Effect of ethanolic extracts of *Justicia neesii* Ramam. against experimental models of pain and pyrexia

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**ABSTRACT**

**Objective:** The main objective of this study is to evaluate the analgesic and anti-pyretic activities of ethanolic extracts of *Justicia neesii* Ramam. by different experimental models.

**Materials and Methods:** The analgesic activity of plant extract was evaluated against thermal and chemical stimulus induced by Eddy’s hot plate and acetic acid respectively in mice. Brewer’s yeast induced pyrexia was used to evaluate the antipyretic activity in rats and TAB vaccine induced pyrexia was used to evaluate the antipyretic activity in rabbits.

**Results:** In the hot plate model 400 mg/kg p.o. dose of *J. neesii* has shown its maximal effect at 3 h. The results are significant (*P* < 0.05) and comparable to the values of standard drug pentazocine (30 mg/kg i.p.). In acetic acid induced writhing model 400 mg/kg p.o. of plant extracts have shown highly significant activity (*P* < 0.001) and better than standard drug indomethacin (10 mg/kg p.o.). The 400 mg/kg p.o. dose of plant extract has given significant results against both yeast induced pyrexia and TAB vaccine induced pyrexia (*P* < 0.01 and 0.05 respectively). These values are comparable to that of paracetamol 100 mg/kg p.o. standard dose.

**Conclusion:** This study shows that the ethanol extract of *J. neesii* has significant analgesic and antipyretic activity.

**KEY WORDS:** Brewer’s yeast, hot plate, pyrexia, TAB vaccine, writhing

**Introduction**

India is a country with huge biodiversity of plants, out of them 1500 medicinal plants are well recognized which are serving the people for treating different ailments with fewer adverse reactions.[1] *Justicia neesii* is one of such plant belonging to the family Acanthaceae and grows in tropical regions of India as a small tropical herb. Some previous studies on this plant have reported the presence of various types of lignans. Three β-apolignans including 1,4-dihydrotaiwanin C, Jusneesiin, Jusneesiinol[2] and two arylnaphthalide lignans including juscicranthin and justirumalin are found to be present.[3,4] The plant was also found to contain diphyllin glycosides such as neesiinoside A and neesiinoside B.[5] In previous studies, it was reported that the lignans are having significant analgesic activities.[6] We observed the use of *J. neesii* for treating fever, wounds, helmenthic infections and headache in tribal areas of East Godavari district, Andhra Pradesh, India. However, the review of scientific literature revealed that there is no significant pharmacological work done on *J. neesii*. This ethno botanical and phytochemical information on *J. neesii* guided us research minds to focus on the screening of antipyretic and analgesic properties.

**Materials and Methods**

**Collection and Identification of Plant**

Plant material was collected from different areas of East Godavari district, Andhra Pradesh during the month of February 2014 on day time. The plant was taxonomically identified by the experts of Botanical Survey of India, Hyderabad (BSI/ DRC/2013-14/Tech./915-A).

**Extraction of Plant Material**

Whole plant parts including leaves, stem, twigs, flowers, seeds, roots were separated and made free from soil matter. They were dried and powdered by using hand pulverizer to a coarse powder. Then, the powder was extracted with ethanol...
by using soxhlet apparatus at a temperature of 50°C–55°C for 8 h. The extracts were concentrated using a vacuum evaporator, and the semisolid mass was dried in vacuum desiccators. The yield of plant extract was found to be 10.58% (w/w).

**Experimental Animals**

Adult Albino rats of either sex weighing between 200 and 250 g, male Swiss Albino mice weighing between 25 and 27 g and albino rabbits of both sex weighing between 1.5 and 1.8 kg were procured from authorized vendors (Mahaveera Enterprises, Hyderabad). Rats and mice were maintained in polypropylene cages and rabbits in iron cages at a temperature of 25 ± 2°C and relative humidity 45–55% with 12:12 light and dark cycle. Animals were given standard diet and water ad libitum. Animals were acclimatized to the laboratory conditions 1-week before the experiment. Animals were fasted over night before the experiment. Experimental protocol was approved by the Institutional Animal Ethical Committee, and the IAEC was approved by CPCSEA (1047/ac/07/CPCSEA).

**Acute Toxicity Study**

The toxicity of plant extract on experimental animal was tested according to the Organisation of Economic Co-operation and Development-423 guideline. Adult nulliparous and nonpregnant female albino rats were selected for the toxicity study, as female rats are more sensitive. Six animals were assigned to each group and fasted overnight prior to the administration of oral doses of test substances at a concentration of 5, 50, 300 and 2000 mg/kg body weight. All the test concentrations are adjusted to below 2 mL volume and administered using oral gavages. The animals were observed for first 30 min and periodically for 24 h. Mortality was not observed at any dose level. The observation was continued for 14 days for toxic signs.

**Analgesic Activity**

**Eddy’s hot plate method**

The rodent paws are very sensitive to the thermal stimulus, and they will show responses like jumping and licking when exposed to moderate heat. In this method, analgesic activity was tested against thermal stimulus. The mice are placed on the copper plate, which is at a temperature of 55°C–56°C and the time between initial placement and a hand lick or a jump was taken as reaction time.[7] Mice with baseline latency more than 20 s are eliminated from the study. Mice are divided into five groups of six animals each. First group served as a negative control and received 1% Tween 80 in distilled water 10 mL/kg (p.o.). Group-2, Group-3 and Group-4 received 100, 200 and 400 mg/kg body weight (p.o.) of ethanolic extracts. Group-5 served as a positive control and received 30 mg/kg (i.p.) of standard drug pentazocine. The basal reaction time was noted at 0 h and then readings were taken at 1 h, 2 h, 3 h and 4 h after the treatment.

**Acetic Acid Induced Writhing Test**

In this method, analgesic activity was tested against chemical stimulus. A stereotyped behavior characterized by constriction of the abdomen, twinning of the trunk and extension of hind limbs is called writhe. Acetic acid will cause abdominal constrictions in mice by irritating the serous membrane, which causes visceral or peritoneal pain. The percentage protection of test and standard drugs against abdominal constriction was taken as an index of analgesic activity.[8] This method is used for testing peripherally acting analgesics. Mice were divided into five groups of six animals each. First group serves as a negative control and received 1% Tween 80 in distilled water 10 mL/kg (p.o.). Group-2, Group-3 and Group-4 received 100, 200 and 400 mg/kg body weight (p.o.) of ethanolic extracts. Group-5 served as a positive control and received 10 mg/kg (p.o.) of standard drug indomethacin. All the drugs are given 30 min before the administration of acetic acid. The writhing and stretching movements were observed and counted for 30 min after the administration of 0.7% acetic acid in a volume of 10 mL/kg (i.p.). The percent reduction in writhing was calculated and compared with the control group.

**Antipyretic Activity**

**Brewer’s yeast induced pyrexia in rats**

In this method, rats were divided into six groups of six animals each. Group-1 serves as a nonpyretic control and receives only vehicle (1% Tween 80 in distilled water) 2 mL/kg (p.o.). Pyrexia was induced to Group-2 to Group-6 by injecting 10 mL/kg (s.c.) of 20% yeast suspension (in normal saline) to each rat and gently massaged for uniform distribution. The rats will become hyperthermic, 18 h after subcutaneous injection of yeast suspension.[9] Then, the rats of Group-2, which served as pyretic control is given with 1% Tween 80 in distilled water 2 mL/kg (p.o.), Group-3, 4, 5 serves as test groups and receives 100 mg/kg, 200 mg/kg and 400 mg/kg (p.o.) of plant extract respectively. Group-6 receives 100 mg/kg (p.o.) of standard drug paracetamol. The rectal temperature was measured by using electric clinical thermometer by inserting it up to one inch for every reading. The values were noted for every 1-h starting from 0 h to 6 h.

**TAB vaccine induced pyrexia in rabbits**

In this method, rabbits were divided into six groups of six animals each. Group-1 serves as a nonpyretic control and receives only vehicle (1% Tween 80 in distilled water) 2 mL/kg (p.o.). Pyrexia was induced to Group-2 to Group-6 by injecting 0.5 mL (i.v.) of TAB vaccine to each rabbit through marginal ear vein. The rabbits will become hyperthermic, 60 min after the injection of vaccine.[10] Then the rabbits of Group-2 which served as pyretic control is given with 1% Tween 80 in distilled water 2 mL/kg (p.o.), Group-3, 4, 5 serves as test groups and receives 100 mg/kg, 200 mg/kg and 400 mg/kg (p.o.) of plant extract respectively. Group-6 receives 100 mg/kg (p.o.) of standard drug paracetamol. The rectal temperature was measured by using electric clinical thermometer by inserting it up to 5 cm for every reading. The values were noted for every 1-h starting from 0 h to 6 h.

**Statistical Analysis**

Data were represented as mean ± standard error of mean and analyzed by one-way analysis of variance, followed by Dunnett’s multiple comparison. *P* < 0.05 was considered as significant.

**Results**

The acute toxicity studies conducted on plant extract did not reveal any toxic signs even at 2000 mg/kg (p.o.) concentration.
The experimental animals did not exhibit any behavioral changes. Hence, the ethanolic extract of *J. neesii* was found to be safe for internal administration.

Table 1 shows the analgesic activity of different concentrations of test solutions against thermal stimulus given by the hot plate. 400 mg/kg (p.o.) dose of plant extract showed a significant effect (*P* < 0.05) and this was comparable to standard pentazocine. The maximum effect was observed at 400 mg/kg (p.o.) dose at 3 h time, which showed a reaction time of 23.55 s, where the standard drug pentazocine (30 mg/kg i.p.) showed a reaction time of 19.78 s. Table 2 shows the analgesic activity of different concentrations of test solutions against chemical stimulus induced by acetic acid. Plant extract at all the concentrations have showed significant results (*P* < 0.001). The maximum decrease in number of writhing was observed for 400 mg/kg (p.o.) dose at a percentage of 88.91; whereas the standard drug indomethacin (10 mg/kg p.o.) showed a reduction of 82.27%.

Figure 1 shows the effect of test and standard solutions on rectal temperature of rats following the administration of yeast suspension. Plant extract at 200 and 400 mg/kg (p.o.) dose shows statistically significant (*P* < 0.05) antipyretic effects. 400 mg/kg dose of plant extract showed more significant effect (*P* < 0.01) and this was comparable to standard paracetamol. Figure 2 shows the effect of test and standard solutions on rectal temperature of rabbits following the administration of TAB vaccine. In this method, 400 mg/kg (p.o.) dose of plant extract only showed a significant effect (*P* < 0.05) and this was comparable to standard paracetamol.

**Discussion**

Hot plate model is a well validated model for screening of opioid analgesics and for drugs acting on spinal cord.[11] In this study the dose dependent analgesic effect was observed for this plant extract, which prolongs the hot plate latency and revealing the centrally acting nature of plant extract. Acetic acid induced writhing is the simple and reliable model for rapid evaluation of peripherally acting analgesics.[1] The acetic acid induced writhing involves the release of bradykinin and prostanoids at peripheral tissues,[12] which acts on local peritoneal receptors. Nonsteroidal anti-inflammatory drugs (NSAIDs) like indomethacin will inhibit the enzyme cyclooxygenase at peripheral tissues and block the release of endogenous substances that exerts pain. The antagonism of prostaglandin receptors or suppression of the formation of the prostanoids may be the possible reason for analgesic effects of plant extract against writhing. From these screenings we observed that effective dose of test compound is varying based on experimental method adopted for evaluation.

It is well-known that most of the analgesic and anti-inflammatory drugs will also act as antipyretics.[13] Several endogenous substances like interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor α (TNF-α) and prostaglandins

![Figure 1: Antipyretic activity of *Justicia neesii* on yeast induced pyrexia in rats](image1)

![Figure 2: Antipyretic activity of *Justicia neesii* on TAB vaccine induced pyrexia in rabbits](image2)

**Table 1:**

| Group | Drugs       | Dose (mg/kg) | Reaction time in seconds at |
|-------|-------------|--------------|-----------------------------|
|       |             |              | 0 h                         | 1 h                         | 2 h                         | 3 h                         | 4 h                         |
| I     | Control     | -            | 04.73±0.16                  | 04.81±0.17                  | 04.80±0.20                  | 04.81±0.10                  | 04.86±0.09                  |
| II    | *J. neesii* | 100          | 05.02±0.08                  | 06.18±0.12                  | 08.24±0.12                  | 11.29±0.15                  | 10.33±0.16                  |
| III   | *J. neesii* | 200          | 04.70±0.18                  | 07.65±0.15                  | 11.65±0.18                  | 17.64±0.15                  | 16.65±0.09                  |
| IV    | *J. neesii* | 400          | 04.57±0.13                  | 08.30±0.14*                 | 14.34±0.08*                 | 23.55±0.18*                 | 21.89±0.04                  |
| V     | Pentazocine | 30           | 04.72±0.13                  | 07.66±0.08*                 | 11.69±0.08*                 | 19.78±0.08*                 | 23.22±0.12*                 |

Values are expressed in mean±SEM, *n*=6; *P*<0.05 statistically significant compared to control group. SEM=Standard error of mean. *J. neesii*=*Justicia neesii*
are evident to produce pyrexia. Yeast induces the pathogenic fever by stimulating the release of prostaglandins and TNF-α, which leads to hyperthermia. TAB vaccine is a sterile suspension composed of $1 \times 10^9$ *Salmonella typhi* and $7.5 \times 10^8$ each of *Salmonella paratyphi* A and B inactivated bacilli per mL. The lipopolysaccharides present in the TAB vaccine may induce hyperthermia by stimulating the release of endogenous pyrogens. The antipyretic effects of NSAIDs will be produced through inhibition of prostaglandin synthetase in hypothalamus. Antipyretic drugs decrease the levels of PGE$_2$ in particular in the hypothalamus region by acting on COX-2. They also mediate their effects by increasing the production of vasopressin and arginine. The antipyretic nature of the plant extract may be through the inhibition of prostaglandin formation particularly at anterior hypothalamic region. The experimental results seem to suggest that the analgesic activity of plant extract was more than the antipyretic activity, as it suppresses the pain even at low concentrations.

It is well known that prostaglandins are having significant role in mediating inflammation, nociception and pyrexia. NSAIDs are the most commonly used drugs in these clinical complications, which are having evidentiary mechanisms in blocking the prostaglandin synthesis. The results of the present experiment show a parallel relationship between the test extract and standard NSAIDs. Hence, we are expecting that the active principles present in test drug is having potential of inhibiting prostaglandin actions and can serve as anti-inflammatory, analgesic and antipyretic medicine.

This experiment concluded that *J. neesii* is having potential analgesic and antipyretic actions. However, the exact phytochemicals responsible for these effects of *J. neesii* have to be identified and further pharmacological studies may be taken up in developing lead compounds and to overcome the limitations of current work.

References

1. Barua CC, Roy JD, Buragohain B, Barua AG, Borah P, Lahkar M. Analgesic and anti-nociceptive activity of hydroethanolic extract of *Drynaria cordata* Willd. Indian J Pharmacol 2011;43:121-5.
2. Rajasekhar D, Subbaraju GV. Justicia lignans-V. Three new beta-apo-lignans from *Justicia neesii* Ramamorthy. Tetrahedron 1998;54:1327-36.
3. Rajasekhar D, Vanisree M, Subbaraju GV. Justicia lignans: Part 4 – Two new arylnaphthalide lignans from *Justicia neesii* Ramamorthy. Indian J Chem 1999;38B: 713-7.
4. Gopalaiah K, Kavithaj, Kanumuri RV, Rajasekhar D, Subbaraju GV. Justicia lignans: Part 9 – Two new lignans from *Justicia neesii* Ramamorthy. (White flower variety) Indian J Chem 2001;40B: 596-600.
5. Subbaraju GV, Rajasekhar D, Kavitha J, Jamez JJ. Justicia lignans: Part 7 – Two new diphyllin glycosides from *Justicia neesii* Ramamorthy. Indian J Chem 2001;40B: 313-9.
6. Borsato ML, Grael CF, Souza GE, Lopes NP. Analgesic activity of the lignans from *Lycnophora ericoides*. Phytochemistry 2000;55:809-13.
7. Eddy NB, Leimbach D. Synthetic analogs. II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther 1953;107:385-93.
8. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959;18:412-6.
9. Loux JJ, DePalma PD, Yankeil SL. Antipyretic testing of aspirin in rats. Toxicol Appl Pharmacol 1972;22:672-5.
10. Saxena PN. Role of prostaglandins in mediation of pyrogen fever. Indian J Med Res 1979;70:499-503.
11. Alhaider AA, Lei SZ, Wilcox GL. Spinal 5-HT3 receptor-mediated antinociception: Possible release of GABA. J Neurosci 1991;11:1881-8.
12. Berkenkopf JW, Weichman BM. Production of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: Its role in the writhing response. Prostaglandins 1988;36:693-709.
13. Hajare SW, Chandra S, Tandan SK, Sarma J, Lal J, Telang AG. Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. Indian J Pharmacol 2000;32:357-60.
14. Kluger MJ. Fever: Role of pyrogens and cytokines. Physiol Rev 1991;71:93-127.
15. Chidambaram K, Albert J, Karpagam K, Sivanesan. Antipyretic activity of *Crateva magna* bark on tab-vaccine induced pyrexia. Int J Pharm Sci Res 2011;2:856-9.
16. Clark WG, Cumby HR. The antipyretic effect of indomethacin. J Physiol 1975;248:625-38.
17. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cytoxigenase-1 variant inhibited by acetaminophen and other analgesics/antipyretic drugs: Cloning, structure, and expression. Proc Natl Acad Sci U S A 2002:99:13926-31.

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### Table 2: Analgesic activity of *J. neesii* by acetic acid induced writhing model

| Group | Drugs     | Dose (mg/kg) | Number of writhing | Percentage reduction |
|-------|-----------|--------------|--------------------|----------------------|
| I     | Control   | -            | 60.17±0.60         | 00.00                |
| II    | *J. neesii* | 100          | 24.17±0.60**       | 60.73                |
| III   | *J. neesii* | 200          | 12.50±0.43***      | 79.22                |
| IV    | *J. neesii* | 400          | 06.67±0.49***      | 88.91                |
| V     | Indomethacin | 10          | 10.67±0.56***      | 82.27                |

Values are expressed in mean±SEM, n=6; **P<0.01 statistically significant compared to control group. SEM=Standard error of mean, *J. neesii*=Justicia neesii.