EVALUATION OF DIURETIC AND LAXATIVE ACTIVITIES OF ETHANOL EXTRACT FROM IXORA PAVETTA LEAF

Sumanta Mondal*, Marouthu.I.P.Pushyami and S. Ganapaty
Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.

ARTICLE INFO

Article history
Received 29/10/2013
Available online
28/11/2013

Keywords
Ixora pavetta Andrews,
furosemide,
agar-agar,
loperamide,
Sodium picosulfate.

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 chronic wounds, urinary diseases, skin infection, pulmonary troubles, liver disorder, hair tonic, sedative, diuretic, laxative, leucorrhoea and venereal diseases. Preliminary phytochemical screening of ethanol extract of Ixora pavetta leaf (EEIPL) showed the presence of flavonoids, tannins, saponins, gums, mucilages, carbohydrates, and proteins respectively. The diuretic and laxative activities of EEIPL were studied in albino rats. EEIPL significantly increased the urinary out put as well as urinary electrolyte concentration at a dose of 400 and 600 mg/kg, p.o., but the effect was found to be the less potent when compared with the reference standard (Furosemide-20 mg/kg, p.o.). Further, the ethanol extract increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increase sodium ion excretion to a greater extent than potassium, which is a very essential requirement of an ideal diuretic with lesser hyperkalaemic side effect. Similarly the extract of I. pavetta leaf (200, 400 and 600 mg/kg, p.o.), produced significant laxative activity and reduced loperamide induced constipation in dose dependent manner and 600 mg/kg, p.o., of the ethanol extract of I. pavetta leaf superior to that of the standard drug agar-agar (300mg/kg, p.o.) and sodium picosulfate (5 mg/kg, p.o.). The results of this studies justify the use of Ixora pavetta leaf in traditional medicine as diuretics and significantly accelerated stool frequency and suitable for constipation.

Please cite this article in press as Dr. Sumanta Mondal et. al. Evaluation of Diuretic and Laxative activities of Ethanol Extract from Ixora Pavetta Leaf. Indo American Journal of Pharm Research. 2013;3(10).

Copy right © 2013 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
INTRODUCTION

Nature has a treasure of medicines to treat all kinds of ailments. When our prehistoric ancestors first roamed the earth in search of food, they perhaps learnt from bitter experience, which plants, and herbs edible and which were not. The importance of this information and experience was vital to the health and to the tribe and so was passed down from generation to generation. Not only did they discover many source of food while foraying but also medicines. This formed their medicinal love for thousands of years to come. Out of this fundamental knowledge came corpus of herbal knowledge, which was grown continuously to the present day [1].

Diuretics which increase the excretion of sodium chloride. Diuretics are prescribed for people suffering from edema, high blood pressure or heart diseases. Women suffering from PMS symptoms such as bloating are often advised to take diuretics to rid the body of excess fluid retention [2]. Diuretics can be found in a variety of food sources, prescription medications and natural remedies whereas now a day natural herbal remedies are often used as a preferred alternative to synthetic diuretics and can provide the same benefits without the negative side effects [3]. Similarly laxatives (or purgatives) are foods, compounds, or drugs taken to induce bowel movements or to loosen the stool, most often taken to treat constipation. Certain stimulant, lubricant, and saline laxatives are used to evacuate the colon for rectal and bowel examinations, and may be supplemented by enemas in that circumstance [4]. Some vegetables and foods can be eaten to cure constipation and act as laxatives, although the effectiveness may vary. These include: apple juice, beets, chicory, almonds, endive, coconut, chocolate, grapes, coffee, chicken broth, flaxseed, liquorice, mangos, papayas, molasses, olives, prunes, plums, pineapple, walnuts etc [5].

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 [6-9]. Similarly, tribes of Nellore district Andhra Pradesh, used root bark infusion as ethnic practice to cures jaundice and burning micturition [10]. 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 [7]. 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, leucorrhoea and venereal diseases [10]. Reports on biological activities of the plant are scarce, like ethnicanolic extract of I. pavetta flower significantly decrease the gastric secretion in the aspirin induced and pylorus ligated rats [10]. 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-xlyopyranoside [11]. The arial parts contain 6, 7-dimethoxycoumarin and the seed oil gave capric, lauric, myristic, palmitic, stearic, arachidic, behenic, oleic and linoleic acids [10].

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 diuretic and laxative activities. So, the present study is investigated to exploring the details of diuretic and laxative action of ethanol extract from Ixora pavetta leaf (EEIPL) by using experimental animal models.

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 leaves was loaded into the soxhlet extractor and subjected to extraction with ethanol (90%v/v) for 18 hr at 55-55°C. After extraction, the solvent was distilled off and filtered under vacuum pressure and the filtrate was concentrated using rotary vacuum evaporator (Evator, Media Instrument Mfg. Co., Mumbai, India) and concentrated to obtain a dark greenish residue (6.8% w/w).

Preliminary phytochemical tests

The ethanol extract of the plant material was screened for various classes of natural products using standard qualitative methods [12]. 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 magnesiam burnings 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, Andhra Pradesh under standard environmental condition of temperature (25°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 continuously for the first 4 h for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any [13].

Diuretic activity [14,15]

In this method, male albino rats weighing between 150-200g deprived of food and water for 18 hours prior to the experiment, were divided into four groups of six rats in each. The animal groups were administered orally either with vehicle (1% Tween-80 in normal saline, 25 ml/kg, p.o.) The first group of animals serving as control, received normal saline (25 ml/kg, p.o.), the second group received furosemide (20 mg/kg, p.o.) in saline (10); Group-III, IV and V received ethanol extract from Ixora pavetta leaf (EEIPL) at doses of 200, 400 and 600 mg/kg.p.o., in a similar manner. Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faces, kept at 200 ± 0.5°C. The volume of urine collected was measured at the end of 5 h. During this period, no food and water was made available to animals. The parameters taken were the body weight before and after test period, total urine volume, concentration of Na⁺, K⁺ and Cl⁻ in the urine. Na⁺ and K⁺ concentrations were determined by flame photometer and Cl⁻ concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator [16,17]. The results are depicted in Table 1.

Laxative activity [18]

Rats of either sex, fasted for 12 h before the experiment, but with water provided ad libitum. The animals were divided into five groups of six each. The animal groups were administered orally with vehicle (1% Tween-80 in normal saline, 2 ml, p.o.), reference standard agar-agar (300 mg/kg, p.o.) in saline and doses of EEIPL (200, 400 and 600 mg/kg, p.o.) in a similar manner. Immediately after dosing, the animals were separately placed in cages suitable for collection of faces. After 8 h drug administration, the faces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 h (Table 2).

Loperamide induced constipation in rats [19, 20]

Rats were placed individually in cages lined with clean filter paper, allowed to fast for 18 hours and divided into five groups of six animals each. Group I received vehicle 1%v/v Tween 80 (2 ml, p.o.), Group II received standard drug sodium picosulphate (5 mg/kg, p.o.), Group-III, IV and V received ethanol extract from Ixora pavetta leaf at doses of 200, 400 and 600 mg/kg,p.o., in a similar manner. After 1 hour treatment, all the group animals received Loperamide (5 mg/kg, p.o.) by oral gavage. The faeces production (total number of normal as well as wet faeces) in all five groups was monitored for 8 h (Table 3).

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference with the help of GraphPad InStat software. 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

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, saponins, etc., that exert a physiological effect. The compounds that are responsible for therapeutic effects are usually the secondary metabolites. A systematic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. The preliminary phytochemical screening of I. pavetta leaf ethanol extract showed presence of flavonoids, tannins, saponins, gums, mucilages, carbohydrates, and proteins respectively.

In acute toxicity study when the ethanol extract of I. pavetta leaf orally administered to mice in graded doses from 100 to 2000 mg/kg, p.o., the extract induced sedation, mild diuresis, and purgation 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.

In present study, we can demonstrate that the ethanol extract of *Ixora pavetta* leaf (EEIPL) significantly increased the urinary out put at a dose of 400 and 600 mg/kg, p.o., but the effect was found to be the less potent in increasing the urinary out put when compared with the reference standard (Furosemide-20 mg/kg, p.o.). However, the test extract at lower dose (200mg/kg) failed to do so. The urinary levels of Na⁺, K⁺ and Cl⁻ ions were significantly increased by 400 and 600 mg/kg, p.o., of test extract. The diuretic activity demonstrated by the test extract at 400 and 600 mg/kg was significantly lesser than the standard drug. The results are compiled in the Table 1.

### Table 1: Diuretic activity of ethanol extract of *I. pavetta* leaf

<table>
<thead>
<tr>
<th>Treatment and Dose</th>
<th>Urine Volume (ml)</th>
<th>Diuretics Index</th>
<th>Concentration of ions (mmol/l)</th>
<th>Na⁺/K⁺ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control (25 ml/kg, p.o.)</td>
<td>3.21±0.15</td>
<td>-</td>
<td>104.11±2.15</td>
<td>94.8±2.33</td>
</tr>
<tr>
<td>Group II: Furosemide (20 mg/kg, p.o.)</td>
<td>8.16±0.41**</td>
<td>2.542</td>
<td>124.06±1.22**</td>
<td>63.05±2.61**</td>
</tr>
<tr>
<td>Group III: EEIPL (200 mg/kg, p.o.)</td>
<td>4.01±0.71</td>
<td>1.249</td>
<td>96.21±3.02</td>
<td>88.11±2.98</td>
</tr>
<tr>
<td>Group IV: EEIPL (400 mg/kg, p.o.)</td>
<td>6.87±0.19**</td>
<td>2.140</td>
<td>117.16±2.11**</td>
<td>76.45±1.08**</td>
</tr>
<tr>
<td>Group V: EEIPL (600 mg/kg, p.o.)</td>
<td>7.98±0.33**</td>
<td>2.485</td>
<td>121.80±2.84**</td>
<td>65.21±3.21**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA. *P<0.05, **P<0.01 when compared to control; Dunnet’s t-test.

The laxative activity of ethanol extract of *I. pavetta* leaf (EEIPL) was studied in albino wistar rats. The activity of the extract was found to be in a dose dependant increase in faecal output of rats at selected dose levels. EEIPL at the doses of 200, 400 and 600 mg/kg, p.o., increased significantly fecal output of rats compared to control group and 600 mg/kg, p.o., of *I. pavetta* leaf extract was found to be superior to that of the standard drug agar-agar (300mg/kg, p.o.). The results are compiled in the Table 2.

### Table 2: Effect of ethanol extract of *I. pavetta* leaf (EEIPL) on Laxative activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Faecal Output (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1% Tween-80 (2 ml, p.o.)</td>
<td>0.898 ± 0.57</td>
</tr>
<tr>
<td>II</td>
<td>Agar-agar</td>
<td>300 mg/kg, p.o.</td>
<td>5.81±0.97**</td>
</tr>
<tr>
<td>III</td>
<td>EEIPL</td>
<td>200 mg/kg, p.o.</td>
<td>2.85±0.33**</td>
</tr>
<tr>
<td>IV</td>
<td>EEIPL</td>
<td>400 mg/kg, p.o.</td>
<td>4.71±0.88**</td>
</tr>
<tr>
<td>V</td>
<td>EEIPL</td>
<td>600 mg/kg, p.o.</td>
<td>6.31±0.43**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA. *P<0.05, **P<0.01 when compared to control; Dunnet’s t-test.

Similarly in the loperamide-induced constipation, EEIPL increased the total number of faeces in a dose dependant manner and the results were statistically significant (p < 0.01) when compare with control group animals (Table 3). The reduction of the loperamide induced constipation at 600 mg/kg, p.o., of the ethanol extract of *I. pavetta* leaf treatment was also found to be superior to that of the standard group treatment by 5 mg/kg, p.o., of sodium picosulfate. In the present study, we demonstrated that the *I. pavetta* leaf significantly accelerated stool frequency and suitable for constipation.

### Table 3: Effect of ethanol extract of *I. pavetta* leaf on loperamide induced constipation in adult Wistar rats

<table>
<thead>
<tr>
<th>Treatment and Dose</th>
<th>Faecal Output (g) upto 8h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: 1%v/v Tween 80 (2 ml, p.o.)</td>
<td>0.508 ± 0.57</td>
</tr>
<tr>
<td>Group II: Sodium picosulfate (5 mg/kg, p.o.)</td>
<td>4.21±0.67**</td>
</tr>
<tr>
<td>Group III: Ethanol extract of <em>I. pavetta</em> leaf (200 mg/kg, p.o.)</td>
<td>1.08±0.09**</td>
</tr>
<tr>
<td>Group IV: Ethanol extract of <em>I. pavetta</em> leaf (400 mg/kg, p.o.)</td>
<td>3.68±0.22**</td>
</tr>
<tr>
<td>Group V: Ethanol extract of <em>I. pavetta</em> leaf (600 mg/kg, p.o.)</td>
<td>4.91±0.88**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA. *P<0.05, **P<0.01 when compared to control; Dunnet’s t-test.
DISCUSSION

Diuretics relieve pulmonary congestion and peripheral edema and are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure [21]. Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that the ethanol extract of *Ixora pavetta* Andrews leaf significantly increased the urinary output as well as urinary electrolyte concentration at a dose of 400 and 600 mg/kg, p.o. but the effect was found to be the less potent in increasing the urinary output when compared with the reference standard. Further, the extract was found to be more effective in enhancing urinary electrolyte concentration for all the three ions tested (Na⁺, K⁺ and Cl⁻). The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extracts increase sodium ion excretion to a greater extent than potassium, which is a very essential requirement of an ideal diuretic with lesser hyperkalaemic side effect. The laxative activity study revealed significant activity of the ethanol extracts up to 8 h of drug administration. The ethanol extract was found to be superior to that of the standard drug. Similarly in the loperamide-induced constipation, EEIPL increased the total number of faeces and accelerated stool frequency and suitable for constipation. The Presence of phytoconstituents like terpenoids, sterols, flavonoids, phenolic compounds, tannins, saponins and alkaloids have been previously found to be responsible for diuretic and laxative activities in plants [22, 23]. Phytochemical screening of the ethanol extract of *I. pavetta* leaf revealed the presence of flavonoids, saponins and tannins. These constituents may be responsible for the diuretics and laxative activities.

CONCLUSION

The results of the present study justify that the use of *Ixora pavetta* Andrews leaf as diuretics and laxative in traditional medicine. Further studies may be directed at characterizing the bioactive ingredients that are responsible for the observed activities in the plant.

ACKNOWLEDGEMENTS

The authors are thankful to Institute of Pharmacy, GITAM University for providing necessary facilities to carry out the research work. The authors are also thankful to the Dr. Venkayyah, Scientist-in-charge, Herbarium Botanist of Andhra University, Visakhapatnam, Andhra Pradesh, India, for helping in identifying and authenticating the plant.

REFERENCES


