

Evaluation of Analgesic Activity of Methanolic Extract of *Bougainvillea spectabilis* Leaves in Experimental Animal Models.

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ABSTRACT

Background: Anti-inflammatory activity of leaves of *Bougainvillea spectabilis* (family Nyctaginaceae) has already been demonstrated in experimental animals. As pain is one of the important components of inflammation, we had set forward a study this find out possible analgesic activity of the same in animal models Objective: Evaluation of analgesic effects of, *Bougainvillea spectabilis* in mice models. **Methods:** 215 gm of fresh dried leaves of *Bougainvillea spectabilis* (BS) were collected from the local area during the flowering season and air dried. Following Methanol extraction, under reduced pressure solvent was removed on a rotary evaporator. The lyophilized extract was collected and the yield was 8 gm. That was used as an emulsion prepared in propylene glycol and orally administered (20 and 50 mg/kg). Central and peripheral analgesic activities of *Bougainvillea spectabilis* (BS) were evaluated by tail flick, tail immersion test and writhing test (acetic acid induced) respectively. Study Design: This is an experimental study designed on animal models. **Results:** *Bougainvillea spectabilis* (BS) had shown no analgesic action in central analgesic model at different hours as the reaction time was less than 10 seconds at all time interval. With regard to peripheral analgesic activity, maximal activity was observed at 50 mg/kg b.w. The mean writhes \pm standard deviation were 42.7 ± 0.9 and 40 ± 0.5 respectively in BS (20 mg/kg) and BS (50 mg/kg) in comparison to standard drug aspirin (33.3 ± 0.4), control mice being 55.3 ± 0.4 . **Conclusion:** Our data indicates that *Bougainvillea spectabilis* (50 mg/kg) has a significant peripheral analgesic activity. Without isolating the active principles it's extremely difficult to pinpoint the mechanisms contributing to the observed analgesic activities of *Bougainvillea spectabilis* and extrapolate that in clinical practice.

Keywords: *Bougainvillea spectabilis*, analgesic, mice.

INTRODUCTION

Bougainvillea spectabilis (BS) is popular as an ornamental plant In India and other tropical countries though it has a long tradition of medicinal use. Scientists are not fully aware of the fact that all parts of plants including roots are used in forms of infusions (mostly flowers). Sometimes decoctions (stems, leaves, roots) and tinctures of the same preparation is preferred.

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Medical practitioners of Tamilnadu has been using this plant product to treat inflammation.^[1] It also

showed some effect in sore throat, flu, fever, diarrhea, and diabetes (it contain insulin like chemical pinitol).^[2,3] It protects liver probably due to esculetin content, used in asthma and bronchitis, to regulate menstruation and stop leucorrhea and treatment of gastrointestinal bleeding and epigastric pain.

It is obvious that like every herb, *Bougainvillea spectabilis* has many chemotypes. It contains anthraquinones, tannins, pinitol, esculetin, glycosides, alkaloids, quercetin, and many other components responsible for variable therapeutic effects. In animal experimental models, water extracts of BS had shown significant lowering of estrogen in female and testosterone level in male mice. Alcohol extract of BS reduces bad cholesterol level and hence the risk of cardiovascular diseases. *Bougainvillea* leaves. (ethanolic extract) showed significant reduction of serum calcium and potassium and increase in phosphate, serum urea and

creatinine concentration. Therefore, continuous usage of ethanolic extract of BS might adversely affect normal functions of kidney and liver.

Mandal et al and Joshi et al observed anti-inflammatory activities of BS (methanolic extract of leaves) in experimental models.^[4,5] Though limited information is available regarding its phytochemistry, pharmacological and toxicological activities of BS are available in plant research. As part of a continuing search for plant derived analgesic agents, this study was undertaken to explore the potential application of methanolic extract of *Bougainvillea spectabilis* (BS) leaves as an analgesic agent in different animal models.

Objectives

Evaluation of analgesic effects of *Bougainvillea spectabilis* (BS) in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice of average weight 15-25 g. of either sex were acclimatized for one week before starting the experiments. Standard laboratory conditions and temperature ($24 \pm 5^\circ\text{C}$) were given to all animals. Animals were provided with free access to water *ad libitum* and commercial pellet feed. 12 hr light/dark cycle was maintained. The study was approved by the Institutional Animal Ethics Committee and followed OECD guidelines. CPCSEA/544 is the registration no of Institutional Animal Ethics Committee of IPGME&R, Kolkata.

Preparation of Plant Product

The identity of the plant was authenticated by the Botanical Survey of India. Fresh leaves from the flowering plant *Bougainvillea spectabilis* (BS) were collected from the local area and air-dried (215.00g). Following methanol extraction solvent was removed on a rotary evaporator under reduced pressure. The yield was 8 gm after the extract was freeze-dried (lyophilized). Propylene glycol was used to prepare an emulsion. *Bougainvillea spectabilis* (BS) in an initial pilot study was administered up to a maximum of 200 mg/kg of b.w. 50kg/kg. of BS leaves had shown maximum analgesic effect.

- Method for evaluation of central analgesic activity.
- D' Armour et al., (1941) invented this method for evaluation of central analgesic activity later modified by Ther et al. (1963).^[6,7] Forty eight rats were taken and divided into four groups of twelve mice each. The groups were treated as follows:

Group (n=12)	Treatment
Group I	Control
Group II	Standard drug, Pethidine (12 mg/kg)
Group III	<i>Bougainvillea spectabilis</i> (BS) (20mg/kg)
Group IV	<i>Bougainvillea spectabilis</i> (BS) ,(50mg/kg)

Tail Flick Test

An analgesiometer was provided by Model Mark I Techno Electronic, Lucknow. The tail of each mouse was placed 2 mm above the nichrome wire of the instrument. 5-amp current was passed and the radiant heat was generated. It was directed to the proximal third of the tail. The reaction time was recorded as the time taken by each animal to withdraw (flick) the tail away from the hot wire. Mice that failed to withdraw its tail within 15 seconds was excluded from the experiment. The normal reaction time was recorded before administration of the test compound or the standard drug. The reaction time was measured at 30, 60, 90, 120 and 150 min after administration of test and standard drugs. The analgesic activity was classified as positive if the mouse failed to withdraw its tail within 15 sec of exposure.

c) Tail immersion method:

This method of the test was adopted by Ramabadrhan *et al.* (1989).^[8] Mice were divided into four groups. Each group contains 12 animals. Tail (lower 5.0 cm) of the mice was then dipped in a beaker of water ($55 \pm 0.5^\circ\text{C}$). The reaction time is the time required to withdraw the tail out of water. The animals discarded were those who could not lift its tail out of the water within 10 seconds. The basal reaction time for all the animals was recorded as the 0-minute observation. The reaction time was measured at different (30, 60, 90,120 and 150) min after administration of test and standard drugs. A normal control, without any treatment was also maintained. The reaction time was noted 30, 60, 90, 120 and 150 min after drug administration.

d) Model for evaluation of Peripheral analgesic activity

Acetic acid induced writhing test

This was based on the method described by Koster *et al.* (1959).^[9] Albino mice of either sex were divided into four groups of twelve animals each. *Bougainvillea spectabilis* (20 and 50 mg/kg of b.w) at doses of 0.1 ml/10 gm, aspirin (30 mg/kg; standard) were orally administered respectively to the three groups. Intraperitoneal injection of 0.6 % v/v acetic acid solution in water at a dose of 10 ml/kg. A control group without any drug treatment was also treated with acetic acid. Immediately after administration of acetic acid, the number of stretches (a syndrome, characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) was counted for 15 min. Evidence for the presence of analgesia was measured by reduction in the number of writhes as compared to the control group. This is expressed as percent inhibition of writhing, which is calculated according to the following formula:

$$\text{Mean number of writhes} - \text{Mean number of writhes}$$

$$\% \text{ Inhibition} = \frac{\text{in control group} - \text{in test group}}{\text{Mean number of writhes in control group}}$$

Statistical Analysis: SPSS-20 was used as software to measure test of significance (evaluated by ANOVA), with $P < 0.05$ implying significance. The results were expressed as mean \pm standard deviation and in terms of percentages.

Toxicity studies:

The potential toxicity of BS was measured in a subacute toxicity model in Swiss albino mice by Ghosh in 1984^[10]. BS was given orally to mice in a stepwise fashion. Minimum dose of 100 mg/Kg to a maximum of 1.5 g/kg b.w. Highest dose was given for an additional two weeks. Renal function tests (urea and Creatinine) and Liver function tests (serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase were carried out using appropriate kits.

RESULTS

Bougainvillea spectabilis (BS) had shown no analgesic action in central analgesic model at different hours. Reaction time was less than 10 seconds at all time interval. [Figure 2], [Figure 3] With regard to peripheral analgesic activity, maximal activity was observed at 50 mg/kg b.w, that remained little bit lesser at 20 mg/kg. The mean writhes \pm standard deviation were 42.7 ± 0.9 and 40 ± 0.5 respectively, control mice being 55.3 ± 0.4 . [Figure 1] The degree of inhibition at these doses were 27.6% and 22.6% respectively and statistically significant as compared to controls ($P < 0.001$). However this analgesic activity was lower than that obtained with aspirin where the mean writhes \pm SD was 33.3 ± 0.4 causing 39.8% inhibition. Algesia is caused acetic acid, which liberates endogenous substances including serotonin, histamine, prostaglandins, bradykinine and substance P.

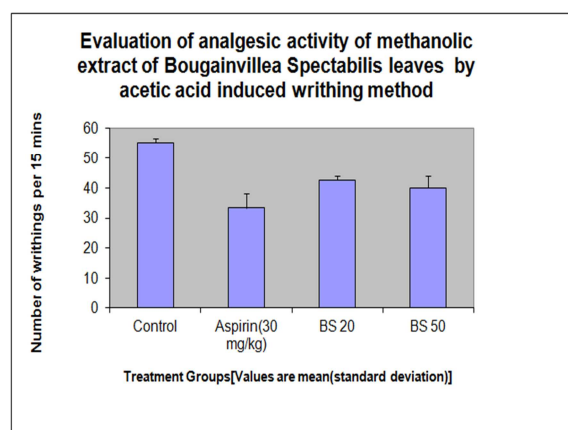


Figure 1: Effects of methanolic extract of *Bougainvillea spectabilis* by acetic acid induced writhing test.

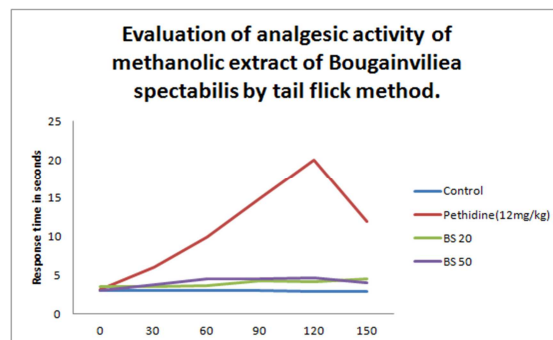


Figure 2: Effects of methanolic extract of *Bougainvillea spectabilis* by tail flick method.

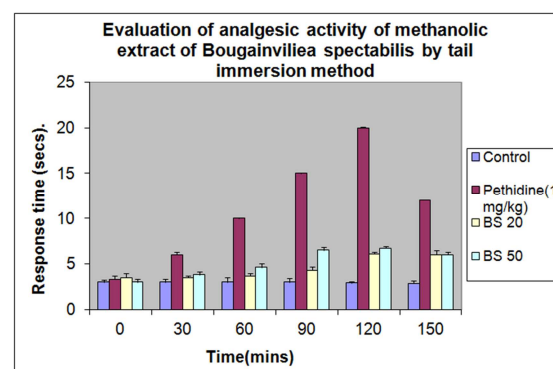


Figure 3: Effects of methanolic extract of *Bougainvillea spectabilis* by tail immersion method.

DISCUSSION

Peripheral nociceptors are responsible for causing sensitization, which may lead to hyperalgesia. It occurs when different chemical products, such as bradykinin, 5-HT, histamine are released near nociceptor terminals after or during tissue inflammation. Algesia is caused by endogenous substances including serotonin, histamine, prostaglandins, bradykinine and substance P liberated by acetic acid. Inflammation may destroy cells near nociceptor when exposed to a noxious stimulus. Proteolytic enzymes are released by dying cells that react with circulating globulins to form bradykinin. Activation of a second messenger system and sensitizing of the nerve ending happened after binding of bradykinin to a receptor on the membrane of the nociceptor (William, 1998b)^[11,12] It also provoked the release of neuropeptides such as substance P, neurokinin A, and CGRP as described by Geppetti, in 1993.^[13] Data in the experiments shown by Mandal et al indicated that BS (50 mg/Kg of body weight) had a certain degree of both anti-inflammatory (acute and chronic) activity, as it was capable of decreasing edema formation in all kinds of anti-inflammatory models. Garcia, in 1978 had shown that bradykinin was more potent than histamine and serotonin as mediator of pain, which was also established by Moncada et al., in 1978. He emphasized the role of prostaglandins in sensitizing the nerve endings to the

effects of bradykinin and other algogens.^[14] Underlying mechanism of nerve ending sensitization is either opening up of the ion channels or by activation of second messenger. Therefore, it is possible that the peripheral analgesic activity of *Bougainvillea spectabilis* leaves (methanolic extract) observed was due to its concomitant anti-inflammatory activity already established by Mandal et al in 2015.

Absence of toxicity of BS in a subacute toxicity model:

Sub-acute toxicity studies using the methanolic extract of BS leaves showed no major toxicity as evidenced by the absence of any hepatic or renal damage; the levels of SGOT, SGPT, Alkaline phosphatase, urea and creatinine of control and treated groups were not significantly different when tested statistically.

CONCLUSION

The results of the present study revealed only peripheral analgesic activity. Without isolating the active principles, it's extremely difficult to pinpoint the mechanisms contributing to the observed analgesic activity of *Bougainvillea spectabilis* and extrapolate that in clinical practice.

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