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Research Article

ANALGESIC EFFECTS OF THE ETHANOLIC EXTRACT OF FLOWERS OF *SPATHODEA CAMPANULATA* STUDIED IN ALBINO MICE

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Abstract: The analgesic effect and possible mechanism of action of 100-500 mg/kg of the ethanolic flower extract of *Spathodea campanulata* were investigated in different experimental models of analgesia using the Acetic acid induced writhing test, formalin test and Tail immersion methods. Oral pre treatment with 100-500 mg/kg of *Spathodea campanulata* caused significant and dose related analgesic effect in the treated rats in all the experimental models used. This analgesia was mediated via central and peripheral mechanisms. Overall, the results showed that *Spathodea campanulata* possesses analgesic effect which lends support to its folkloric use in the local management of pain.
Key words: Analgesic activity, *Spathodea campanulata,* ethanolic extract, pain.

Introduction

Analgesics are defined as the substances which decreases pain sensation by increasing pain threshold to external stimuli. Noxious pain stimuli can be developed by thermal, chemicals and physical pressure.[1] Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs present well known side and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000 - 4000 compounds are to be synthesized, screened and tested where the cost of development ranges from 0.5 to 5 million dollars.[2]

Pain is an unpleasant sensation localized to a part of the body. It is both sensation and emotion. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distension, or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system.[3]

Spathodea campanulata P.Beauv. species belonging to the Bignoniaceae family. It is a native of West Africa. This spectacular flowering tree is abundantly planted throughout the tropics and has naturalized in many parts of the Pacific. It is a large tree that can reach 50ft in height. The flared, funnel shaped flowers appear in 8 to 10cm long racemes on the tips of the branches**;** leaves grow to 40cm long**;** fruit is a long pod**;** very small seeds with transparent wings.

Several medicinal properties have been reported on it including flavonoids of eight bignoniaceous plants.[4] *Spathodea campanulata***:** An experimental evaluation of the analgesic and anti inflammatory properties.[5] Hypoglcemic, anti-HIV and antimalarial activity were also observed in stem bark extracts.[6] and the presence of anthocyanins in flowers of *Spathodea campanulata.*[7] Hence, in the present study, to evaluate the *in vivo* analgesic activity of the ethanolic extract of *Spathodea campanulata* flowers.

Materials and methods

Plant Collection and Identification

The Plant species namely *Spathodea campanulata* flowers were collected in Mannargudi and around Thiruvarur (Dt), Tamil Nadu. The plant was identified and authenticated by Dr.Soosairaj, Department of botany, St. Joseph's college, Trichirappalli. [Voucher number of the specimen: SJCBOT 1563/2013].

Preparation of Plant Powder

The flowers were air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder mater was used further *in vivo* Analgesic Activity.

Extraction of Plant Material

Ethanol and aqueous extracts were prepared according to the methodology of Indian pharmacopoeia (anonymous, 1996). The coarse powder material was subjected to soxhlet extraction separately and successively with Ethanol and distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40°C-50°C) the ethanol and aqueous extracts put in air tight container stored in a refrigerator.

Drugs and Chemicals

Acetic acid, Formalin, Aspirin were purchased for Sigma chemical company, Mumbai. All other chemicals used in this study were of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Animals

Male albino rats of Wistar strain approximately weighing 120 -150g were used in this study. They were healthy animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under experimental conditions (Temperature 27 ± 2°C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum.* They were acclimatized to the environment for one week prior to experimental for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.02%), Ash (8.02%) and sand silica (1.02%).

Acetic Acid induced writhing model

The method described by Koster *et al.*, was used.[8] Thirty mice were fasted for 6 hours and divided into five groups of six animals per group. Groups 2-4 received the extract orally (100, 250 and 500 mg/kg), respectively. The first Group received saline while the fifth group was given aspirin (150mg/kg) Sixty minutes later 0.6% acetic acid (10ml: kg) was injected (i.p.). The numbers of abdominal constrictions during the following 15 minutes period were counted. A reduction in the writhing number as compared to the control group was considered as evidence for the presence of analgesia.

Formalin test

This study was based on the method described by Shibata *et al.*, was used.[9] Thirty mice were fasted for 6 hours and divided into five groups of six animals per group. Groups 2-4 received the extract orally (100, 250 and 500 mg/kg), respectively. The first Group received saline while the fifth group was given Indomethacin (10mg/kg b.wt.) was administered 45 minutes, before formalin injection. 20µl of 1% formalin was injected subcutaneously into the right hind paw of rats. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 minutes after formalin injection (First Phase) and 15-30 minutes after formalin injection (second phase).

Tail – immersion test

The method described by Janssen *et al.*, was used.[10] Thirty mice were fasted for 6 hours and divided into five groups of six animals per group. Groups 2-4 received the extract orally (100, 250 and 500 mg/kg), respectively. The first Group received saline while the fifth group was given Morphine (10 mg/kg). The lower 5 cm portion of the tail was immersed in a beaker of water maintained at 55 ± 0.5°C. The time in second for tail withdrawal from the water was taken as the reaction time, with a cut – off time of immersion set at 10 second. The reaction time was measured 1 hour after oral administration of ethanolic extract (100, 250 and 500 mg/kg). Morphine (10 mg/kg) was administered orally.

Results and discussion

Acetic Acid induced writhing model

The effect of *Spathodea campanulata* extract on acetic acid induced writhing in rats was shown Table 1 and Figure 1. *Spathodea campanulata* at the doses of 100, 250 and 500 mg/kg body weight produced a very significant($P < 0.001$) reduction writhing induced by acetic acid when compared to control. The % inhibitions of writhes or % protection were found to be 23.91 %, 48.56 % and 74.62 % for extracts dose of 100, 250 and 500 mg/kg body weight, respectively. The highest dose (500 mg/kg) produced a significant ($P < 0.001$), percentage inhibition of 74.62 % while Aspirin (150 mg/kg) gave 50.73 %, a value slightly lower than that of the extract.

Acute and chronic tests were employed in evaluating the analgesic effect of the ethanolic extract of *Spathodea campanulata*. It is necessary to apply tests which differ with respect to stimulus quality, intensity and duration, to obtain as complete a picture as possible of the analgesic properties of a substance using behavioral nociceptive tests.[11] The results obtained indicate that the extracts possess a moderate dose dependent analgesic effect on the various pain models used.

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics.[12] In general, acetic acid cause pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response.[13] The method has also been associated with prostanoids in general, that is, increased level of PGE2 and PGF2α in peritoneal fluids, as well as lipooxygenase products.[14] The significant reduction in acetic acid induced writhes by *Spathodea campanulata* suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Formalin Test

There was a significant dose-dependent inhibition of both phases of the formalin induced pain response in rats were shown in Table 2 and Figure 2, with a more potent effect on the first phase than the second phase. Indomethacin (10 mg/kg body weight) also inhibited both phases of the pain significantly($P < 0.001$) when compared to control group. Among the various doses, 500 mg/kg has significant reduce the pain response in both phases. The % inhibitions were found to be in first phase 48.57 %, 55.71 % and 74.28 %, 33.77 %, 37.74% and 51.65% in second phase, for extracts dose of 100, 250 and 500 mg/kg body weight, respectively. The highest dose (500 mg/kg) produced a significant ($P < 0.001$), percentage inhibition of 74.28 % while Indomethacin (10 mg/kg) gave 67.14 %, a value slightly lower than that of the extract in first phase.

The extract gave a similar effect on the formalin test inhibiting both the first and the second phase. Formalin test is biphasic and measures pain of both neurogenic (first phase) and of inflammatory origin (second phase). The first phase (0 – 5 minutes) being a result of direct stimulation of nociceptors measures centrally mediated effects and is

insensitive to anti inflammatory agents while the second phase (15 – 30 minutes) which is qualitatively different from the first phase is dependent on peripheral inflammation and changes in central procession due to chemical mediators release from damaged cells that stimulate nociception and thus induced pain.[15] In general, the test measures the response to a long lasting nociceptive stimulus similar to clinical pain and is recommended as a tool in basic pain research for studying the mechanisms of analgesic agents because of its connection to tissue injury. Agents that act primarily on the CNS inhibit both phases equally while peripherally acting drugs inhibit the late phase. The ability of *Spathodea campanulata* to inhibit both phases of the formalin test indicates its both central and peripherally mediated activity, probably by prostaglandin synthesis inhibition, as well as central inhibition mechanism.

Tail immersion Test

The effect of ethanolic extract on tail immersion tests were shown in Table 3 and Figure 3. These extract showed a dose dependent inhibition of pain. Morphine (10 mg/kg body weight) also inhibited the pain significantly ($P < 0.001$) when compared to control group. Among the various doses, 500mg/kg has significant reduce the pain response. The % inhibitions were found to be 58 %, 125 % and 230 % for extracts dose of 100, 250 and 500 mg/kg body weight, respectively. The highest dose (500 mg/kg) produced a significant ($P < 0.001$), percentage inhibition of 230 % while Morphine (10 mg/kg) gave 400 %, a value slightly higher than that of the extract.

The extracts had a significant effect in the tail immersion tests. The significant increase in pain threshold produced by *Spathodea campanulata* in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems.[16] The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes and other endogenous substances that are key players in pain. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The effect of the extract on this pain models indicates that it might be centrally acting.

From the present study, it is concluded that ethanolic extract of *Spathodea campanulata* flowers possesses potent analgesic effect against different stimuli. This is evidenced by significant increase in the reaction time by stimuli in different experimental models.

Table 1: Analgesic Activity of the *Spathodea campanulata* on Acetic Acid Writhing in Rats

Groups Treatment	Dose(mg/kg)	No. of Writhing	% of Inhibition
Group I	-	23 ± 3.74	-
Group II	100	17.5 ± 3.08	23.91
Group III	250	11.83 ± 2.48	48.56
Group IV	500	5.83 ± 2.31	74.62
Group V	150	11.33 ± 2.94	50.73

Values are expressed as mean ± SD. (P<0.001)

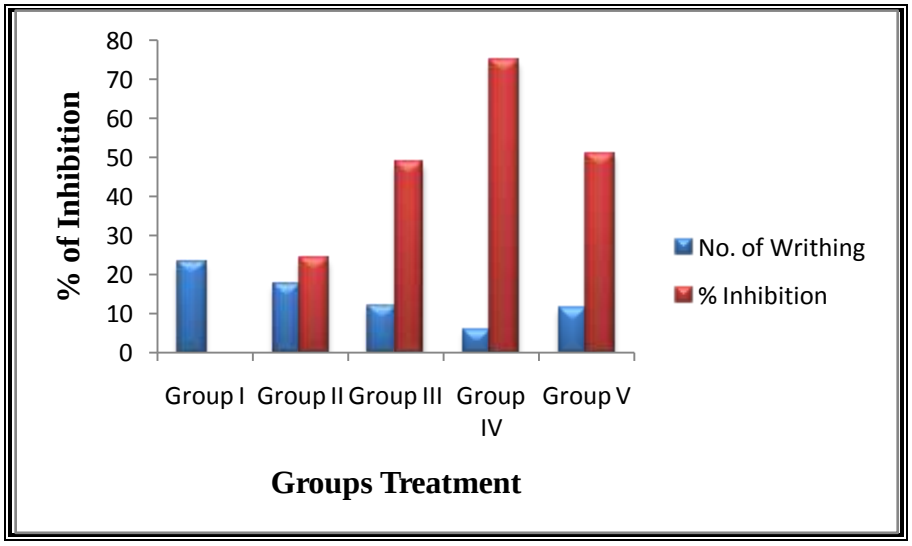

Figure 1: Analgesic Activity of *Spathodea campanulata* on Acetic Acid Writhing in Rats

Table 2: Analgesic Activity of *Spathodea campanulata* on Formalin – Induced Pain in Rats

Groups Treatment	Dose (mg/kg)	0-5 (mins)	% of Inhibition	15 -30 (mins)	% of Inhibition
Group I	-	70.5 ± 5.31	-	151.16 ± 4.49	-
Group II	100	36.66 ± 1.21	48.57	100.33 ± 2.73	33.77
Group III	250	31 ± 2.36	55.71	94.83 ± 3.31	37.74
Group IV	500	18.83 ± 2.99	74.28	73.5 ± 4.63	51.65
Group V	150	23.16 ± 2.31	67.14	115.16 ± 3.68	23.84

Values are expressed as mean ± SD. (P<0.001)

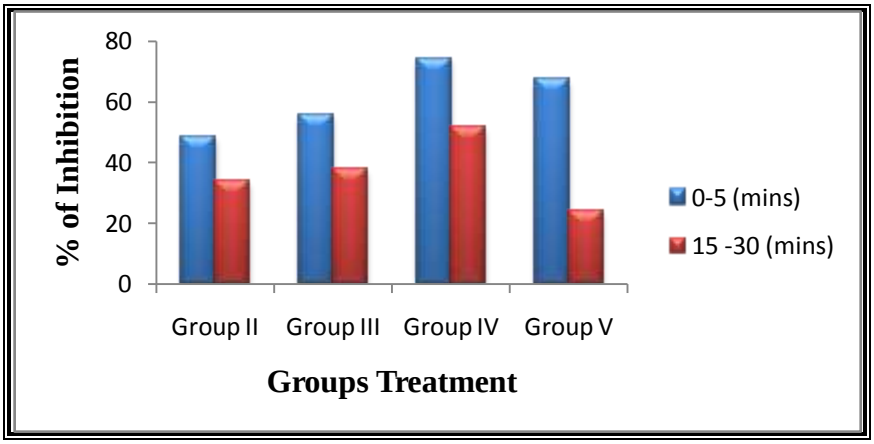

Figure 2: Analgesic Activity of *Spathodea campanulata* on Formalin – Induced Pain in Rats

Table 3: Analgesic Activity of *Spathodea campanulata* on Tail Immersion Test in Rats

Groups Treatment	Dose (mg/kg)	Reaction Time (Sec)	% of Inhibition
Group I	-	2 ± 0.89	-
Group II	100	3.16 ± 1.16	58
Group III	250	4.5 ± 1.87	125
Group IV	500	6.66 ± 2.80	230
Group V	150	10.5 ± 1.87	400

Values are expressed as mean ± SD. (P<0.001)

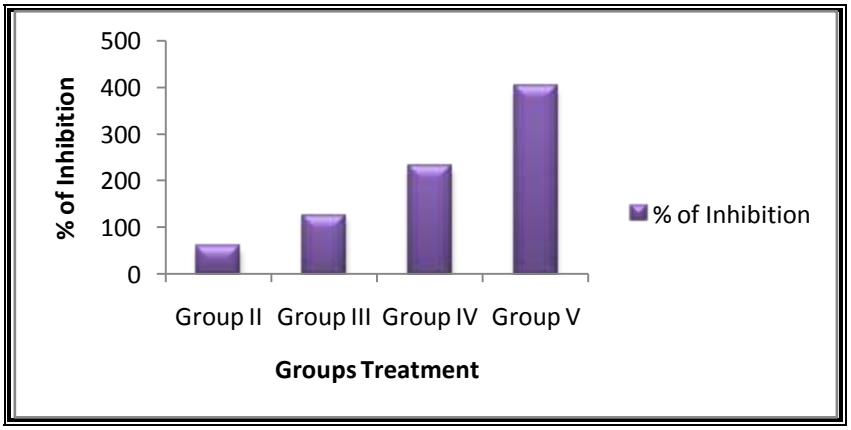

Figure 3: Analgesic Activity of *Spathodea campanulata* on Tail Immersion Test in Rats

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References

1. Tripathi KD. Essential of medical Pharmacology. 5th - edn. Jayapee Brother Medical Publisher. **2003**; 219-220.
2. Besra SE, Sharma RM and Gomes A. anti inflammatory effect of petroleum ether extract of leaves of Litchi chinensis Gaertn(Sapinadaceae). *J.ethnopharmacology*, **1994**; 54:1-6.
3. Fields HL, Martin JB, Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL and Jameson JL. Pain pathophysiology and management In: Harrison's Principles of internal medicine. 17th ed. New York, McGraw Hill. **2008**; 81-86.
4. Sankara Subramanian S, Nagarajan S and Sulochana N. Flavonoids of eight bignoniaceous plants. *Phytochemistry*. **1972**; 11: 1499.
5. Emmanuel EI and Peter AA. *Spathodea Campanulata*: an Experimental Evaluation of the Analgesic and Anti-inflammatory Properties of a Traditional Remedy. *Asian Journal of Medical Sciences*. **2009**; 1(2): 35-38.
6. Niyonzima G and Laekeman G. hypoglycaemic, anticomplement and anti HIV activities *Spathodea campanulata* stems bark. *Phytomedicine.* **1999**; 6(1): 45-49.
7. Banerjee A and De B. Anthocyanins in some flowers of west Bengal. *Journal of Medicinal and aromatic plant science.* **2001**; 23: 493-496.
8. Koster R, Anderson M and De Beer EJ. Acetic acid analgesic screening. *Federation proceedings.* **1959**;18: 418-420.
9. Shibata M, Ohkubo T and Takahashi H. Modified formalin test; characteristic biphasic pain response. *Pain*. **1989**;38: 347-352.
10. Janssen PAJ, Niemegeer CJE and Dony JGH. *Arzneim Forsch Drug Res.* **1963**; 6: 502.
11. Ghule BV, Murugananthan G and Yeole PG. Analgesic and antipyretic effects of *Capparis zeylanica* leaves. *Fitoterapia.* **2007**;78: 365-369.
12. Gene RM, Segura L and Adzet T. *Heterotheca inuloides*: anti inflammatory and analgesic effects. *J. Ethnopharmacol.* **1998**; 60: 157-162
13. Bently GA, Newton SH and Starr J. Studies on the Anti-nociceptive action of agonist drugs and their interaction with opioid mechanisms. *Br.J. Pharmacol.* **1983**;79: 125-134.
14. Roberts LJ and Morrow JD. Analgesic, antipyretic and anti inflammatory agents and drugs employed in the treatment of Gout. In: Eds. Goodman and Gilman's The pharmacological Basis of Theraprutics. 10th ed. McGraw-Hill. New York. **2001**; 687-732.
15. Hunskaar S and Hole K. The formalin test in rats: Dissociation between inflammatory and non inflammatory pain. *Pain*. **1987**; 30: 103-114.
16. Wigdor S and Wilcox GL. Central and systemic morphine-induced antinoniception in rats: Contribution of descending serotonergic and noradrenergic pathways. *J Pharmacol Exp Ther*. **1987**;242: 90-95.