IXORA JAVANICA DC FLOWERS METHANOLIC EXTRACT ANTI-UROLITHIATIC ACTIVITY ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

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ABSTRACT:

Urolithiasis is a most common health problem seen in many individuals. When kidney stones made of calcium and oxalate crystals return, the majority of the population has urolithiasis, which is a prevalent health issue. Although there are several traditional treatments for urothialisis, it is impossible to prevent kidney stone recurrence and drug side effects. The demand for polyherbal formulations has increased as a result of an increase in the prevalence of stone formation that is accompanied by inflammation and excruciating pain. The anti-urolithiatic action of *Ixora javanica* DC was determined by ethylene glycol induced lithiasis. Experimental animals were given ethylene glycolated water (0.75% v/v) for 28 days in order to induce kidney stone formation. *Ixora javanica* methanolic extracts were administered starting on the first day as an inhibiting agent. While test extract reduced the elevated level of these ions in urine, ethylene glycol boosted urinary parameters like calcium, oxalate, and inoarganic phosphate. *Ixora javanica* methanolic extract also enhanced urine magnesium content, and the experiment revealed a reduction in the elevated serum creatinine concentration. Histological research revealed that the methanolic extract of the test medication performed noticeably better. These findings supported *Ixora javanica* flower extract has ability to treat urolithiasis.

Keywords: Anti-urolithiatic, *Ixora javanica*, Ethylene glycol, methanolic extract

INTRODUCTION:

Urolithiasis is a widespread metabolic disease with a prevalence of rising risk of renal failure and considered as major health issues worldwide[1-2]. Since decades, urolithiasis recurrence has been a significant issue, and about 12% of the population suffers from urinary tract stones, with males at higher risk than females[3-4]. Traditional usage of medicinal herbs is a supplemental therapeutic alternative for treating stone formation with fewer side effects. Researchers made an effort to outline the mechanisms of different herbal plants in the treatment of urolithiasis and to provide future investments and guidance in the creation of formulations for urolithiasis based on plants. Males are more likely than females to suffer from urolithiasis, with a ratio of 11:2, because oestrogen inhibits stone formation whereas testosterone increases the potency of stone creation[1].

The different herbal plant extracts contain active phytoconstituents such flavonoids, alkaloids, saponins, and sterols[5-7], which have bioactive properties like diuretic, ant-inflammatory, analgesic, and antioxidant.

MATERIAL AND METHODS:

Procurement of chemicals: All the chemicals used were of Analytical grade. Crystalloid forming solutions, i.e., solution of calcium acetate and sodium oxalate (for calcium oxalate) were prepared in distilled water. Cystone used as standard, was obtained from Himalaya herbal health care.

Plant material & extract

Collection and Authentication:

2 kg of fresh flowers of *I. javanica* DC were collected from the local areas of Hyderabad and washed with water properly, to remove dust, drained well and then shade dried.

The plant was authenticated by Dr. Bhadraiah, Head of the Department, Department of Botany, Osmania Univeristy, Hyderabad. The voucher specimen with No. 01712 was stored in Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad.

Fig. 1: *Ixora javanica* DC (Orange red) flowers

Experimental Procedure

Extraction and Purification:

The dried flowers were grinded to coarse powder and then subjected to continuous successive soxhilation with the different solvents of increasing polarity. First petroleum ether (60-80 °C) extraction, followed by ethyl acetate and finally methanol extraction were done. The powdered material is divided into batches each of 250 gm and used in a soxhlet extractor.

After complete extraction, the solvents were distilled off and finally dried under reduced pressure to dryness in flash evaporator. The air dried extracts were weighed and percentage yields of extraction were calculated. The colour and consistency of the extracts were recorded. The solvents were evaporated in vacuum and the extracts were stored in desiccators[8-9].

The preliminary phytochemical studies were performed for testing the presence of different chemical groups in different extracts.

Preliminary Phytochemical Investigation *(I. javanica* DC flowers)

Phyto-constituents	**Petroleum ether (60-80 ºC) extract**	**Ethyl acetate extract**	**Methanol extract**
Steroids	+	-	+
Triterpenoids	+	+	+
Saponins	-	-	+
Glycosides	-	-	+
Carbohydrates	-	+	+
Alkaloids	-	-	+
Flavonoids	-	+	+
Tannins	-	-	+
Proteins	-	-	-

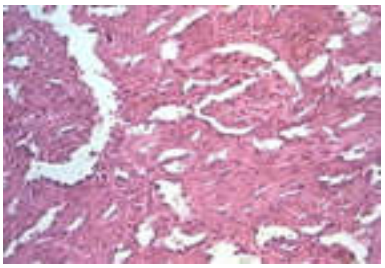
Animals

Wistar albino rats weighing between 150-200 gm were used for the work. They were held in standard environmental conditions and fed with standard rat food and water *ad libitum*. All animal experimental procedures were conducted accordingly CPCSEA guidelines and approved by the IAEC. (Reg.No. MRCP/CPCSEA/IAEC/2022-23/Ph/2).

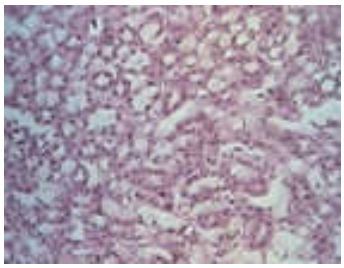
Evaluation for anti-urolithiatic activity:

Five groups, each with six albino rats, were involved in the 28-day treatment. Group I served as the control group and received drinking water and food for rats. Group II received 1% of the 80 solutions, which served as the lithiatic group. Group III received standard medication, Cystone (750 mg/kg), from the 15th to the 28th day. Groups IV and V received the two test dosages of methanolic extract, which were 300 mg/kg and 500 mg/kg, respectively. Except for group I, all groups received 0.75% ethylene glycol water to produce kidney stones. Rats were housed in individual metabolic cages. On the 28th day, urine samples were collected and the volume of the urine was measured. It was then preserved at 4ºC with a drop of concentrated hydrochloric acid added. Numerous biological markers, including calcium, creatinine, phosphate, magnesium, and oxalate concentration, were measured in a urine sample[10– 20].

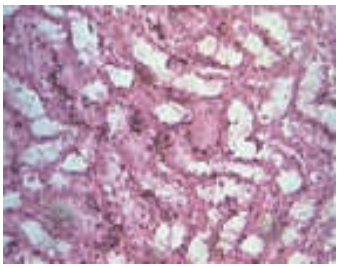
Histopathology[21-22]: After sacrificing rats, kidneys were removed from each group and examined. After a saline wash, they were preserved in formalin that was 8–10% phosphate buffered. Using the paraffin procedure, sections were cut, and eosin and hemotoxylin were applied to stain them. To find histopathological alterations, a light microscope was utilized, and pictures were obtained.



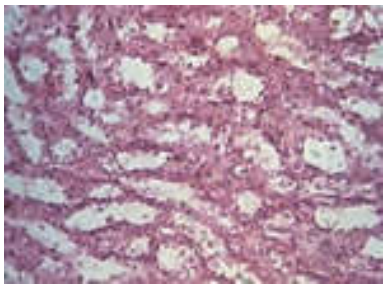
Group I Normal



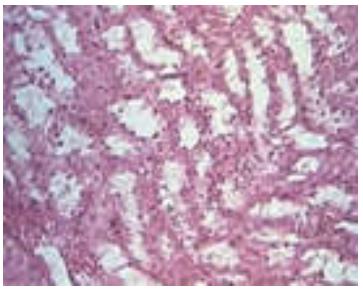
Group II Lithiatc control



Group III Cystone (Std)



Group IV Extract- 300 mg/Kg



Group V Extract- 500 mg/Kg

Statistical Analysis:

The results were expressed as Mean ± SEM. Statistical calculation was done by one way ANOVA followed by Tukey test. $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION:

The results of phytochemical investigation of petroleum ether extract showed the presence of steroids, triterpenoids, whereas, ethyl acetate extract shown the occurrence of flavonoids, triterpenoids and carbohydrates and finally methanol extract indicated the presence of steroids, triterpenoids, flavonoids, alkaloids, saponins, carbohydrates, tannins and glycosides.

Activity: The group II model, control had a higher weight of kidney than the normal group I. However, the use of a herbal medication and cystone as a standard during treatment led to a significant (P<0.05) decrease in the dry kidney weight when compared to Group II. The model group's body weight was found to be much lower than that of the normal group; however the animal body weights of the herbal extract therapy groups showed a notable increase when compared to the model group. At the conclusion of the trial, treatment with standard and herbal extract 500 mg/kg produced a notable increase in urine volume; however, the model group's urine volume increased significantly when treated with a 300 mg/kg dose of herbal extract. When compared to normal control groups, Group II's urine calcium levels showed a statistically significant (P<0.05) increase when ethylene glycol (0.75%) was added to drinking water.

Table1:Effect on physiological parameters

Groups	Group-I	Group-II	Group-III	Group-IV	Group-V
Wet kidney Weight(g)	0.89±0.05	1.02±0.20	0.85±0.12	0.92±0.03	0.95±0.04
Dry kidney Weight(g)	0.77±0.04	1.24±0.14	0.72±0.06	0.71±0.04	0.73±0.03
%change Body weight	8.02±2.14	-17.14±5.17	10.32±2.32	-6.63±2.21	-4.87±2.61
Urine volume	10.93±0.58	3.58±0.47	8.9±0.64	3.94±0.34	6.4±0.14

Values expressed are mean ±S.E.M. Statistical analysis was done by one way ANOVA test for multiple comparisons followed by Tukey test & $P<0.05$ was considered as significant.

Table 2: Effect of *Ixora javanica* flower methanolic extact on Urinary biological parameter

Therapy groups	Calcium	Urinary excretion parameter (mg/24hrs)		Magnesium
		Oxalate	Phosphate	
Group-I (Normal)	2.61±0.27	6.78±0.14	3.15±0.03	5.95±0.49
Group-II (Lithiatic control)	18.20±0.23	12.34±0.37	6.82±0.18	1.87±0.06
Group-III (Standard)	12.54±0.30	4.18±0.08	3.38±0.16	5.87±0.21
Group-IV(Test-300mg/kg)	16.37±0.17	7.2±0.29	6.20±0.19	13.38±1.22
Group-V(Test-500mg/kg)	13.82±0.59	5.8±0.43	2.79±0.25	6.21±0.68

Values expressed are mean ± S.E.M. Statistical analysis was done by one way ANOVA test for multiple comparisons followed by Tukey test & $P<0.05$ was considered as significant.

Table 3: Effect of methanolic extracts of *Ixora javanica* flowers on serum & urinary creatinine level

Biological parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
Serum Creatinine(mg/dl)	1.42±0.28	5.18±0.18	0.38±0.07	0.57±0.52	0.54±0.13
Urine Creatinine(mg/24hrs)	0.57±0.04	0.07±0.01	0.58±0.11	0.73±0.08	0.72±0.10

Values expressed are mean ±S.E.M. Statistical analysis was done by one way ANOVA test for multiple comparisons followed by Tukey test & $P<0.05$ was considered as significant.

By using Cystone showed a significant (P<0.05) decrease in calcium levels in the urine. Compared to lithiatic control, therapy with herbal drug extract, 500 mg/kg, in Group V showed significantly (P<0.05) reduced urine calcium levels. Group V experienced a notable reduction in urine calcium levels after receiving a 500 mg/kg dosage of herbal medicine extract. When compared to the normal control group I, the model control group II animals had significantly (P<0.05) greater urine oxalate levels. Urinary oxalate levels were significantly (P<0.05) decreased after 28 days of pre-therapy with standard medication and natural medicine extracts (Table 2). Figure 3 illustrates the statistically significant (P<0.05) decrease in urinary creatinine levels in animals compared to normal control when ethylene glycol (0.75%) was added to drinking water. Urine samples from Groups III-V were found to have significantly (P<0.05) higher creatinine levels. The lithiatic group's serum creatinine levels were found to be significantly (P<0.05) higher than those of the group I control animals. When compared to the model group, pretherapy with Cystone and herbal medicine extract showed a significant (P<0.05) decrease in serum creatinine levels (Table 3).Comparing Group II's urine inorganic phosphate levels to those of the normal control group, Group I, a significant increase was noted. Phosphorous levels were significantly (P<0.05) lower in the groups treated with the standard medication Cystone and the herbal drug extract (500 mg/kg) than in the model group II. Group IV, which received the herbal drug extract (300 mg/kg), showed a notable fall in phosphorus levels. Comparing Group II's urine magnesium levels to those of the normal control Group I, a notable (P<0.05) fall was seen (table 2). A noteworthy (P<0.05) increase was noted in every therapy group. The model group treated with ethylene glycol showed significant tubule dilatation and crystal deposition in the histopathology examination. But tubular dilatation and crystal deposition were significantly decreased by cystone and herbal medicine extract therapy (figure 1). The findings clearly show that urolithiasis was caused in the experimental animals by 0.75% ethylene glycol in drinking water. The standard medicine Cystone 750 mg/kg therapy increased urine magnesium and creatinine and lowered urinary calcium, oxalate, inorganic phosphate, and serum creatinine levels (P<0.05). Increased urine volume is evidence of its potent diuretic action. A 500 mg/kg dose of herbal extract significantly reduced (P<0.05) the levels of serum creatinine, calcium, oxalate, and inorganic phosphate in the urine. Significant increases in creatinine and magnesium levels were found in the urine. Its anti-urolithiatic action was demonstrated by the notable decrease in dry kidney weight and increase in urine volume that the 500 mg/kg dose caused. Urinary oxalate and serum oxalate were both considerably (P<0.05) reduced by 300 mg/kg of herbal extract. Creatinine levels, while it raised remarkably urinary creatinine levels. Within significant effect on rest of the parameters, it may be concluded that high dose of the herbal drug extract produce anti-urolithiatic effect.

CONCLUSION:

The methanolic extract of *Ixora javanica* DC flowers has shown significant antiurolithiatic effect, hence further research has to be carried out by isolating the active ingredient from the extract by column chromatography and structure determination of the various fractions obtained. The most active compound obtained can be further used for formulating a dosage form.

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