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Antinociceptive activity of aqueous leaf extracts of *Boussingaultia gracilis*

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ABSTRACT

The aqueous leaf extracts of *Boussingaultia gracilis* is used for hepatoprotective, ulcer, fever and rheumatism. The present study was undertaken to evaluate the analgesic activity of the aqueous extract of *Boussingaultia gracilis* (Bassellaceae) aqueous leaf (BGPE) was investigated in chemical models of nociception in mice. BGPE at doses of 100, 200 and 400 mg/kg p.o produced an inhibition of 29.22%, 51.80% and 79.87% of the abdominal writhes induced by acetic acid in mice. In the formalin test, the administration of 100, 200 and 400mg/kg p.o had no effects in the first phase (0–5 min) but produced a dose-dependent analgesic effect on the second phase (15–40 min) with inhibitions of the licking time of 31.54%, 45.27% and 60.52% respectively. Based on the results of this study, we suggest that the peripheral analgesic effect of *Boussingaultia gracilis* may be attributed to inhibition of prostaglandin release and other mediators involved. Further studies are needed to evaluate the mechanism of action of the analgesic activity of the *Boussingaultia gracilis*. The result obtained shows that the *Boussingaultia gracilis* possesses an adequate significant analgesic activity which confirms the claims of traditional uses of the plant leaf.

Keywords: Antinociceptive activity, *Boussingaultia gracilis*, Traditional medicine.

INTRODUCTION

Etiology of pain is the processing of noxious stimuli and encoding by the nervous system. When a noxious stimulus is applied to normal tissue, physiological nociceptive pain occurs and withdrawal reflexes are activated. Pathophysiological nociceptive pain occurs when tissue is inflamed or injured; it may appear as spontaneous pain, as hyperalgesia and can be effectively treated with nonsteroidal anti-inflammatory drugs and opiates [1,2]. But development of dependence and incidence of side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new analgesic drugs, which includes herbal drugs.

Boussingaultia gracilis under the family Bassellaceae often species of ornamental succulent vine species found rarely in India often cultivated regularly as an ornamental plants found in Malaysia and Taiwan. The fresh leaves of Madeira vine are frequently used as vegetables in Taiwan and as folk medicine as a hypoglycemic agent, an anti-inflammatory analgesic, and a stomachics. Recent studies have demonstrated that B. gracilis ethanol extracts (BGEs) have inhibitory activities against spasmogen-induced contractions of the isolated gastric fundus and ethanol-induced gastric lesions in rats. In addition, hot water extracts of B. gracilis have antiviral activities against herpes simplex virus and adenovirus type 3. It has also been reported that the butanolic fraction from B. gracilis exhibited antidiabetic effects in alloxan-induced mice. *Boussingaultia gracilis* are often cultivated as ornamentals; however, under favorable conditions they can become troublesome weeds.

MATERIALS AND METHODS

Collection of Plant material

The aqueous leaf of *Boussingaultia gracilis* was collected from Kurnool District, A.P, India. The plant was authenticated by Dr.Madhava Chetty, Botanist. Chittoor, A.P. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected fresh leaf was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with distilled water (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 18.3%w/w.

Animals used

Male albino mice (20-25g) were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given ad libitum.

Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute Toxicity Study

The acute toxicity aqueous extract of *Boussingaultia gracilis* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, $1/20^{th}$ (100mg/kg), $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [4].

Antinociceptive activity

Acetic acid-induced writhing in mice

The writhing test was carried out as described by Koster et al. [5]. Male Albino mice weighing 20 to 25 gm were divided into five groups of six animals each. Groups I was received vehicle control (1 % gum acacia, 1ml/100 g) whereas Group-II, III and VI received aqueous extract of the *Boussingaultia gracilis* (BGPE) (100, 200 and 400 mg/kg body weight) p.o Group-V received standard drug (indomethacin, 10mg/kg) p.o, respectively. The muscular contraction was induced by an intraperitoneal injection of 0.6% acetic acid solution (0.25 ml/animal) 30 min after the treatment. The responses of extract treated groups were compared with those of animals receiving indomethacin 10mg/kg (as standard drug), as well as with the controls.

Formalin induced pain

The formalin test was carried out as described by Hunskaar and Hole [6]. Male Albino mice weighing 20 to 25 gm were divided into five groups of six animals each. All groups of animals were injected subcutaneously with 20µl of formalin into the dorsal hind paw. The Group I received vehicle control (1 % gum acacia, 1ml/100 g) whereas Group-II, III and IV received aqueous extract of the *Boussingaultia gracilis* (100, 200 and 400 mg/kg body weight p.o) and Group-V received standard drug (indomethacin, 10mg/kg) p.o, respectively 30 min before formalin injection.

The time the mice spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by Tjolsen et al. [7], two distinct periods of intensive licking activity were identified and scored separately. The first period (early phase) was recorded 1- 5min after the injection of formalin and the second period (late phase) was recorded 20–40 min after the injection. The percentage inhibition of licking was calculated by the formula: (C−T)/C×100 where C represents the vehicle treated control group value for each phase and T represents the treated group value for each phase.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Acetic acid-induced writhing in mice

Table-1 shows the pain behavior of writhing response, which is presented as cumulative abdominal stretching response. The treatment of animals with BGPE extract (100 and 200mg/kg/ body weight p.o) produced a significant and dose dependent inhibition of the control writhes. The inhibition writhing response by BGPE (400 mg/kg) (79.87%) was more than to that produced by Indomethacin (10 mg/kg) (82.77%).

Formalin induced pain

BGPE (200 mg/kg, 400 mg/kg) produced significant ($P < 0.01$) inhibition in the late phase of formalin induced pain (45.27 and 60.52%) respectively (Table-2). The positive control indomethacin (10 mg/kg) also produced significant ($P < 0.01$) inhibition in the late phase (65.86%).

DISCUSSION

The aqueous extract of *Boussingaultia gracilis* (BGPE) given orally at doses of 100, 200 and 400 mg/kg significantly inhibited the acetic acid-induced writhing response in a dose-dependent manner. Acetic acid, which is used as an inducer for writhing syndromes and, causes analgesia by releasing of endogenous substances, which then excites the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins [8]. It is well known that after administration of acetic acid there is the liberation of several mediators such as cytokines and eicosanoids. Also the arachidonic acid is liberated from membrane after phospholipase A2 activity leading to the production of prostaglandins and leukotrienes [9]. In this method extracts could act inhibiting phospholipase A2, or even blocking cyclooxigenases (COX-1 and/or COX-2). This result indicates that the analgesic effect of BGPE might be mediated by its peripheral effect such as blockage the stimulus propagation in the pain nervous fibers or blockage in the eicosanoid system.

The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic effect. Experimental results demonstrated that substance P and bradykinin participate in the early phase, while histamine, serotonin, prostaglandins, nitric oxide and bradykinin are involved in the late phase of the formalin test. Centrally acting drugs such as opioids inhibit both

phases equally [10]. But peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [11, 12]. The effect of *Boussingaultia gracilis* on the late phase of formalin test suggests that its activity may be resulted from its peripheral action or anti inflammatory action when compared with indomethacin activity.

Based on the results of this study, we suggest that the peripheral analgesic effect of *Boussingaultia gracilis* may be attributed to inhibition of prostaglandin release and other mediators involved. Further studies are necessary to fully elucidate the mechanism of action of the analgesic activity of the *Boussingaultia gracilis*.

Table-1. Analgesic effect of aqueous extract of *Boussingaultia gracilis* (BGPE) in the acetic acid-induced writhing test [a]

Group	Design of treatment	Number of writhings[b]	Inhibition (%)
I	Control (1% gum acacia, 1ml/100g)	35.52±1.33	–
II	BGPE (100mg/kg b.w, p.o)	25.14 ± 1.27	29.11
III	BGPE (200mg/kg b.w, p.o)	17.12 ±1.28*	51.10
IV	BGPE (400mg/kg b.w, p.o)	7.15 ± 0.22**	79.32
V	Indodomethacin (10mg/kg b.w, p.o)	6.12 ±0.29**	82.14

[a] After 30 minutes BGPE treatment, mice were injected i.p. with 0.6% (v/v) acetic acid. [b] Values are mean±S.E.M. (N=6). * P<0.05, ** P<0.01 significant compared with control values (ANOVA followed Dunnett's test).

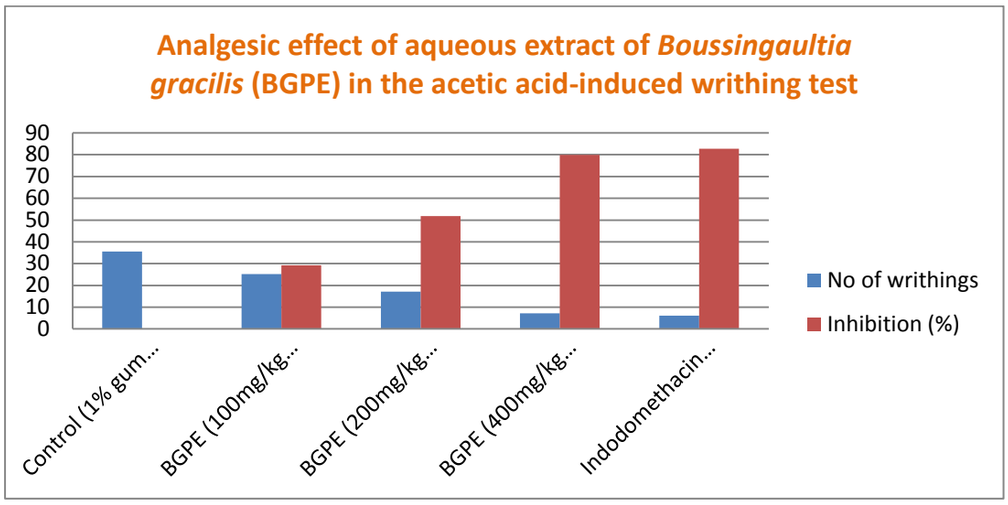
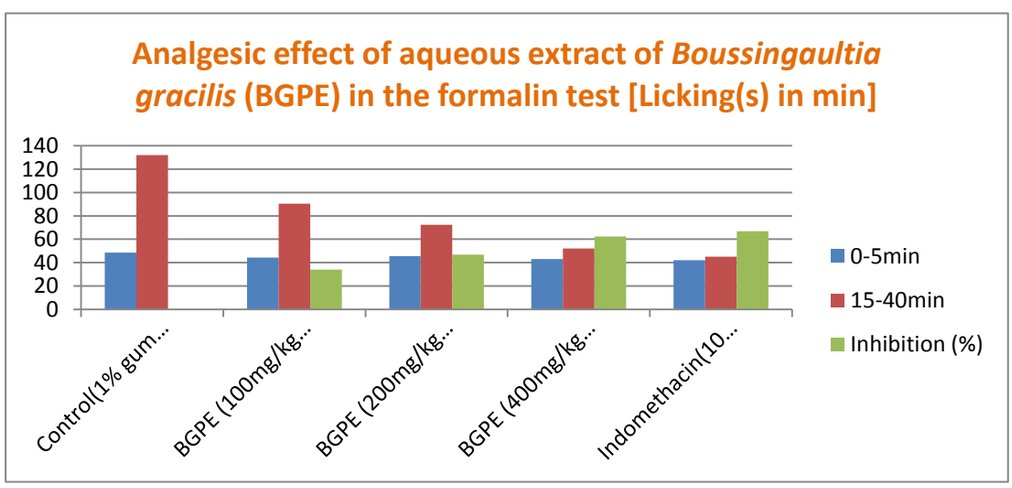


Table 2. Analgesic effect of aqueous extract of *Boussingaultia gracilis* (BGPE) in the formalin test [a]

		Licking(s) [b]		
Groups	Design of treatment	0-5min	15-40min	Inhibition (%)
I	Control(1% gum acacia,1ml/100g)	48.61± 1.92	132.18±2.67	–
II	BGPE (100mg/kg b.w, p.o)	44.22±1.58	90.49±2.16*	34.12
III	BGPE (200mg/kg b.w, p.o)	45.48±1.5	72.33±2.42**	46.71
IV	BGPE (400mg/kg b.w, p.o)	43.19± 1.49	52.19±2.36**	62.50
V	Indomethacin(10mg/kg b.w, p.o)	42.15± 1.33	45.12± 2.18**	66.97

[a] After 30 minutes BGPE treatment, mice were injected s.c with 20µl of formalin into the dorsal hind paw. [b] Values are expressed as mean±S.E.M. (N=6).* P<0.05, ** P<0.01 significant compared with control values (ANOVA followed y Dunnett's test).



CONCLUSION

The present study has demonstrated with pharmacological models that *Boussingaultia gracilis* has analgesic effect attributed to the plant in folklore medicine. However, more experiments were needed to find out the mode of action and identify the compounds responsible for the analgesic effect.

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