent reduction in volume of exudate in ml was observed by BVAE extracts and the potency of anti-inflammatory activity was evaluated using percentage inhibition of inflammation brought about by BVAE. It was found to be:
BVAE 200> BVAE 150 > BVAE 100 > BVAE 50. So it can be concluded BVAE exhibits anti-inflammatory activity in a dose dependent manner.

Conclusion

The study was taken up to evaluate aqueous extract of *Bauhinia variegate* root for analgesic and anti-inflammatory activities.
The acute toxicity study conducted for aqueous extracts indicated that they are safe up to 1000 mg/kg body weight.
Aqueous extract of *Bauhinia variegate* root produced significant analgesic activity in both Hot plate and acetic acid induced writhing models in mice. In hot plate method percentage increase in reaction time was determined where as in acetic acid induced writhing model percentage decrease in writhing was determined.
Evaluation of anti-inflammatory activity was done by Formalin induced paw edema model, Croton oil ear edema model and turpentine oil induced granuloma model respectively. In Formalin induced paw edema model mean change in paw volume and percentage protection were calculated. In croton oil ear edema model the difference between untreated ear and treated ear were determined which indicated degree of inflammatory edema. In carrageenan induced paw edema model the change in paw volume and percentage protection were calculated. It was evident from the results that aqueous extract of *Bauhinia variegate* root produced significant anti-inflammatory activity in all the three models.
From the results obtained it can be concluded that aqueous extract of *Bauhinia variegate* root possess analgesic and anti-inflammatory activities.

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