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EXPLORING THE ANTI-UROLITHIC AND ANTIOXIDANT PROPERTIES OF COCCINEA LEAVES: A COMPREHENSIVE EVALUATION

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ABSTRACT

Exploiting the leaves of *Ixora coccinea* Phytochemical, Pharmacological aspects with special emphasis on anti urolithiatic activity. The phytochemical screening result shows Alkaloids, Carbohydrates, Proteins and aminoacids, Tannins and phenolic compounds, Phytosterols, Flavanoids, Saponins, etc. In the present study, the urine amount augmented in the treated group's animals than that of the control and it abridged in the untreated lithiatic animals when comparing to the standard and urinal concentration of an assortment of ions investigated varied drastically. The antioxidant activity of the Ethanolic extract was estimated by five methods. DPPH radical scavenging activity, reducing power determination, scavenging of hydrogen peroxide, Assay of nitric oxide scavenging activity and compared with different standards like Ascorbic acid, Quercetine and Epicatechine. *Ixora coccinea* are having antioxidant activity. It was evaluated by five methods. In the Hydrogen peroxide scavenging activity method, the IC50 values are calculated and compared with the standard Ascorbic acid, it depicts 140 µg of *Ixora coccinea* of is equivalent to that of 12.5 µg of ascorbic acid. In the present study, Ixora coccinea are having good antioxidant and antiurolithiatic activity, it was proved obviously in this juncture. Meticulous percevings of the patho-physiology of illness and method of action of these herbal medicines have great importance in improvement of effective and safe antiurolithiatic agent. The antioxidant action was calculated as free radical scavenging activity technique, Nitric oxide scavenging, DPPH method, Reducing control determination technique, Hydrogen peroxide method. All the methods depict good response due to thepresence of phenolic compounds and flavonoids in three species. The herbal drugs exert their urolithiatic consequence by varying the ionic content of urine lessening the ca+ and oxalate ion strength or escalating magnesium and citrate excretion and also with diuretic activity. In this respect this information provides a fruitful area for scientific research by willing investigators. An attempt may be made to develop new herbal formulation to treat Kidney stone by Ixora coccinea plants. From this present study we can conclude by using this Ixora coccinea, we can go for herbal formulation development to treat Kidney stone.

KEYWORDS: Anti-Urolithic, Antioxidant, Coccinea Leaves, Phyto chemical evoluation.

INTRODUCTION

Kidney stones are one of the most painful urologic disorders. Renal stone affects 5 to 15% of adults. Epidemiological studies revealed that nephrolithiasis is more common in men (12%) than in women (6%) and is more prevalent between the ages of 20 to 40 in both sexes.[1] Urinary stones affect 10–12% of the population in industrialized countries.[2] The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing.[3] With a prevalence of > 10% and an expected recurrence rate of ~ 50%, the stone disease has an important effect on the healthcare system.[4] The aetiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices. Most calculi in the urinary system arising from a common component of urine, e.g. calcium oxalate representing up to 80% of analyzed stones. In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with the loss of kidneys or renal damage. Also, nearly 15% of the population of northern India suffers from kidney stones.[5] Urinary calculi is the third prevalent disorder in the urinary system. Urolithiasis is a common disease with an increasing incidence and prevalence worldwide that appears even more pronounced in industrialized countries.[6] Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened.[7] Features associated with recurrence include a young age of onset, positive family history, infection stones and underlying medical conditions.[8] Urolithiasis or nephrolithiasis represents the clinical condition of kidney stone disease. Stone formation in the urinary tract has been recognized for thousands of years, but during

the last few decades, the pattern and incidence of the disease have changed markedly.

Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physico-chemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract.[9] Ethylene glycol (EG) is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase/ aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxylic acid, which, in turn, is further oxidized to oxalic acid by glycolate oxidase. High doses of EG (>2,500 mg/kg body wt), particularly when given as an oral bolus, cause the saturation dependent accumulation of glycolic acid in the plasma. So, glycolate oxidase (GO) is one of the rate-limiting enzymes in the metabolism of EG.[10]

The mechanism of stone formation is a complex process which results from several physicochemical properties including supersaturation, crystal nucleation, precipitation, crystal growth, aggregation of crystals and retention of urinary stone constituents within tubular cells. There are various types of kidney stones which include cystine stones, calcium oxalate stones, calcium phosphate stones, struvite stones and uric acid stones. However, it is evident that crystal retention, cell apoptosis, renal cell injury, and associated stone promoters or inhibitors play important roles for kidney stone formation.[11]

Most of the plants are used in the alternative system of medicine but they are not systematically standardized. According to WHO guideline all the customary drugs should be standardized before going for the formulation. One of the WHO assembly resolutions emphasized the need to ensure quality control of medicinal plants products using modern techniques and establishment of required standards of quality for herbal medicines. So the objective of the project is to evaluate the phytochemical characters and pharmacological activity of the selected plants by advanced techniques. Exploiting the leaves of *Ixora coccinea* Phytochemical, Pharmacological aspects with special emphasis on anti urolithiatic activity.

MATERIALS AND METHODS

Authentication of plant materials

The leaves of *Ixora coccinea* available locally were collected in and around collected from Chittor district in AP. The voucher specimens had been submitted and preserved in herbarium for future reference.

Processing of plant material

The plant materials were collected and shade dried at room temperature and was subjected to size reduction to get course powder of desired particle size. Then powdered and passed through mesh size 40 and stored in air tight containers. These powdered materials were subjected to successive extraction. Each (1kg) powdered drugs were extracted with ethanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and solvents were evaporated under abridged pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts were calculated and stored in desiccators and used for further experiments.

Preliminary phytochemical analysis

The Ethanolic and aqueous extracts of the plant materials were separately prepared and subjected to chemical tests for the identification of its chemical constituents. Chemical tests were carried out on the aqueous and ethanol extracts and on the powdered specimens using standard procedures to identify the constituents.

Experimental design

Animal selection

Healthy Inbred Albino rats of *Wistar* strain, of male, aged around 2 to 3 months and weighing 150-200 g were selected for the antiurolithiatic activity used. The animals were acclimatized to standard laboratory conditions (temperature:25±2 ◦C) and maintained on 12-h light-dark cycle, relative humidity of 45-55%, and maintained on 12–hour light: 12–hour dark cycle in animal house. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum. The animal care and investigational protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose. The acute oral toxicity study was carried out as per the OECD guidelines usin For acute toxicity studies, Wistar albino mice of either sex weighing between 25 and 30 g were selected and employing the up and down method prior to evaluating all the extracts for antiurolithiatic activity. One-tenth of the median lethal dose (LD50) was taken as an effective dose.

Antiurolithiatic activity study

Chemicals

All the chemicals and reagents were purchased from Merck, Mumbai, India. Solventsand all the reagents used were of analyticalgrade. The creatinine kit purchased from (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kits from (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid level.

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats. Animals were divided into nine groups containing six animals in

each.

Treatment protocol

The grouped animals received the treatment as follows

Group I – Received normal diet and served as controls.

Group II - *Lithiatic control:* The animals were given normal diet and 1% Ethylene glycol indrinking water, for 28 days.

Group III - Received 1% ethylene glycol in drinking water and then treated with Ethanolic extract of IC at a dose of 200mg/kg orally, for 28 days.

Group IV - Received 1% Ethylene glycol in drinking water and then treated with Aqueous extracts of IC at a dose of 200mg/kg orally, for 28 days.

All extracts were given once daily by oral route.

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 14^{th}, and 28^{th} day of calculi induction treatment. The volume and calcium content of urine were measured. Calcium in urine was estimated using kit by COBAS MIRA PLUS auto analyzer. Urine was analyzed for oxalate, magnesium, phosphate, uric acid, citrate and total protein.

Serum analysis

The blood was collected from the retro-orbital sinus under anaesthetic condition and serum was separated by centrifugation at 10,000rpm for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid levels respectively.

Kidney histopathology

The abdomen was cut open to remove both kidneys from each animal. Isolated Kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and Stained with H and E (Haematoxylin and Eosin) for histopathological examination. The slides were examined under light microscope to study microscopic network of the kidney and calcium oxalate sediments.

Enzyme assays

A portion of kidney was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffererd KCL (pH 7.4)for the estimation of glutathione(GSH), Super oxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA).

Antioxidant activity

Renal cellular exposure to oxalate (Ox) and/or CaOx crystals leads to the production of Reactive Oxygen Species (ROS), development of oxidative stress followed by injury and inflammation. Renal injury and inflammation appear to play a noteworthy role in stone formation. An overproduction of ROS and a reduction in cellular antioxidant capacities, due to down-regulated expression of the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glucose-6 phosphate dehydrogenase) as well as radical scavengers (vitamin E, ascorbic acid, abridged glutathione) leads to the development of Oxidative Stress (OS) (Ying, W. M, 2002). Oxidative stress followed by renal cell injury and inflammation due to lipid peroxidation (Udupa. K.N., Singh R.H.1995). Loss of membrane integrity subsequently facilitates the retention of calcium oxalate crystals and growth of stones in renal tubules. Recent studies have provided evidence that CaOx kidney stone patients malondialdehyde (MDA) in their urine, indicating ROS in kidneys of CaOx stone patients. Urinal excretion of these MDA is considered as a marker of renal epithelial cell injury.

Recent studies evidenced that treatment with anti-oxidants and free radical scavengers abridged CaOx crystal induced renal injuries. Pre-treatment with vitamin E along with mannitol abolished the deposition of CaOx crystals in the kidneys of rats injected with sodium oxalate (A.Helen, K.Krishnakumar 2003). Alanine-induced deposition of CaOx crystals in rat kidneys was blocked by dietary supplementation with vitamin E plus selenium. These antioxidant therapies restore the activity of antioxidant enzymes and free radical scavengers (Zima, T.S. 2001). Therefore, treatments with natural antioxidants and free radical scavengers, seems to possible thereupatic strategy for ameliorating hyperoxaluria induced oxidative stress and renal cell injury in urolithiasis. Herbal medicine or plants are rich source of natural antioxidants, can be used in treatmentof hyperoxaluria induced oxidative stress and urolithiasis. Protective effect of herbals in hyperoxaluric oxidative stress and CaOx crystal deposition is due to their potentialantioxidant activity depicts reduction in oxalate-induced renal tubular epithelial cellinjury in cell culture due to their antioxidant activity.

DPPH radical scavenging activity

The free radical scavenging activity was measured by (Kumaran A 2007)method, the decrease in absorbance of Ethanolic solution of DPPH. A stock solution of DPPH ($33mgL^{-1}$) was prepared in methanol and 5ml of this stock solution was added to 1ml of the plant extract solutions at different concentrations(25, 50, 75, 100, 150, 200, 250, $2500ug/ml^{-1}$). After30min, absorbance was measured at 517nm and compared with the standard ascorbic acid ($10\text{-}50ugml^{-1}$) pH 7.4. Percentage of DPPH scavenging activity of the plant extracts and the standard was calculated. The percentage extract of inhibition was calculated by the formula [(Ao-A1)/Ao] x100, when Ao is the absorbance of the control & A1 is the absorbance of the extract/standard.

Reducing power determination

Reducing power determination using potassium ferricyanide (Oyaiz*u et al., 1986*). The reducing power of the extracts was determined with different concentrations

of extracts/standard (50-250mgm/ml) in methanol were mixed with phosphate buffer (PH 6.6) and incubated with (2.5ml) of potassium ferricyanide solution (1%w/v) at 50°C for 20 min. Then 2.5 ml of trichloroacetic acid was added to the mixture and which was then centrifuged for 10 min. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Scavenging of hydrogen peroxide

Capability of three extracts to forage hydrogen peroxide was determined by ((Ruch.R.J.1984) method. A solution of H_2O_2 (2mol/l) has been prepared in phosphate buffer-PH 7.4. Hydrogen peroxide concentration was determined by spectrometric ally absorbance at 230nm.Extracts were prepared at the concentration of 50-250mgm/ml and added to the H_2O_2 solution (0.6ml). Blank contains phosphate buffer with without H_2O_2. Each concentration a particular blank sample was utilized taking the reading. The % of inhibition effect was calculated from the formula $[(A_0 - A_1)/A_0]$ X 100. Where as $A_1$is the absorbance of extract/standard and A_0 is the absorbance of the standard and.

Assay of nitric oxide scavenging activity

The nitric oxide scavenging activity of the samples was calculated as per the scheme of (Sreejayan and Rao., 1997). 3ml of Sodium nitropruside in 0.2 M in phosphated saline (pH 7.4) with special strenght of Ethanolic extracts and incubate at room temperature for 2 hours. Same reaction component devoid of the ethanol extract but the equivalent amount of ethanol served as the control. After the period of incubation, 0.5 ml of reagent Griess was added. The absorbance of the chromophore was measured at 546nm. Quercetin and the same mixture of the reaction without the sample extracts were employed as positive and negative control. The range or degree of radical scavenging activity was measured as

% NO radical scavenging activity = (control OD - sample OD / control OD) × 100.

The analysis was done three times. Sample strength lending 50% inhibition (IC 50) under the assay condition was measured from the pictorals of inhibition percentage aligned with sample concentration.

Statistical analysis

The consequences were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Newmannkeul's multiple range tests and $p < 0.05$ was considered noteworthy.

RESULT AND DISCUSSION

The Phytochemical screening results are as follows

Table 1: Phytochemical screening.

S. no.	Chemical test	Ethanol
1	Alkaloids	
A	Mayer's test	+
B	Dragendroff's test	+
C	Wagner's test	+
D	Hager's test	+
2	Carbohydrates	
A	Molisch's test	+
B	Fehling's test	+
C	Barfoed's test	+
3	Proteins and Free Amino acids	
A	Ninhydrin test	-
B	Biuret test	+
C	Xantho Protein test	+
4	Tannins and Phenolic Compounds	
A	Ferric chloride test	+
B	Lead acetate test	+
C	Gelatin test	+
5	Phytosterols	
A	Libermann Burchard test	+
B	Salkowski test	+
6	Flavanoids	
A	Shinoda test	+
7	Saponins	+
8	Glycosides	-
9	Terpenoids	-

The phytochemical screening result shows ethanolic extract contain Alkaloids, Carbohydrates, Proteins and aminoacids, Tannins and phenolic compounds, Phytosterols, Flavanoids, Saponins, etc.

S. no.	Chemical test	Aqeous
1	Alkaloids	
A	Mayer's test	+
B	Dragendroff's test	+
C	Wagner's test	+
D	Hager's test	+
2	Carbohydrates	
A	Molisch's test	+
B	Fehling's test	+
C	Barfoed's test	+
3	Proteins and Free Amino acids	
A	Ninhydrin test	_
B	Biuret test	+
C	Xantho Protein test	+
4	Tannins and Phenolic Compounds	
A	Ferric chloride test	+
B	Lead acetate test	+
C	Gelatin test	+
5	Phytosterols	
A	Libermann Burchard test	-
B	Salkowski test	-
6	Flavanoids	
A	Shinoda test	+
7	Saponins	+
8	Glycosides	-
9	Terpenoids	-

The phytochemical screening result shows Aqous extract contain Alkaloids, Carbohydrates, Proteins and aminoacids, Tannins and phenolic compounds, Flavanoids, Saponins, etc.

Antiurolithiatic activity

In the present study, the urine amount augmented in the treated group's animals than that of the control and it abridged in the untreated lithiatic animals when comparing to the standard and urinal concentration of the an assortment of ions investigated varied drastically, subsequent ethylene glycol treatment in the lithiatic control. The oxalate, calcium, uric acid, creatinine and phosphate excretion were notably increased on day 14^{th} & 28^{th} respectively for GROUP-II following ethylene glycol treatment. Management with Ethanolic and aqueous extracts of *Ixora coccinea* abridged the excretions notably on 14^{th} day of treatment and supplementary abridged on 28^{th} day, like standard. In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were augmented notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. Treatment with Ethanolic and aqueous extracts of Ixora coccinea abridged all above mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol action**.** After management with Ethanolic and aqueous extracts of *Ixora coccinea* the magnesium level was restored near to regular and standard levels.

Low urinal magnesium content is a general attribute in stone formers. An alike condition was observed in the (GROUP-II) rats. Management with Ethanolic and aqueous extracts of *Ixora coccinea* elevated the urinal magnesium level, and consequently, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been distinguished in hyperoxaluric rats and stone formers.

In this study before estimating the ionic concentration of the urine on 14^{th} and 28^{th} day, the total quantity of urine excreted by the 9 groups were estimated. The treated groups depicted augmented the amount of urine volume. The consequences were expressed in table no1. In the 14^{th} and 28^{th} day after treatment the Urine parameters such as calcium, uric acid, creatinine, oxalate, phosphate and magnesium levels were estimated. Then the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were estimated on 28^{th} day of the treatment. The kidney was prepared for the estimation of glutathione (GSH)Super oxide dismutase (SOD) catalase (CAT) and malondialdehyde (MDA) that enzyme level was documented in the table.

Table 2: Effect of *Ixora coccinea* on urinal output in urolithiasis induced rats.

Days	Group-I	Group -II	Group-III	Group-IV
0	7.25±0.52	7.30±0.60	7.35±0.66	7.90±0.70
14	7.89±0.60	5.35±1.36	6.45±1.50	6.20±1.20

Table 3: Effect of *Ixora coccinea* on urinal Biochemical parameters on the 14th day.

Groups	Protein(mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	70.90±3.76	4.42±0.58	6.15±0.70	3.30±0.62	0.90±0.08	16.30±1.50	33.60±2.26
Group -II	158.40 ±7.30	1.35 ±0.11	22.15 ±1.60	13.60±1.32	1.86±0.24	48.20 ±4.45	78.66±4.26
Group -III	93.12 ±5.78	2.95 ±0.38	12.65 ±0.95	8.40 ±0.86	1.22 ±0.16	24.38 ±2.68	44.82 ±3.32

Table 4: Effect of *Ixora coccinea* on Urinal Biochemical parameters on 28th day.

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	65.96±2.86	4.20±0.52	5.63±0.54	3.22±0.65	0.80±0.08	15.80±1.83	32.90±2.20
Group -II	152.22 ±6.30	0.98 ±0.14	20.15±1.98	12.5 ±1.62	1.56 ±0.14	32.65 ±3.42	73.60 ±4.26
Group -III	86.20 ±4.20	2.50 ±0.36	9.45 ±1.10	5.5 ±0.94	0.92 ±0.09	23.22 ±2.76	43.55 ±3.73
Group -IV	82.66 ±3.55	2.88 ±0.40	8.90 ±0.92	4.20 ±0.85	0.86 ±0.11	19.30 ±2.32	37.80 ±3.15

Table 5: Effect of *Ixora coccinea* on serum Biochemical parameters on 28day.

Groups	Magnesium (mg/dl)	Calcium(mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	4.80±0.86	9.40±1.32	3.45±0.40	0.56±0.03	6.6±0.57	12.06±1.43
Group –II	1.38±0.25	18.30±2.34	9.7±1.10	1.01±0.13	12.60±1.61	26.01±3.25
Group -III	3.28±0.46	11.85±1.88	4.55±0.55	0.91±0.09	8.45±0.88	20.10±2.65
Group –IV	3.67±0.52	11.22±1.60	4.10±0.46	0.80±0.07	8.12±0.78	19.85±2.05

GP_1- Normal;
GROUP-II- Lithiatic Control;
GP_3- EEIC (200mg/kg);
GP_4- AEIC (200mg/kg);
GP- Cystone herbal tablets (100mg/kg)

- Values are expressed in ml/24 h urine sample mean ± SEM
- Values were originate out by by means of ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were notably different from normal control (GP_1) at P< 0.01
- **(b) Values were notably different from Lithiatic control (GROUP-II) at P<0.01

The abdomen was cut open to take away both kidneys from each animal. Secluded Kidneys were made tidy away from extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was prepared and stained for histopathological examination. The slides were examined under light microscope to study microscopic design of the kidney and calcium oxalate deposits. The photo from the histopathological examination gave a clear consequence ,that depicts the reduction in the stone development and the inflammations also abridged when comparing with the standard drug and the untreated group depicted large quantity of microcrystal deposition and stern dilation of most tubules and mass tubulointerstitial inflammatory infiltrationwith lesion area.

Histopathological study

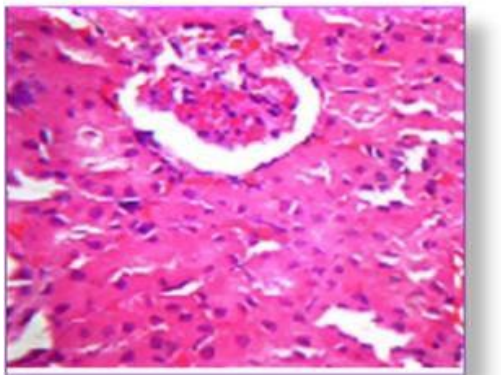

Fig. 1: Section of kidney glomerului-I.

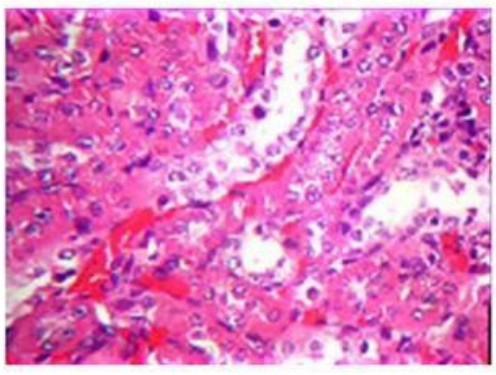

Fig. 2: Section of kidney glomerului-II.

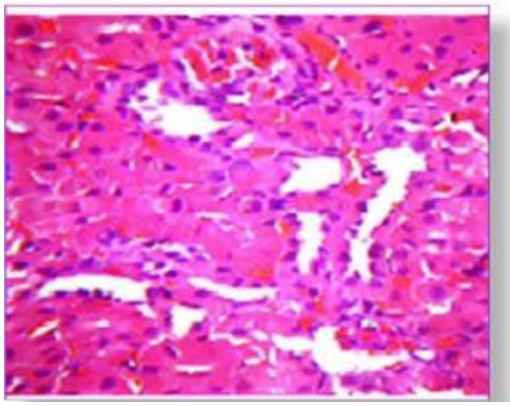

Fig. 3: Section of kidney glomerului-III.

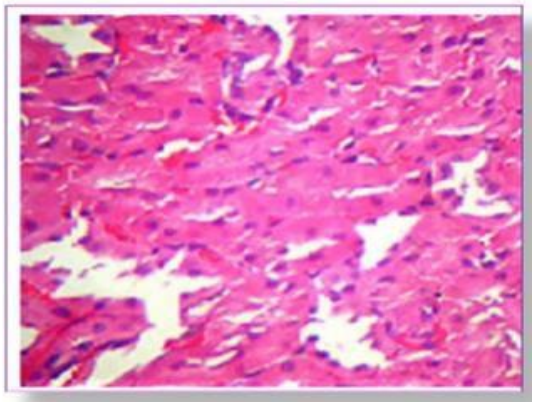

Fig. 4: Section of kidney glomerului-IV.

In vivo antioxidant activity

For *invivo* antioxidant action EtOH treatment augmented MDA (P<0.01) and lessened GSH (p<0.01) SOD (p<0.01) and CAT (0.01) levels in control animals. Aqueous and ethanolic extracts of Ixora coccinea at a dose of 200mg/kg produced noteworthy (p<0.001) reduction in MDA and augmented GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone.

Table 6: Effect of Aqueous and Ethanolic extracts of *Ixora coccinea* on antioxidantenzymes in renal tissue.

Treatment	Catalase A/protein	SOD B/mgprotein	GSH nmoles/mgprotein	MDA nmoles/mgprotein
Normal control	3.20±0.18	9.20±0.18	3.72±0.16	3.88±0.28
Ethylene glycol	0.98±0.02	3.68±0.07	0.52±0.06	10.05±0.46
Cystone	2.80±0.16	7.05±0.11	2.68±0.14	4.20±0.30
AEIC 200mg/kg	2.32±0.09	6.55±0.10	2.12±0.10	5.68±0.35
EEIC 200mg/kg	2.28±0.08	6.60±0.12	2.20±0.11	5.76±0.45

- Values are expressed as Mean± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul'smultiple range tests.
- **(a)values were notably different from normal control (GP_1)at P< 0.01
- **(b) values were notably different from Lithiatic control (GROUP-II)at P<0.01

Antioxidant activity

All these three plants are having antioxidant action. It was evaluated by five technique. In the Hydrogen peroxide scavenging action method the IC50 values are calculated and comparing with the standard Ascorbic acid, it depicts 140 µg of *Ixora coccinea* is corresponding to that of 12.5 µg of ascorbic acid. The anti-haemolytic activity method, IC50 values are calculated and compared with the standard Quercetine. The consequence depicts 211.29µg of *Ixora coccinea* is corresponding to 57.06 µg of Quercetine. Flavonoids are phenolic components, abundant in several plants, which inhibit lipid per oxidation and lipoxygenases *invitro* and in presence of Fe^{3+}.In the lessening power action method the IC50 values are calculated and distinction with the paradigm Epicatechine. The outcome depicts 114 µg of *Ixora coccinea* is equivalent to 12.5 µg of Epicatechine action.

NO is an imperative component intermediary generated by endothelial structures, macrophages, neuronals and troubled in the directive of an assortment of physiological processes. Excess concentration of NO is implicated in the cytotoxic trait pragmatic in an assortment of disorders like AIDS, cancer, Alzheimer's and arthritis. Oxygen reacts with the surplus NO to manufacture nitrite and peroxy nitrite anions, which act as free radicals.[23] From consequences of Nitric oxide technique, it proved that the aqueous leaf extract of *AC* had effectual anti oxidant action.

These extract fight with oxygen to act in retort with NO and accordingly inhibit the generation of the nitrite and peroxy nitrite anions. In NO scavenging action technique the IC50 values are calculated and compared with the standard Quercetine IC50 value. It depicts 294.16 µg of *Ixora coccinea* is corresponding to 25 µg of Quercetine. Free radical scavenging action of the aqueous leaf extract of *AC* is concentration dependent, as the concentration of the test compounds raised, the radical scavenging activity increases. In the DPPH scavenging action method the IC50 values are calculated and it lends a good consequence of 384µg of *Ixora coccinea* is equivalent to 24µg of Ascorbic acid.

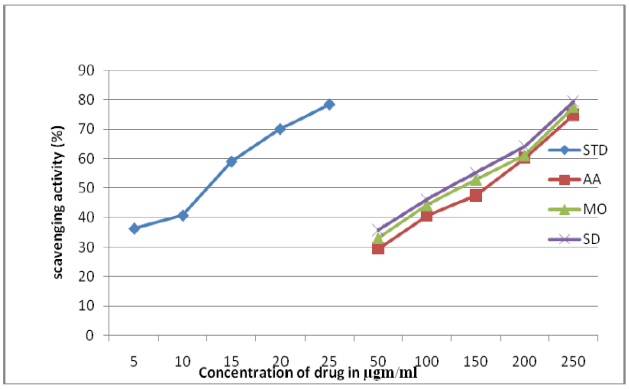

Fig. 5: Hydrogen peroxide method.

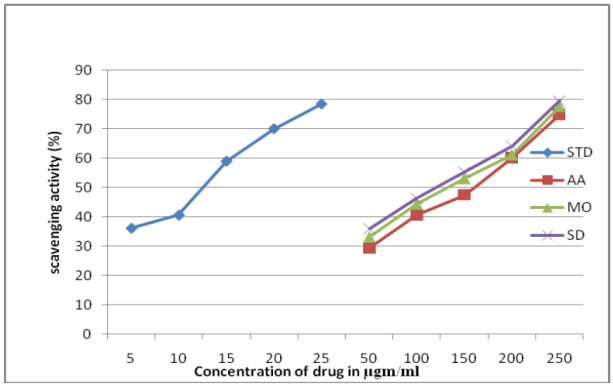

Fig. 6: Scavenging of hydrogen peroxide.

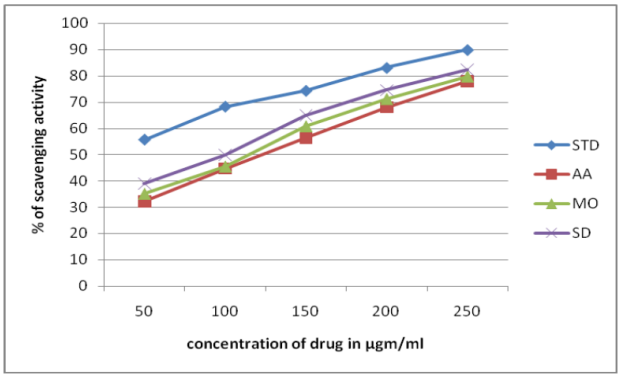

Fig. 7: Reducing power determination.

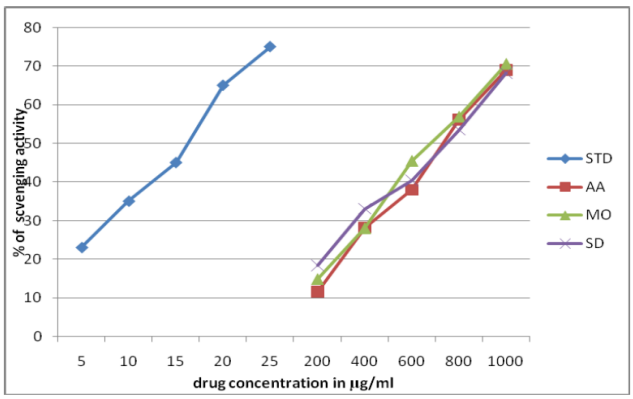

Fig. 8: Nitric oxide scavenging activity.

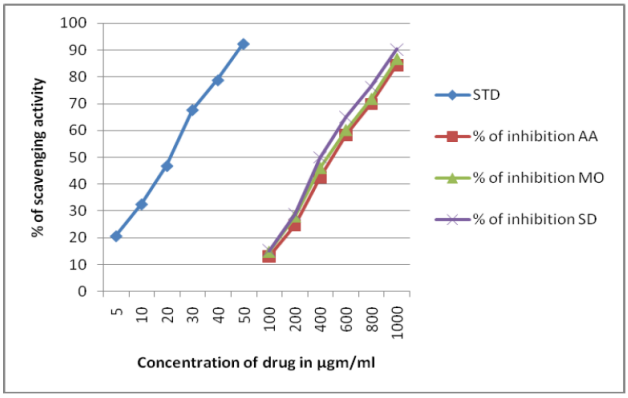

Fig. 9: DPPH radical scavenging activity.

SUMMARY AND CONCLUSION

This present study evaluated *Ixora coccinea* for urolithiasis and related problems in human. As conventional medicines are typically taken by the oral route, same course of administration was used for assessment of antilithiatic consequence of the extracts of *Ixora coccinea* at a dose of 200mg/kg and Cystone herbal tablet at a dose of 100mg/kg aligned with ethylene glycol induced urolithiasis in rats. In the present study, male rats were selected to persuade urolithiasis because the urinal system of male rats resembles that of humans and also the quantity of stone deposition in female rats was drastically fewer. Substantiation in preceding studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi encompassed mainly of calcium oxalate. The biochemical mechanisms for this progression are related to a raised in the urinal concentration of oxalate. Stone development in ethylene glycol fed animals is caused by hyperoxaluria, which causes augmented renal retention and emission of oxalate. In the present study oxalate and calcium excretion progressively augmented in calculi- induced animals, since it is customary that hyperoxaluria, is a far more risk reason in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are comparatively much more important than those of calcium. Augmented urinal calcium is a feature favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and consequent crystal growth. Conversely, extracts of *Ixora coccinea* treated animals lowered the levels of oxalate as well as calcium excretion.

In the present study, *Ixora coccinea* are having good antioxidant and antiurolithiatic activity, it was proved obviously in this juncture. Meticulous percevings of the patho-physiology of illness and method of action of these herbal medicines have great importance in improvement of effective and safe antiurolithiatic agent. The antioxidant action was calculated as free radical scavenging activity technique, Nitric oxide scavenging, DPPH method, Reducing control determination technique, Hydrogen peroxide method. All the methods depict good response due to thepresence of phenolic compounds and flavonoids in three species. The herbal drugs exert their urolithiatic consequence by varying the ionic content of urine lessening the ca+ and oxalate ion strength or escalating magnesium and citrate excretion and also with diuretic activity. In this respect this information provides a fruitful area for scientific research by willing investigators. An attempt may be made to develop new herbal formulation to treat Kidney stone by *Ixora coccinea* plants. From this present study we can conclude by using this *Ixora coccinea*, we can go for herbal formulation development to treat Kidney stone.

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