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A phyto-pharmacological screening for whole plant of *Ixora pavetta*

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Abstract
The world population is facing major problem with liver and kidney disorders. Despite its frequent occurence, high morbidity and high mortality, its medical management is currently inadequate, no exact therapy has been successfully prevented the progression of nephritis and hepatic diseases. The plant *Ixora pavetta* belongs to family Rubiaceae. Many researchers have screened for analgesic, anti-inflammatory, anti-ulcer, anti-oxidant, anti-microbial activities on leaves and flowers of Ixora pavetta. The selected plant is traditionally used to treat the visceral obstructions, haemorrhoids, dropsy, piles, hepatotoxicity etc. The genus *Ixora* has been reported to possess different classes of phytochemical constituent's mainly aromatic acrid oil, tannin, saponin, carbohydrates, flavonoids. Since there is a claim that the flavanoids present in the plants are responsible for the hepatoprotective activity. The present study is performed to reveal that the hepatoprotective and nephroprotoxicity activities of *Ixora pavetta* in rifampicin induced hepatotoxicity and nephrotoxicity in rat model, to prove its claims in folklore practice against liver and kidney disorders.

Keywords: Hepatotoxicity, *Ixora pavetta*, nephrotoxicity, rifampicin, whole plant

Introduction
The largest organ in the human body, liver plays a very important role in the metabolism of foreign compounds entering the body. The exposure to the foreign compounds may be through consumption of alien/contaminated foods, from exposure to chemical substances in the occupation environment or through synthetic drugs consumed for various pathological conditions. These compounds have many toxic manifestations on the human liver [1]. The liver gets injured also by viruses, chemicals, alcohol and autoimmune diseases. Liver diseases remained one of the serious health problems, and medicinal plants and herbs have been in use for treating these in the Indian traditional systems of medicine, especially in Ayurveda. The present modern age demands proof on a scientific basis to justify the various medicinal uses of herbs [2]. Mohana Lakshmi S *et al*. (2013) [59] evaluated the anti-inflammatory potential of methanol extract of *Pavetta indica.* Vinod Kumar *et al*. (2013) [60] tested the antimicrobial activity of aqueous and organic extracts of *Ixora pavetta* leaves against Bacillus subtilis, E.coli, Saccharomyces cerviceae using disc diffusion assay. Leaf extract showed bactericidal activity against *B.subtilis.* Kharat A.R *et al.* (2013) [61] reviewed the phytochemical and pharmacological activity of genus *Ixora* and attempted to cover the available literature on *Ixora* genus with respect to pharmacognostic characters, traditional uses, chemical constituents and summary of various pharmacological activities. Celestian Baboo *et al.* (2011) [64] analyzed the ethanolic flower extract of *Ixora pavetta* by GC-MS technique. The compounds *viz*. 3-Butyn-2-ol, 3-Butyn-1-ol, Amyl nitrite, 2Octyn-1-ol, 1, 9-Decadiyne and Butyl glyoxylate were identified from the study. Sedative property is one of the traditional uses of *Ixora pavetta* was found to be due to the presence of Amyl nitrite in the extract.
It is evident from the literature review that extensive research has been carried out on various parts of *Ixora pavetta* and established that, this plant is highly useful in treatment of various disorders. Though some of the plants are reputed in the indigenous system of medicine for their activities, they require scientific evaluation. The literature survey revealed that, no scientific studies were carried out to investigate hepatoprotective and anti-oxidant activity of methanolic extract of whole plant of *Ixora pavetta.*

Materials and Method
Collection and preparation of extracts
The plant material was collected from the plant *Ixora pavetta,* which are collected during the month of June at Dept. of botany, Kakatiya University, Warangal (Dist.) of Telangana.

Then it was authentified by Dr. P. Satyanarayana Raju, professor, Department of Botany, Kakatiya University. The whole plant part of *Ixora pavetta* was dried at room temperature and grounded into powder and passed hrough 60# sieve. The powder (500gm) was extracted successively in soxhlet by methanol, ethanol and ethyl acetate. The sediments were filtered and the filtrate was dried at 40°C in an oven to get dried product. The different fractions obtained were prepared and used for hepatoprotective and nephroprotective activities.

Chemicals and instruments:
All chemicals used in the study were pure. Reference standard Rifampicin obtained from Symed Pharm. Pvt. Ltd, Hyderabad.

Preliminary phytochemical screening:
The extract was subjected to preliminary phytochemical screening was performed by using standard protocol. 5-7
The methanolic extract of the whole plant part of *Ixora pavetta*, carried out test tube qualitative reactions gave positive results for alkaoloids, flavonoids, glycosides, saponins and tannins, phenols, steroids and tri-terpenoids. And also carried out TLC study for the methanolic extract.

Acute toxicity studies
Although medicinal plants may produce several biological activities in humans, but generally very little is known about their toxicity. Though, safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems, several drugs produce acute and obvious signs of toxicity which are used in the traditional medicine. The present study was undertaken to investigate acute toxicity of methanolic extract which is prepared from whole plant of *Ixora pavetta.*

Animal model
The methanolic extract of *Ixora pavetta* was pharmacologically screened for its toxic and biological effects in selected animal models. All animal studies were performed as per the guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC). CPCSEA Reg. No: 769/2011/CPCSEA. Albino mice of either sex weighing between 16-25 g procured from Sainath Agencies, Hyderabad - 48, for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry conditions.

Experimental design
A total of 36 animals were divided into 6 groups (6 no's in each group). Group 1 is served as normal control received only vehicle for 28 days (distilled water, p.o) whereas groups 2,3,4,5 and 6 received 20% ethanol (3.76 g / kg / day) to induce hepatotoxicy for 28 days on every alternate day. Group 1 was served as normal control; Group 2 received ethanol (3.76 g / kg) orally. Group 3 received silymarin (25 mg / kg) standard drug orally. Groups 4 – 6 received ethanol (3.76 g / kg) orally on every alternate day and three different doses of MEIP (100, 200 and 400 mg) (were prepared by dissolving in distilled water and administered orally) respectively. After oral administration, the blood samples were withdrawn by retro-orbital puncture at 8th, 15th, 22nd and 29th days respectively. The collected blood samples were centrifuged at 2500 rpm for 15 min to get clear serum and were used to analyse the biochemical parameters such as serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alanine amino transaminase (ALP), bilirubin, and total protein. Finally on the 29th day the animals were sacrificed using high dose of ether and the livers of all experimental animals were isolated.

Histopathological Evaluation
The liver and kidney organs were fixed in neutral buffered formalin for 24 h. Sections of tissue from liver and kidney organs were examined histopathologically to study the hepatoprotective and nephroprotective activity of methanolic extract of whole plant of *Ixora pavetta*. The liver and kidney organs were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-µm.

Result
Acute toxicity studies were carried out on whole plant of methanolic extract of *Ixora pavetta* up to the dose of 2000 mg/kg which demonstrated that the extract did not show any sign of toxicity and mortality. However, there was a decrease in physical activity, which was observed only at the dose of 2000 mg/kg. Thus, the present doses regime (100 and 200 mg/kg) was chosen for further studies. The results of hepatoprotective and nephroprotective activities of crude methanolic extracts of this plant at a dose of 100 mg/kg, 200mg/kg and 400mg/kg on rats intoxicated with standard silymarin (25 mg/kg) were illustrated in the table 1, fig-1and 2. The tables also showed the comparison of effects among the untreated (control) and alcohol treated (negative control) group with the drug treated group of rats. Treatment with whole plant of methanolic extract of *Ixora pavetta* at different dose levels (100, 200 and 400 mg/kg) and standard silymarin (25 mg/kg) significantly decreased the enzymes serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alanine amino transaminase (ALP) and bilirubin levels as compared to disease control group. Oral administration of methanolic extract of *Ixora pavetta* at different dose levels (100, 200 and 400 mg/kg) and standard silymarin (25 mg/kg) significantly increases the enzymatic antioxidants like superoxide dismutase, catalase, glutathione levels and reduces the lipid peroxidise levels when compared to the disease control group. All the above parameters indicating the hepatoprotective activity of methanolic extract of *Ixora pavetta* against rifampicin-induced liver cell damage.
Whole plant of methanolic extract of *Ixora pavetta,* extract at 400 mg/kg produced a significant reduction in the elevated serum biochemical parameter (Bilirubin, SGPT, SGOT and ALP) levels and elevated the decreased enzymatic anti-oxidant (SOD, catalase, glutathione) levels in Rifampicin induced hepatotoxic rats. These effects were found to be comparable and even more than that of the standard Silymarin (25 mg/kg). Oral administration of alcohol significantly decreased the body weight and increased the serum biochemical parameters such as blood urea nitrogen, serum creatinine and serum uric acid. Whole plant of methanolic extract of *Ixora pavetta* at different dose levels (100, 200 and 400 mg/kg) significantly increased the body weights and lowers the serum bilirubin biochemical parameters Table-1.
The nephroprotective effect produced by methanolic extract of *Ixora pavetta* at 400 mg/kg was found to be more than that of the standard Silymarin (25 mg/kg). Histological changes such as cortical glomerular, peritubular blood vessel congestion and interstitial inflammation were observed in disease control group. The methanolic extract of *Ixora pavetta,* extract at 400 mg/kg had significantly prevented these histological changes.

Table 1: effect of the methonolic extract (whole plant) of *Ixora Pavetta* on bilirubin levels (mg/dl) in alcohol induced hepatotoxic rats

Time in Days	Normal Control Group							Diseased Control Group							Silymarin(25mg/kg)						
	A	B	C	D	E	F	MEAN± SEM	A	B	C	D	E	F	MEAN± SEM	A	B	C	D	E	F	MEAN± SEM
0	0.7	0.8	0.6	0.9	0.7	0.6	0.71±0.04	2.2	2.3	2.1	2.2	3.3	2.2	2.38±0.18	2.3	2.2	2.4	2.4	2.3	2.7	2.38± 0.07
8	0.6	0.7	0.9	0.7	0.8	0.9	0.76±0.04	2.6	2.7	2.5	2.7	2.6	2.6	2.61±0.03	1.9	2.1	2.2	1.8	1.9	2.1	2 ±0.06
15	0.8	0.6	0.7	0.6	0.9	0.9	0.75±0.05	3.0	3.1	2.9	3.1	2.9	2.9	2.98±0.04	1.7	1.8	1.5	1.6	1.6	1.5	1.61± 0.04
22	0.6	0.6	0.9	0.8	0.7	0.7	0.71±0.04	3.3	4.2	3.2	3.4	3.3	3.4	3.46±0.14	1.3	1.2	1.1	1.3	1.2	1.2	1.21 ±0.03
29	0.9	0.7	0.8	0.9	0.6	0.8	0.78±0.04	3.5	3.7	3.6	3.8	3.7	3.6	3.65±0.04	0.7	0.9	0.7	0.8	0.9	0.8	0.8 ±0.04

n=6 significant at p<0.05*, 0.01**, 0.001***

Table 2: Effect of the Methonolic extract (Whole Plant) of *ixora pavetta* on SGPT levels (IU/l) in alcohol induced hepatotoxic rats

Time in Days	Normal Control Group							Diseased Control Group							Silymarin(25mg/kg)						
	A	B	C	D	E	F	MEAN±SEM	A	B	C	D	E	F	MEAN±SEM	A	B	C	D	E	F	MEAN±SEM
0	25	28	27	25	26	27	26.33±0.49	64	70	72	69	71	68	69±1.55	66.5	66.6	68.1	69.2	68.5	71.0	68.32± 0.69
8	27	26	28	28	25	26	26.3±0.49	69	71	73	70	72	69	70.50±0.619	61.2	63.2	60.5	60.8	59.5	59.6	60.80± 0.55
15	28	29	30	22	23	29	26.83±1.4	74	76	73	75	72	76	74.33±0.66	51.2	53.6	52.6	50.5	51.2	53.5	52.10 ±0.53
22	29	29	28	27	24	26	27.16±0.79	79	80	77	78	80	77	78.5±0.56	42.5	44.6	42.6	44.1	43.9	43.3	43.5 ±0.34
29	28	30	24	28	26	27	27.2±0.83	84	87	88	86	84	85	85.66±0.66	29.5	28.7	26.8	26.2	30.3	29.5	28.5 ±0.66

n=6 significant at p<0.05*, 0.01**, 0.001***

Table 3: Effect of the methonolic extract (whole plant) of *Ixora Pavetta* on total protein levels (mg/dl) in alcohol induced hepatotoxic rats

Time in Days	Normal Control Group							Diseased Control Group							Silymarin(25mg/kg) treatment						
	A	B	C	D	E	F	MEAN±SEM	A	B	C	D	E	F	MEAN±SEM	A	B	C	D	E	F	MEAN±SEM
0	6.8	6.9	6.72	6.8	6.4	6.5	6.68±0.07	5.1	5.3	5.2	5.1	5.3	5.1	5.18±0.04	4.5	4.6	4.4	4.3	4.4	4.7	4.5± 0.06
7	6.9	6.9	6.8	6.7	6.6	6.8	6.78±0.04	5.0	5.2	5.1	5.0	5.1	5.0	5.06±0.03	4.8	4.9	4.7	4.8	4.9	5.3	4.9 ± 0.08
14	6.6	6.7	6.5	6.59	6.5	6.6	6.58±0.03	4.9	5.1	5.0	4.8	4.8	4.7	4.88±0.06	5.3	5.2	5.1	5.3	5.4	5.4	5.3 ± 0.04
21	6.8	6.78	6.79	6.86	6.9	6.8	6.82±0.02	4.7	4.6	4.8	4.7	4.6	4.6	4.66±0.03	5.6	5.7	5.5	5.7	5.6	5.7	5.6 ± 0.03
28	7.0	6.8	6.85	6.7	6.75	6.9	6.8±0.04	4.3	4.5	4.4	4.3	4.4	4.6	4.41±0.04	6.1	5.9	6.0	6.1	5.9	6.4	6.1 ± 0.1

n=6 significant at p<0.05*, 0.01**, 0.001***

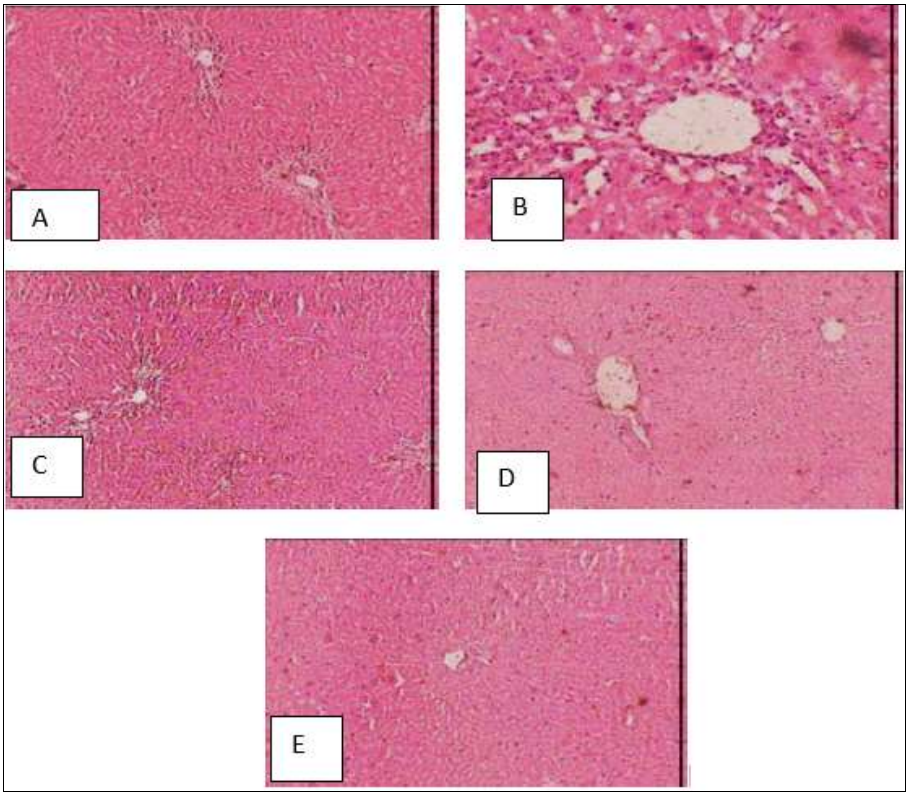

Fig 1: a. Group I (Normal control), b. Group II (alcohol) Section of liver with normal cell structure Section of liver showing centriolobular necrosis, c. Group III (Standard-Silymarin), d.Group IV (*Ixora pavetta* -200), e. Group V (*Ixora pavetta* -400) Section of liver showing significantly reduced Necrotic area

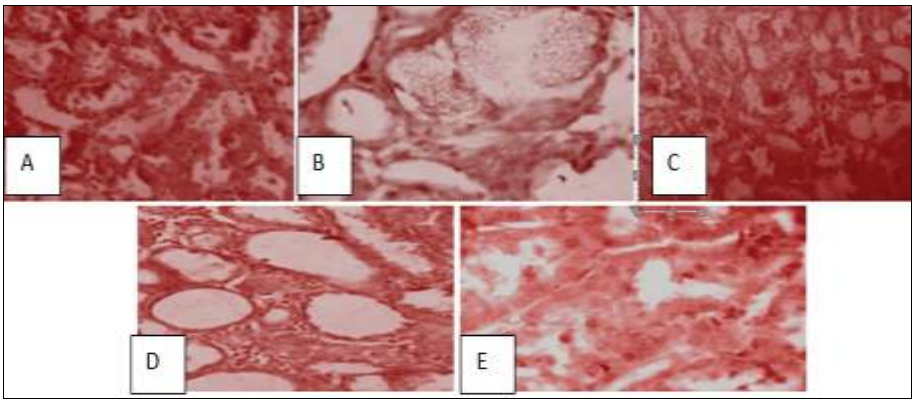

Fig 2: a. Group I (Normal control), b. Group II (alcohol induced Necrotic area), c. Group III (Standard-Silymarin), d. Group IV (*Ixora pavetta* -200), e. Group V (*Ixora pavetta* -400) Section of liver showing significantly reduced Necrotic area

Conclusion

The plant *Ixora pavetta* belongs to the family Rubiaceae. The whole plant parts of the above plants are selected for the investigation of hepatoprotective and anti-oxidant activity. The methanolic extract of the whole plant of *Ixora pavetta* gave positive results for flavonoids, glycosides, saponins and tannins, phenols and triterpinoids. An LD_{50} study of methanolic extract was conducted up to a dose of level of 2 mg/kg and no mortality was observed in any of the animals which induced the practically nontoxic nature and safety of the extract. There was a significant dose related decrease in serum biochemical parameters of liver like SGPT, SGOT, ALP, bilirubin and increase in total protein levels at different dose levels (100, 200 and 400 mg/kg). Oral administration of methanolic extract of whole plant of *Ixora pavetta* at different dose levels (100, 200 and 400 mg/kg) produced a significant dose dependent increase in the enzymatic antioxidants of liver like superoxide dismutase, catalase and glutathione levels. From the Pharmacological screening, we can conclusively state that the methanolic extract of whole plant of *Ixora pavetta* has hepatoprotective activity and were comparable with that of standard silymarin. The hepatoprotective and anti-oxidant activity produced by whole plant of methanolic extract of *Ixora pavetta* may be due to the presence of flavonoids, glycosides, saponins and tannins, phenols and triterpinoids.

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