ANALGESIC ACTIVITY OF *BAUHINIA VARIEGATA* LINN

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Summary

The aim of the present study was to evaluate the analgesic activity of the ethanolic and aqueous extracts of the root of *Bauhinia variegata* Linn. The central and peripheral analgesic activity of the extracts was evaluated by Eddy's hot plate and acetic acid induced writhing models respectively. The reaction time and number of writhes produced were the parameters recorded in hot plate and acetic acid induced writhing models respectively. The study was conducted at two doses, 200 and 400mg/kg body weight for both aqueous and ethanolic extracts. The data obtained were statistically analysed. Both the extracts especially ethanolic extract at 400 mg/kg produced significant ($P<0.05$) analgesic activity from 45 min and continued till the end of the experiment when compared with the standard drug Tramadol. Both the extracts produced decrease in the number of writhes induced by acetic acid. Ethanolic and aqueous extracts at 400mg/kg produced significant ($P<0.01$) reduction in writhes when compared to the standard drug Indomethacin. From the study it can be concluded that *Bauhinia variegata* Linn. produces significant dose dependent analgesic activity.

Key words: *Bauhinia variegata* Linn., Analgesic activity, Eddy's hot plate, acetic acid induced writhing models

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Introduction

Bauhinia variegata Linn. (Ceasalpiniaceae) is a medium sized deciduous tree found throughout India. It is traditionally used in bronchitis, leprosy and tumours. The stem bark is used as astringent, tonic and anthelmentic. Infusion of the leaves is used as a laxative and for piles. Dried buds are used in the treatment of worm infestations, tumours, diarrhoea and piles. The stem bark is used in ayurveda for its antidiabetic activity [1,2]. The stem bark has been investigated and reported to have antitumour, antibacterial, antifungal, antiulcer and hepatoprotective activity. Flavanone glycoside from root is reported to have anti-inflammatory activity [3,6]. The stem bark is reported to contain 5,7 dihydroxy and 5,7 dimethoxy flavanone-4-O-α-L rhamnopyrosyl-β-D-glycopyranosides, Kaempferol-3-glucoside, lupeol and betasitosterol. Seeds contain protein, fatty oil containing oleic acid, linoleic acid, palmitic acid and stearic acid. Flowers contain cyanidin, malvidin, peonidin and kaempferol. Root contains flavanol glycosides [1,7].

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.

The present investigation aims at evaluation of ethanolic and aqueous extracts of root of *Bauhinia variegata* Linn. for analgesic activity.

Materials and Methods

Plant material: Root of *Bauhinia variegata* Linn. was procured and authenticated from Regional Research Institute, Bangalore. The authenticated root was dried in shade and powdered coarsely.

Preparation of the plant extract: The authenticated root was shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. Coarse powder of the root (1Kg) was soxhlet extracted with 90% ethanol. The aqueous extract was prepared using the same marc by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanol extract (4.2%) and the aqueous extract (2.4%).

Animals: Swiss albino mice weighing between 18 and 25g were procured from registered breeders (Venkateshwara Enterprises, Bangalore). The animals were housed under standard conditions of temperature (25 ± 2^0C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animals Ethics Committee (IAEC) of K. L. E. Society's College of Pharmacy, Bangalore was taken for conducting analgesic activity.

Analgesic activity:

Hot plate method [8]**:** Albino mice were randomly divided into six groups, each having six animals. Group I served as control. BVE (*Bauhinia variegata* Linn. ethanolic extract) and BVA (*Bauhinia variegata* Linn. aqueous extract) at 200 and 400 mg/kg body weight, p.o., and tramadol at 5 mg/kg body weight i.p., were 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 by the formula,
Percentage protection against thermal pain = $(Ta - T_b) \times 100 / T_b$

Where, T_a is reaction time of test and T_b is reaction time of control

Abdominal writhing test using acetic acid in mice [9]: Albino mice were used for the study and were divided into six groups comprising of six animals each. Group I served as control. The II to VI group animals received BVE and BVA at 200 and 400 mg/kg body weight and Indomethacin 10 mg/kg body weight by oral route respectively. Writhings were induced 30 min later by intra peritoneal injection of 0.1 ml of 0.6 % acetic acid to all the animals of the various groups. The number of writhes were counted for 20 min, starting immediately after acetic acid injection. Percentage protection against writhes was calculated for all the groups as follows:
Percentage protection = (WC – W T) X100/ W C
Where, WC = Writhings in control and WT = Writhings in Test

Statistical Analysis: The results are expressed as mean ± SEM. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by the Dunnet and Tukey's as post tests. P-value <0.05 was regarded as statistically significant.

Results

The aqueous and ethanolic extracts of root of *Bauhinia variegata* Linn. were evaluated for analgesic activity by hot plate and acetic acid induced writhing models. The results obtained are presented as follows:

The ethanolic and aqueous extracts significantly and dose dependently protected the mice against thermally induced pain stimulus. Both the extracts at various time intervals at which they were tested for, produced increase in reaction time. At 30 min, only BVA 400 produced analgesic activity comparable (*P<0.05*) to that of standard. The percentage protection against thermally induced pain stimulus by BVA 400 and the standard drug, Tramadol was 85.35±5.21 and 69.84±6.75 respectively. At 45 min BVA 400 produced analgesic activity comparable (*P<0.05*) to that of Tramadol, the percentage protection was 75.91±7.00 and 81.41±5.30 respectively. At 60 min BVA 200 and 400 produced analgesic activity comparable (*P<0.05*) to that of Tramadol. At 90, 120 and 180 min, BVE and BVA at both the doses produced analgesic activity better (*P<0.01*) than Tramadol (Table No. 1).

Results of acetic acid induced writhing response in mice indicate that both aqueous and ethanolic extracts produced analgesic activity in a dose dependent manner. BVA 400, BVE 200 and 400 and Indomethacin produced significant (*P<0.01*) decrease in writhings induced by acetic acid when compared to control. BVA 400 produced maximum (*P<0.01*) decrease in the number of writhes when compared with all other groups.

The percentage decrease in writhings by various extracts was compared to that of the standard drug Indomethacin. BVA 400 produced maximum inhibition (*P<0.01*) of writhing and therefore produced maximum analgesic activity. It produced maximum percentage decrease in writhings which was better (*P<0.01*) than that of standard where as, BVE 400 produced decrease in writhing comparable (*P<0.05*) to that of standard. Both aqueous and ethanolic extracts at lower dose did not produce significant decrease in writhing when compared to standard (Table No. 2).

Table No. 1 Effect of *Bauhinia variegata* root aqueous (BVA) extract, ethanolic (BVE) extract and Tramadol in rats using Hot plate method .

Treatment	Dose (mg/kg)	% increase in reaction time					
		30 min	45 min	60 min	90 min	120 min	180 min
Standard (Tramadol)	5	69.84 ±6.75	81.41 ±5.30	78.74 ±6.40	71.77 ±7.00	66.45 ±7.47	31.69 ±8.07**
BVA	200	46.97 ±15.05	30.30 ±19.31	58.08 ±19.06	80.30 ±6.67**	80.87 ±8.38**	66.67 ±14.91**
BVA	400	85.35 ±5.21*	45.45 ±19.21	53.64 ±13.64	85.35 ±5.21**	86.29 ±5.19**	47.12 ±18.48**
BVE	200	41.75 ±11.26	64.44 ±8.73	81.49 ±6.81*	83.44 ±7.00**	80.30 ±6.67**	66.87 ±12.79**
BVE	400	58.33 ±5.93	75.91 ±7.00*	88.38 ±5.66*	92.80 ±4.63**	92.73 ±10.12**	65.51 ±5.38**

n=6,values represent mean ±SD where, BVA 200 and 400, BVE 200 and 400 indicates *Bauhinia variegata* root aqueous and alcoholic extracts at doses 200 and 400 mg/kg body weight respectively.*Symbols represent statistical significance.** $P < 0.01$., *$P < 0.05$. as compared with Tramadol.

Table No. 2 Effect of *Bauhinia variegata* root extracts and Indomethacin on acetic acid induced writhes in rats.

Treatment	Dose (mg/kg)	Number of writhes in 20 min	% inhibition of writhings
Control	-	39.67± 3.18	-
BVA	200	36.3± 1.36	7.23 ± 3.48
BVA	400	7.33 ±1.96**a, **b	81.28 ±5.01**c, **d
BVE	200	21.83 ±2.92**a	44.25 ± 7.46
BVE	400	8.83 ±3.37**a,	77.45 ± 8.60*c
Indomethacin	10	9.16 ±1.47**a	76.60 ± 3.76

n=6, values represent mean ±SD. where, BVA 200 and 400, BVE 200 and 400 indicates *Bauhinia variegata* root aqueous and alcoholic extracts at doses 200 and 400 mg/kg body weight respectively. *Symbols represent statistical significance. ** $P < 0.01$., *$P < 0.05$. 'a' as compared with control, 'b' is comparision of BVA 400 with other treatment groups, 'c' as compared with Indomethacin and 'd' is comparision of BVA 400 with other treatment groups.

Discussion

Antinociceptive or analgesic activity of *Bauhinia variegata* Linn. 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.

The ethanolic and aqueous 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 analgesic activity which was comparable to that of Tramadol at 30, 45 and 60 min and was better than Tramadol at 90, 120 and 180 min. This indicates that the extracts exhibit analgesic effect by central action. Thermal induced nociception indicates narcotic involvement [10]. The ability of the extracts to prolong the reaction latency to thermally induced pain in mice further suggests central analgesic activity. Thermal nociceptive tests are sensitive to opioid μ receptors [11].

Acetic acid induced writhing test is very sensitive and is able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test [12]. However the test is not specific as it does not indicate whether activity is central and/or peripheral. The intraperitonial injection of acetic acid produces abdominal writhing response due to sensitization of chemosensitive nociceptors by prostaglandins [13]. Acetic acid releases PGE_2 and $PGF_2\alpha$ as well as lipooxygenase products into the peritoneal fluid. BVE and BVA at both the doses produced decrease in number of writhes. The percentage decrease in writhes ±SEM by BVA 400, BVE 400 and Indomethacin was found to be 81.28±2.04, 77.45±8.60 and 76.60±1.53 respectively. This indicates that the analgesic activity of BVA 400 being better than Indomethacin and that of BVE 400 comparable to Indomethacin. The abdominal constriction 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 algogenic substances or the inhibition at the central level of the transmission of painful impulses

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. Flavonoids, tannins, alkaloids and saponins were found to be present in the extracts during phytochemical tests, they may be responsible for the analgesic activity either singly or in combination. Further studies are needed to isolate the active constituents responsible for the observed effect and reveal the possible mechanism of action responsible for analgesic activities of *Bauhinia variegata* Linn.

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