INVESTIGATIONS OF PHYTOCHEMICAL, ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC EFFECTS OF *IXORA PAVETTA* ANDREWS LEAF

Sumanta Mondal*, S. Raja, P.N.V.S.S Prasad and Padilam Suresh
GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.

ABSTRACT

Ixora pavetta Andrews, (var.: *I. Parviflora* Vahl.) is a small tree or evergreen shrub belongs to the family Rubiaceae, and is used for many ailments, especially for the treatment of to treat chronic wounds, urinary diseases, skin infection, pulmonary troubles, liver disorder, hair tonic, sedative, diuretic, diarrhoea, dysentery, leucorrhoea and veneral diseases. Preliminary phytochemical screening of ethanol exract of *I. pavetta* leaf showed the presence of alkaloids, flavonoids, tannins and saponin glycoside. In the analgesic activity, ethanol extract provoked a significant reduction of the number of writhes in acetic acid-induced writhing response, also significantly reduced the licking time in both phases of the formalin test and highest analgesia in hot-plate test ($P < 0.01$) compared to the control group. The anti-inflammatory effects were investigated employing the both carrageenan and arachidonic acid -induced hind-paw oedema in rats and results of the study revealed the extract to have significant ($P < 0.05$) anti-inflammatory effects at a dose of 400 and 600 mg/kg p.o., rats in both the models. The antipyretic activity was evaluated using Brewer's yeast induced pyrexia in rats and the extract showed significant lowering of body temperature in rats at doses of 400 and 600 mg/kg p.o., and no promising results with 200 mg/kg dose level. These findings suggest that ethanol extract of *I. pavetta* leaves possess potent anti-inflammatory, analgesic and antipyretics effects.

Key words: *Ixora pavetta* Andrews; analgesic; anti-inflammatory; antipyretic

*Correspondence author
Sumanta Mondal
GITAM Institute of Pharmacy,
GITAM University, Visakhapatnam,
Andhra Pradesh, India.

INTRODUCTION

A number of natural sources are used in the traditional medical systems in many countries. Many medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. In analysis the research during the last decades, it is estimated that the analgesics, anti-inflammatory and antipyretics are one of the highest therapeutic categories on which research efforts are concentrated. Analgesic drugs act in various ways on the peripheral and central nervous systems and they are distinct from anesthetics, which reversibly eliminate sensation. The research into plants which are employed as pain-relievers in traditional ethnomedicine is therefore one of the productive and logical strategies in the search for new analgesic, anti-inflammatory and antipyretics drugs. Natural products are believed to be an important source of new chemical substance with potential therapeutic applicability. Several plant species traditionally used as analgesics, anti-inflammatory and antipyretics.

Ixora pavetta Andrews, (var.: *I. Parviflora* Vahl.) is a small tree or evergreen shrub belongs to the family Rubiaceae. It is commonly grown in India in gardens, Burma and the Andamans. Generally tribes of Paderu division of Visakhapatnam district of Andhra Pradesh, India, used the leaf and roots during chest and muscle pain (1-4). Similarly, tribes of Nellore district Andhra Pradesh, used root bark infusion as ethnic practice to cures jaundice and burning micturition (5). Flowers is used for Whooping cough, decoction of the stem barks is also used for anaemia and general debility, roots and fruits juice are given to females when urine is highly coloured (2). Various parts of this plant is also used traditionally in malnutrition, locally to treat-chronic wounds, urinary diseases, skin diseases, pulmonary troubles, Liver disorder, hair tonic, sedative, diuretic, diarrhoea, dysentery, leucorrhoea and veneral diseases (6). Reports on biological activities of the plant are scarce, like ethanolic extract of *I. pavetta* flower significantly decrease the gastric secretion in the aspirin induced and pylorus ligated rats (5). However, only a few phytochemical have been reported on this plant in the literature like ixoral, beta-sitosterol, rutin and kaempferol-3-rutinoside was isolated from leaves and flower; Ethanolic extract of *I. pavetta* leaves also contain 3-Butyn-2-ol, 3-Butyn-1-ol, amyl nitrite, 2-Octyn-1-ol, 1, 9-Decadiyne and Butyl glyoxylate. Stems gave a flavoneglycoside, chrysin 5-O-beta-D-xylopyranoside (6). The arial parts contain 6, 7-dimethoxycoumarin and the seed oil gave capric, lauric, myristic, palmitic, stearic, arachidic, behenic, oleic and linoleic acids (5).

Literature available from all possible scientific sources revealed very little research work on this selected medicinal plant, whereas tribes claim that *I. pavetta*, were used in treatment of various diseases and ailments, and they claim for their promising activity but there is no inbuilt scientific proof in support of the utility of this plant or plant products against analgesic, anti-inflammatory and antipyretics diseases. So, the present study is investigated to explore analgesic, anti-inflammatory and antipyretics activities of ethanol extract from *Ixora pavetta* leaf (EEIPL) by using experimental animal models.

MATERIALS AND METHODS

Plant material

The fresh leaves of plant materials were collected from the young and matured plants from in and around East Godavari dist., Andrapradesh, India and authenticated by Dr. Venkayyah, Scientist-in-charge, Herbarium Botanist of Andhra University, Visakhapatnam, Andhra

Pradesh, India. A voucher specimen [Sp. No: AU/ I-I / (255)/2011Tech.II] has been kept in our research laboratory for further reference. The collected materials were washed, shade dried and pulverized by using a mechanical grinder to obtain coarse powder.

Preparation of the extract

The powdered plant materials were defatted with petroleum ether (60^0-80^0C) in a soxhlet extractor. The marc was then air-dried and extracted with ethanol (90%), excess solvents were removed by rotary vacuum evaporator (Evator, Media Instrument Mfg. Co., Mumbai, India) and concentrated to obtain a dark greenish-brown residue (7.0% w/w).

Preliminary phytochemical tests

The ethanol extract of the plant material was screened for various classes of natural products using standard qualitative methods as described by Harborne 1973 (7). Detection for any sterols and terpenes in the extract involved treatment of the extract with petroleum ether and followed by extraction with chloroform. The subsequently acquired chloroform layer was treated with acetic anhydride and concentrated HCl. The change of pink to purple and green to pink colors was indicative of presence of terpenes or sterols, respectively. For alkaloids, the test was carried out by subjecting 1 g ethanolic extract in 10 ml 1% HCl, boiled, filtered and Mayer's reagent. Steroids were screened by adding 1ml of acetic anhydride to 0.25 g ethanolic extract of each sample with 1 ml sulphuric acid. The color changed from violet to blue or green in some samples indicating the presence of steroids. The test for anthraquinones was performed by adding 1 g of extract to 2 ml benzene, filtered and ammonia solution added. For detecting coumarins, a piece of filter paper was moistened in NaOH and then kept over a test tube with boiling plant extract solution. If the filter paper later showed any yellow fluorescence under UV light, that was taken to indicate a positive test for coumarins. Diterpenoids were detected by spraying TLC with ceric sulphate reagent. The presence of flavonoids was determined by dissolving the extract in ethanol, and one piece of magnesium turnings was added followed by conc HCl, added drop wise to that, and heated. Appearance of magenta color indicated the presence of flavonoids. The test for tannins was carried out by subjecting 1 g of each plant extract in 2 ml of distilled water, filtered and ferric chloride reagents added to the filtrate. The extract was carried out to frothing test for the identification of saponins.

Animals

Adult Wistar rats (150–250 g) and Swiss albino mice (18–25 g) of either sex were maintained in the animal house at GITAM institute of pharmacy, GITAM University, Visakhapatnam, Andrapradesh under standard environmental condition of temperature (25^0 C) and light/dark cycles (12/12 h). All experimental protocols were approved by the Institutional Animal Ethics committee of GITAM Institute of Pharmacy, Visakhapatnam, Andhra Pradesh, India (Regd. No.1287/ac/09/CPCSEA and protocol No: IAEC/GIP-1287/M Pharm/IP/PMK-PNVSSP/08/2011-12). Experiments were performed according to the guide for the care and use of laboratory animals.

Acute toxicity study

The acute toxicity studies were conducted as per OECD guidelines 420, where the limit test dose of 2000 mg/kg, p.o., used. Observations were made and recorded continously for the

first 4 h for any behaviorial changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any (8).

Evaluation of analgesic activity

By writhing method

The test was performed according to *Mondal et al.*,2009 (9). Writhing was induced in mice by single intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 min period. Group I serve as control received only vehicle (3 ml/kg, p.o.), the second group received aspirin (200 mg/kg, p.o.), used as reference standard for activity comparison; group III, IV and V received ethanol extract of *I. pavetta* leaves (200, 400 and 600 mg/kg, p.o.). The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition was calculated.

Formalin test

The method of Dubuisson and Dennis 1977 (10), as modified by *Tjolsen et al.*, 1992 (11), was used. Briefly, 0.05 ml of formalin (2.5% formaldehyde) was injected into the plantar surface of the rat hind paw, 30 min after treating the rats with the ethanol extracts (200, 400 and 600 mg/kg i.p.) and standard, aspirin (150 mg/kg, i.p). Behavioral responses detected were recorded as scores in the following manner: rat walking or can stand on injected paw, 0; paw partially elevated, 1; total elevation of injected paw, 2; injected paw licking or biting, 3. Scores of the first 10 min after formalin were recorded as the first phase of analgesia, while the period between 15 and 60 min was recorded as the late phase of pain.

By Eddy's hot plate method

Hot-pate was used to measure response latencies according to the methods of *Reanmongkol, et al.*, 2007 (12). In this study, the hot-plate was maintained at 55 ± 1^0 C and the mice were individually placed on the heated surface. The time in seconds between placement and shaking, paw licking and jumping off the plate was recorded as response latency. Five groups of six animals each the first group received vehicle (3 ml/kg, p.o.); the second group received tramadol (5 mg/kg, i.p.); other groups received doses of ethanol extract *I. pavetta* leaves (200, 400 and 600 mg/kg, p.o.) respectively. Measurements were taken at zero, 60 and 90 minutes after the treatment of animals.

Evaluation of anti-inflammatory activity

Carrageenan and arachidonic acid-induced hind paw edema in rats

Male rats weighing 200–250 gm were used. Paw edema was induced by an intradermal injection of carrageenan (1% in normal saline solution) (9) or 0.5% arachidonic acid in 0.2M carbonate buffer, pH 8.4 (13) into the plantar surface of the right hind paw of the rats at a volume of 0.05 or 0.1 ml, respectively. The animals were divided into five groups. The control group was treated with vehicle (2 ml/kg, p.o.) through oral route. Other groups received indomethacin (10 mg/kg, p.o) or the test extract at doses of 200, 400 and 600 mg/kg, p.o., in a similar manner. Carrageenan (0.1 ml of 1% solution in normal saline) was administered to the rats into the planter surface of the right hind limb to induce paw oedema. Test drugs were given 1 h prior to carrageenan or 2 h prior to arachidonic acid injection. The edema volume was determined using a plethysmometer prior to and 1, 2 and 3 h after

carrageenan injection or 1 h after arachidonic acid injection paw swellings were compared with control.

Evaluation of antipyretic activity

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats (14). Fever was induced by injecting 10 ml/kg (s.c.) of 20% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature of at least 0.7^0 C were selected for the study and animals were divided into five groups. The control group received vehicle (2 ml/kg, p.o.) through oral route. Other groups of animals received one of the following in a similar manner: Aspirin (300 mg/kg) or the test extracts (200, 400 or 600 mg/kg). The rectal temperature was measured at 1, 2 and 4 h after treatment.

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A P-value < 0.05 were considered to be significant. All the values were expressed as mean ± SEM.

RESULTS

Preliminary phytochemical tests revealed presence of alkaloids, sterols, flavonoides, tannins, saponins, phenol and phenolic compounds in the ethanol extract of *I. pavetta*. However the crude extract was found negative for the presence of coumarins.

Acute toxicity study

When orally administered to mice in graded doses from 100 to 2000 mg/kg, p.o., the ethanol extract of *I. pavetta* leaf induced sedation, mild diuresis, purgation and analgesia at all tested doses. However, there was no mortality in any of the above doses at the end of the 14 days of observation, the 1/5th of the preceding dose i.e 400 mg/kg body weight, p.o., was taken as the testing dose for pharmacological evaluation and lower upper dose of 200 and 600 mg/kg body weight, p.o., also tested to find out whether there is any dose dependent pharmacological effect or not.

Analgesic activity

Oral administrations of ethanol extract from *I. pavetta* leaf (EEIPL) showed significantly ($P < 0.01$) reduce the writhings induced by acetic acid in mice; the activity was compared with that of aspirin (Table I). Analgesic studies against thermal noxious stimuli the extract shows dose dependent analgesic effect (Table III). The extract showed a significant ($P < 0.05$) inhibition of the formalin noxious stimulation on both early and late phases of pain at doses of 200, 400 and 600 mg/kg, i.p., respectively, in rats (Table II).

In Eddy's hot method, EEIPL at 400 and 600 mg/kg, p.o., showed significant activity (3.21±0.29; 4.31±0.64) ($P < 0.05$) after 60 mins, whereas at a dose of 200 mg/kg, p.o., showed significant analgesic activity from 90 mins respectively, Tramadol (5 mg/kg, i.p.) used as standard drug, which showed significant activity throughout the course of study.

Anti-inflammatory activity

Effects on Carrageenan-induced hind paw edema in rats

The inhibitory activity on carrageenan-induced rat hind paw edema, caused by the oral administration of ethanol extract of *Ixora pavetta*, at various assessment times after carrageenan injection is shown in Table No IV. The extract showed maximum inhibition of 25.96 % and 33.65% at the dose of 400 and 600 mg/kg, p.o., after 3 h of drug treatment, whereas the extract at a dose of 200 mg/kg, p.o., does not possess significant activity throughout the course of study, standard drug indomethacin (10 mg/kg, p.o.) showed 37.01% of inhibition after 3 h of drug treatment in carrageenan-induced paw edema.

Effects on arachidonic acid-induced hind paw edema in rats

The injection of 0.5% arachidonic acid into the plantar side of the right hind paw significantly produced edema formation by 1 h after challenge. The results depicted in Table V. Indomethacin, a dual inhibitor of arachidonic acid metabolism, at the dose of 10 mg/kg body weight exhibited significant inhibitory activity on the edema formation. Similarly, ethanol extract of *I. pavetta* at the doses of 200, 400 and 600 mg/kg body weight significantly exerted inhibitory effect on arachidonic acid-induced hind paw edema.

Effect of ethanol extract of *I. pavetta* and aspirin on brewer's yeast induced pyrexia in rats.

The ethanol extract of *I. pavetta* leaves showed significant protection effect on yeast-induced fever in rats at doses of 400 and 600 mg/kg, p.o. The reference drug aspirin suppressed the fever induced by yeast in rats from 1st hour of drug administration. On the other hand, the ethanol extract produced significant activity at 4th hour of test sample administration and no promising results with 200 mg/kg, p.o., dose level (Table VI).

DISCUSSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects (15,16).

Ethanol extract of *I. pavetta* leaf (EEIPL) protected against both thermal and chemical induced stimuli, which were evidence from hot-pate and acetic acid induced writhing test. The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways. Test extract also produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways (17). In the radiant heat hot-pate test the plant extract prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center. The results of the formalin study, showed that both the aphasic (early) and tonic pain (late phase) were blocked by the extract.

Carrageenan-induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs, since the relative potency estimates obtained from most drugs tend to reflect clinical experience (18). The carrageenan-induced hind paw edema in rat is known to be sensitive to cyclooxygenase inhibitors, but not to lipoxygenase inhibitors, and has been used to evaluate the effect of non-steroidal anti-inflammatory agents

which primarily inhibit the cyclooxygenase involved in prostaglandins synthesis. It has been demonstrated that the suppression of carrageenan-induced hind paw edema after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents (19). The pretreatment of animals with ethanol extract of *Ixora pavetta* resulted in a significant inhibition of carrageenan-evoked hind paw edema. The significant inhibitory effect of EEIPL on carrageenan-induced paw edema at the third hour, suggests that the main mechanism of action of EEIPL may involve the prostaglandin biosynthesis and/or may also possess some influence on the other inflammatory mediators, e.g. histamine, serotonin, and pro-inflammatory cytokines which are released during the first hour after carrageenan injection.

Arachidonic acid-induced paw edema in rat is a widely used method for evaluating the anti-inflammatory activity of lipoxygenase inhibitors and other agents with a mechanism of action different from cyclooxygenase inhibitor. The lipoxygenase pathway utilizes arachidonic acid by
5-lipoxygenase to produce the lipoxygenase products which are also involved in inflammatory reactions as proinflammatory mediators. Leukotrienes, cause edema together with increased microvascular permeability. Owing to the contribution of leukotrienes to the pathogenesis of many inflammatory processes, they also represent an important target for therapeutic regulation. The paw edema induced by arachidonic acid is perceptibly reduced by lipoxygenase inhibitors and dual inhibitors of arachidonic acid metabolism (20). The present study shows that ethanol extract of *I. pavetta* leaf produced marked inhibition of the paw edematous response induced by arachidonic acid injection and exerted inhibitory effect on the edema formation in this animal model as well.

Baumann et al. (1980) showed that among the flavonoids are inhibitors of lipoxygenase (21). The results of this studies suggest that both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism are inhibited by EEIPL due to presence of flavonoids content. The EEIPL also showed significant effect on yeast-induced fever in rats while the reference drug aspirin suppressed fever induced by yeast in rats by inhibiting the synthesis of prostaglandin E2(22, 23).

CONCLUSION

In conclusion, this study demonstrates that the ethanol extract of *Ixora pavetta* leaves possesses analgesic, anti-inflammatory and antipyretic activities which may explain the folkloric use of acute pain and its positive effects could be produced by the flavonoids. Information of the present study may help the future researchers. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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