Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

EFFECT OF ETHANOLIC EXTRACT OF PORTULACA OLERACEA LINN. ON ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED UROLITHIASIS

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Received: 13 Dec 2012, Revised and Accepted: 29 Jan 2013

ABSTRACT

Objective: This study was aimed to evaluate the antiurolithiasis activity of the ethanolic extract of aerial parts of *Portulaca oleracea* Linn.

Methodology: The activity of the plant was studied using the ethylene glycol (0.75% v/v) and ammonium chloride (2% w/v) induced urolithiasis model in albino rats. Several parameters were used including urinary volume, urine pH, urine and serum parameters to assess the activity. The ethanolic extract of *Portulaca oleracea* was administered in doses of 100, 200 and 400 mg/kg body weight orally for 15 days. Standard drug used was cystone.

Results: Treatment with the extract restored all the elevated biochemical parameters including serum and urine (calcium, creatinine, urea, BUN), restored the urine pH to normal and increased the urine volume significantly ($P < 0.05$) when compared to disease control group. The histopathological studies confirmed the induction of lithiasis as microcrystal deposition was observed in section of kidney from animals treated with ethylene glycol and ammonium chloride. This was reduced, however, after treatment with the extract.

Conclusion: These observations enable us to conclude that the ethanolic extract of *Portulaca oleracea* is effective against ethylene glycol and ammonium chloride induced urolithiasis in albino rats.

Keywords: *Portulaca oleracea,* Urolithiasis, Ethylene glycol, Ammonium chloride, Antiurolithiatic, Ethanolic extract.

INTRODUCTION

Urolithiasis constitutes one of the commonest afflictions requiring surgical intervention in our country and by conservative estimates there are about 5-7 million patients suffering from urinary calculus disease in India. It is not only the high prevalence which requires early attention, but rather the more problematic, high rate of recurrence after surgical removal. It is for these reasons that the Indian Council of Medical Research has classified this disease as one of the refractory diseases and stressed that efficient effort should be made to find out the cause/s of the disease and to search for suitable drugs for its cure. [1]

Although there are a few recent reports of beneficial effects of medical treatments in enhancing clearance of stones in the distal ureters, de facto there is still no satisfactory drug to use in clinical therapy, especially for the prevention or the recurrence of stones. In this regard, many plants have been traditionally used to treat kidney stones and have been shown to be effective. Unlike allopathic medicines which targets only one aspect of Urolithiasis Pathophysiology, most plant based therapy have been shown to be effective at different stages of stone pathophysiology.[2][3]

Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple mechanisms.[4]

Portulaca oleracea L. (Portulacaceae) is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term Global Panacea.[5][6]

In folk medicine, it is utilized as an antipyretic, anti-scorbutic, antiseptic, antispasmodic, diuretic, and anthelmintic and for treatment of urinary disorders. The aerial parts of the plant are used medicinally for reducing pain and swelling. Recent pharmacological studies have shown muscle relaxant activity, reduction in locomotor activity, increase in the onset time of pentylenetetrazole-induced convulsion, analgesic, anti-inflammatory effects and antioxidant properties. It is reported that extracts of *P. oleracea* has inhibitory effect on lipopolysaccharide (LPS) and interferon-γ, (IFN- γ) induced NO production.[7]

The present study plans to systematically evaluate *Portulaca oleracea* to verify the folklore use of the plant for diseases of the kidney and bladder [8], as literature survey showed that no scientific work was carried out to support the above use. In view of this, an attempt has been made to investigate the effectiveness of the ethanolic extract of the aerial parts of *Portulaca oleracea* as a preventive agent against the development of kidney stones.

MATERIALS AND METHODS

Collection and Authentication

The **weed** was collected from local areas of Hyderabad, during July-2011 and authenticated by Dr. Bhadriah, PhD, Head of Department of Botany, Osmania University.

Preparation of Extract [9] [10]

The aerial parts of *Portulaca oleracea* were cleaned, shade dried and powdered by mechanical grinder. For the ethanolic extract, 100gm powder was macerated in 800 ml of ethanol (80% v/v) for 3 days and the mixture was subsequently filtered and concentrated at 40°C. Extract was dried and preserved in a dessicator.

Some part of the total extract was used for phytochemical investigation and rest of the extract was used for pharmacological screening. For pharmacological screening the extract was suspended in 0.3% Carboxy Methyl Cellulose (CMC).

Methodology

Experimental animals

Healthy adult male Wistar albino rats weighing between 150-200 g were selected for the antiurolithiatic activity. They were procured from Small Animal House Shadan Medical College, Peerancheru, Hyderabad. The animals were acclimatized to standard laboratory conditions (temperature: 25± 2°C, relative humidity 65±10%) and maintained on 12 hr light: 12 hr dark cycle. The animals were fed with standard pellet diet (Hindustan Lever Ltd., Bangaluru, India) and drinking water ad libitum. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (/SES/IAEC/019/2011).[11]

Chemicals used

Ethylene glycol (AR Grade), Merck Laboratories, Mumbai, India, Aluminium chloride, Merck Laboratories, Mumbai, India, Cystone®tablets (Himalaya Drug Company, Batch No.A021019B); [12]

Extracts used

Ethanolic extract of aerial parts of *Portulaca oleracea* was insoluble in water. Hence, it was suspended in 0.3% Carboxy Methyl Cellulose

(CMC) just before administration to rats.[10] Calculated quantity of extract was given to each animal in corresponding group, once daily by gavage (intra-gastric administration).

In all cases, the concentrations were prepared in 1 ml/100gm of body weight. The test substances were administered in a single dose using a gastric intubation tube.

Acute Toxicity Study

The acute oral toxicity study was carried out as per the guidelines set by Organization for economic co-operation and development (OECD) (Guidelines 425). The animals were fasted overnight prior to the experiment. The first group was treated after fasting overnight with oral dose of 1000 mg/kg body weight with the ethanolic extract of the aerial parts of *Portulaca oleracea* Linn. The extracts were given in two different groups and the animals were observed continuously for 2-3 hrs for general, behavioural, neurological, autonomic profiles and finally death after 24hrs. If there was no mortality and no sign of toxicity and the extract was found to be safe at this dose level, then a higher dose of 2000mg/kg body weight of the ethanolic extract was administered in another 2 groups. If no mortality was observed, the maximum tested dose level was taken as 2000 mg/kg body weight. The doses for pharmacological studies were taken as 400, 200, 100 mg/kg body weight i.e. $1/5^{th,}$ $1/10^{th}$, $1/20^{th}$of the maximum tested dose (i.e. 2000mg/kg).

Ethylene glycol induced urolithiasis model in albino rats**[**12][13]

The rats were divided into six groups comprising six animals in each group. Each group underwent a different treatment protocol.

All animals had free access to regular rat chow and drinking water *ad libitum* for 15 days. Renal calculi were induced in group **2 to 6** by supplementing with 0.75% v/v ethylene glycol and 2% w/v ammonium chloride in drinking water *ad libitum*. Group IV, V and VI were treated with plant extracts starting from 1^{st} day to 15^{th} day.

During the entire course of investigation, rats were housed in polypropylene metabolic cages having provision of separating the feacal as well as urine samples.

Group 1: Normal control-Carboxymethyl cellulose (0.3 %) from 1^{st} day to 15^{th} day

Renal calculi were induced in group **2 to 6** by supplementing with 0.75% v/v ethylene glycol and 2% w/v ammonium chloride in drinking water *ad libitum*

Group 2: Calculi induced or Disease control- Carboxymethyl cellulose (0.3 %) from 1^{st} day to 15^{th} day

Group 3: Standard drug treated- Cystone tab. (750mg/kg b.w., p.o.) from 1^{st} day to 15^{th} day

Group 4: Ethanolic extract treated- Ethanolic extract of aerial parts of *Portulaca oleracea* (100mg/kg b.w., p.o.)

Group 5: Ethanolic extract treated- Ethanolic extract of aerial parts of *Portulaca oleracea* (200mg/kg b.w., p.o.)

Group 6: Ethanolic extract treated- Ethanolic extract of aerial parts of *Portulaca oleracea* (400mg/kg b.w., p.o.)

Assessment of Antiurolithiatic Activity

Collection and analysis of urine

All the animals were kept in individual cages and urine samples of 24 h were collected on 15^{th} day. Animals had free access to water during the urine collection period. A drop of concentrated HCl was added to the urine before being stored at 4°C. The urinary volume and pH was determined. Urine was analyzed for calcium, and creatinine using calcium and creatinine diagnostic kits (Span Diagnostics Ltd,). The sample was also subjected to microscopic examination for calcium oxalate crystals and for the confirmation of urolithiasis.

Urine was centrifuged at 2500 rpm for 5 min to pool the crystals and observed under simple microscope at 5X or 10X. Shape and number of crystals were observed and are reported in Table 1.

Serum analysis

After the 15 day experimental period, the rats were anaesthetized and blood was collected from the retro-orbital region, centrifuged and the serum collected and analyzed for level of calcium, creatinine, urea and BUN using calcium, creatinine, urea [12] [14] and BUN [15] diagnostic kits (Span Diagnostics Ltd.).

Histopathological Examination [16] [17]

For microscopic evaluation, kidneys were fixed in 10% neutral formalin solution. Following dehydration in ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5µm were stained with hematoxylin-eosin. A minimum of 10 fields for each kidney slide were examined for necrosis and presence of calcium oxalate crystals. Tissue slices were photographed using optical microscope at 10X magnification.

Statistical Analysis [18]

The data were expressed as mean ± S.E.M. The differences were compared using one-way ANOVA followed by Dunnett's test using GraphPad Prism software (version 5). The results were considered significant when $P < 0.05$.

RESULTS

Acute Oral Toxicity

The ethanolic extract of *Portulaca oleracea* was found to be safe and no mortality was observed up to a dose of 2000 mg/kg body weight, p.o. The maximum tested dose was taken as 2000 mg/kg body weight. The doses for pharmacological studies were taken as 400, 200, 100 mg/kg body weight, p.o. i.e. $1/5^{th,}$ $1/10^{th}$, $1/20^{th}$of the maximum tested dose i.e. 2000 mg/kg body weight

Antiurolithiatic Activity

Urine Analysis

Microscopic Examination of Urine**:** The microscopic examination (at 5X and 10X), of the urine of normal group animals was devoid of any crystal or similar structure. In calculi induced rats (Group II), the urine sample showed abundant, large crystals of CaOx with characteristic rectangular shape. The cystone treated animals showed very less number of crystals. However, crystals were seen in all the three test groups viz. group IV, V, VI but the number of crystals in group V and VI were very less when compared to the lithiatic control group II(Table 1). These results indicate the efficacy of the ethanolic extract in preventing the formation of calcium oxalate calculi in the kidney.

Urinary volume and pH: The urinary pH in normal animals was between 8.0-8.5. On induction of calcium oxalate stones, the pH reduced to 6.0 - 6.2. After completion of the study, Group IV, V and VI groups treated with the ethanolic extracts showed an increase in the urinary pH (8.4 - 9.1) when compared to the control group II.

Urine volumes were significantly increased ($P<0.05$) by the ethanolic extract of the plant when compared to the disease control (Group II), (Table 2, Graph No. 1 and 2).

Estimation of Calcium and Creatinine

Table No.3 shows details of 24 h urinary excretion of calcium and creatinine in normal and treated animals. Ethylene glycol and ammonium chloride administration for 15 days resulted in significant ($P<0.05$) hyperoxaluria as compared to normal animals (Group I). Urinary excretion of calcium and phosphate was also significantly ($P<0.05$) increased in-group II. On treatment with ethanolic extract for 15 days, a significant decrease in the urinary excretion of calcium and creatinine was observed in Group IV, V, and VI as compared to the disease control (Group II) (Table 3). These results of the urinary excretion data clearly support that the ethanolic extract of the plant can reduce supersaturation of urine with calculogenic ions (Graph No. 3 and 4).

Table 1: Microscopic Examination of Urine for Calcium oxalate Crystals

Group	Treatment	No. of Calcium oxalate Crystals
Normal control	Sodium carboxymethyl cellulose (0.6 %) for 15 days	Nil
Disease control	Ethylene glycol (EG) and Ammonium chloride(AC) 0.75% EG + 2% AC in distilled water	20-25
Standard	Ethylene glycol(EG) and Ammonium chloride(AC) 0.75% EG + 2% AC in distilled water+ Cystone	3-4
Ethanolic extract treated	Ethylene glycol (EG) and Ammonium chloride (AC) 0.75% EG + 2% AC in distilled water + Ethanolic extract (100mg/kg b.w., p.o.)	10-15
Ethanolic extract treated	Ethylene glycol(EG) and Ammonium chloride(AC) 0.75% EG + 2% AC in distilled water+ Ethanolic extract (200mg/kg b.w., p.o.)	8-10
Ethanolic extract treated	Ethylene glycol(EG) and Ammonium chloride(AC) 0.75% EG + 2% AC in distilled water + Ethanolic extract (400mg/kg b.w., p.o.)	4-5

Table 2: Effect of Ethanolic Extract of *Portulaca oleracea* on pH and Urine Volume

Group	Dose (mg/kg body weight, p.o.)	pH of Urine	Urine volume (ml)
Normal Control	0.3%CMC(Vehicle)	8.21 ± 0.03	2.70 ± 0.10
Disease Control	0.75% EG +2% AC	6.36 ± 0.09	1.58 ± 0.12
Standard(Cystone)	750 mg/kg	7.52 ± 0.11	5.45 ± 0.16***
Ethanolic Extract	100 mg/kg	9.10 ± 0.03	4.12 ± 0.04***
Ethanolic Extract	200 mg/kg	8.40 ± 0.06	5.40 ± 0.08***
Ethanolic Extract	400 mg/kg	8.60 ± 0.02	6.63 ± 0.26***

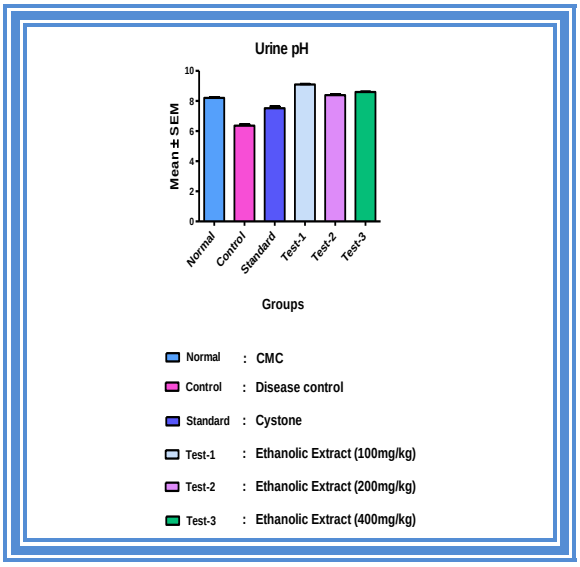
Table 3: Effect of Ethanolic Extract of *Portulaca oleracea* on Urine Parameters

Group	Treatment	Dose (mg/kg body weight, p.o.)	Calcium	Creatinine
I	Normal Control	0.3%CMC(Vehicle)	1.50±0.12	7.68 ± 0.09
II	Disease Control	0.75% EG +2% AC	6.30±0.03	20.55 ± 0.14
III	Standard(Cystone)	750 mg/kg	2.52±0.11***	7.93 ± 0.01***
IV	Ethanolic Extract	100 mg/kg	4.40±0.15***	10.64 ± 0.03***
V	Ethanolic Extract	200 mg/kg	3.80±0.01***	9.70 ± 0.14***
VI	Ethanolic Extract	400 mg/kg	3.43±0.17***	8.60 ± 0.12***

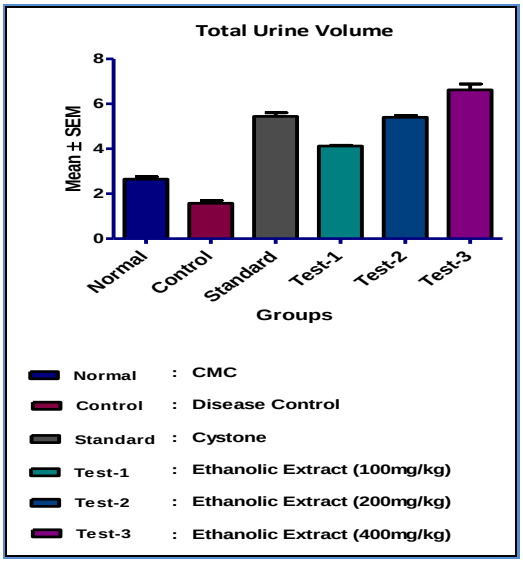
Table 4: Effect of Ethanolic Extract of *Portulaca oleracea* on Serum Parameters

Group	Dose (mg/kg body weight, p.o.)	Calcium	Creatinine	Urea	BUN
Normal Control	0.3%CMC(Vehicle)	7.1 ± 0.05	0.76 ± 0.03	43.97±1.01	20.54±0.47
Disease Control	0.75% EG +2% AC	9.7 ± 0.3	1.18 ± 0.05	78.11±1.05	36.48±0.49
Standard(Cystone)	750 mg/kg	6.9 ± 0.2***	0.86 ± 0.01***	46.27±0.79***	21.61±0.37***
Ethanolic Extract	100 mg/kg	9.2 ± 0.2***	0.93 ± 0.01***	77.35±1.17 ns	36.12±0.37 ns
Ethanolic Extract	200 mg/kg	8.5 ± 0.2***	0.88 ± 0.01***	66.22±2.72 ns	30.95±1.26 ns
Ethanolic Extract	400 mg/kg	7.6±0.08***	0.81 ± 0.01***	51.79±1.03***	24.19±0.48***

Histograms showing the Effect of Ethanolic Extract of *Portulaca oleracea* Linn. On Urine pH and Urine Volume

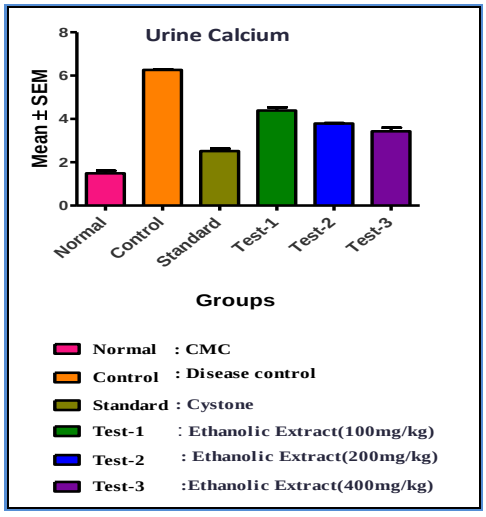


Graph No. 1

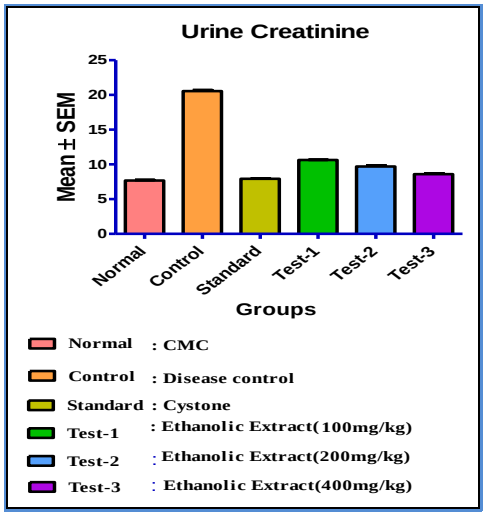


Graph No. 2

Histograms showing the Effect of Ethanolic Extract of *Portulaca oleracea* Linn. On Urinary Calcium and Creatinine Level

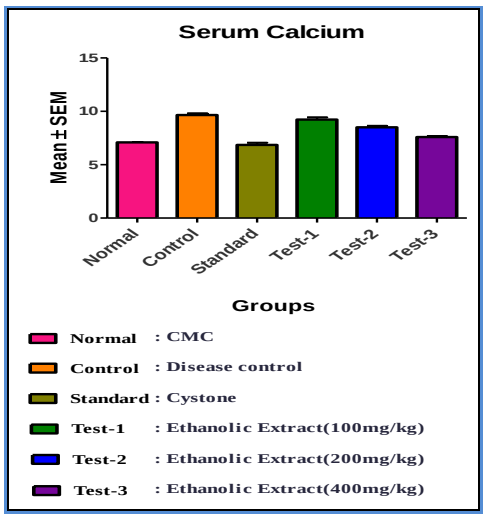


Graph No. 3

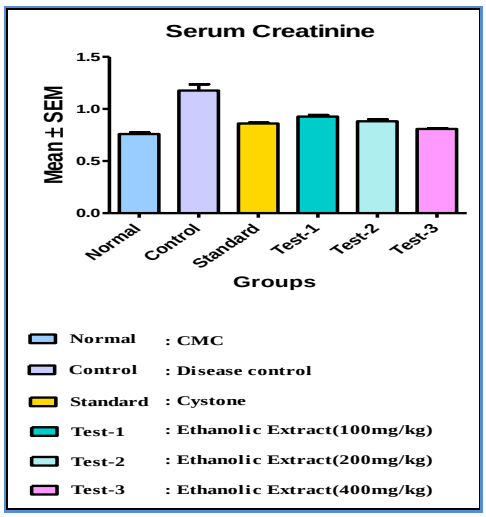


Graph No. 4

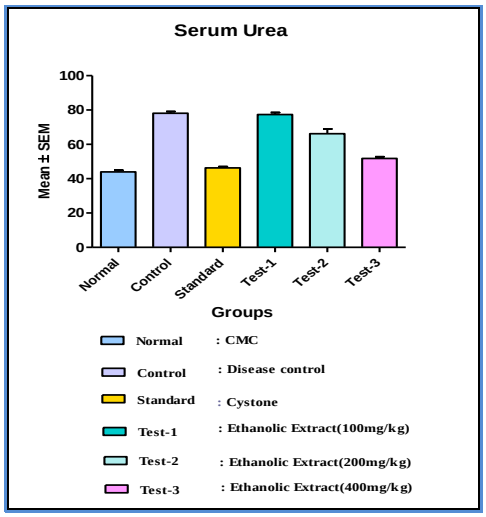
Histograms showing the Effect of Ethanolic Extract of *Portulaca oleracea* Linn. On Serum Calcium, Creatinine, Urea and Blood Urea Nitrogen (BUN)



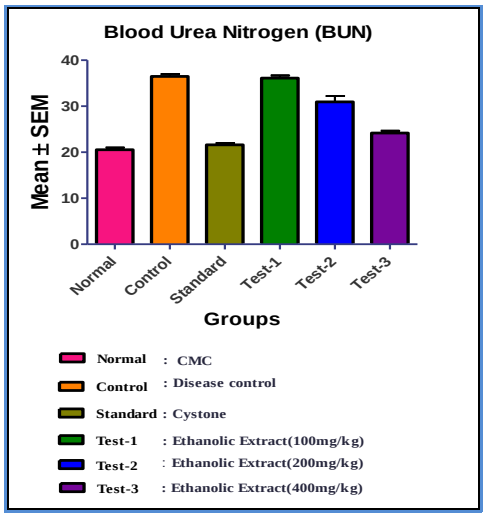
Graph No. 5



Graph No. 6



Graph No. 7



Graph No. 8

Serum Analysis

The serum urea and BUN were remarkably increased in calculi-induced animals (Table 4, Group II). On treatment with ethanolic extract of *Portulaca oleracea,* the elevated serum levels of calcium, creatinine, urea, and BUN were significantly (P<0.05) reversed and the serum levels of group treated with 400mg/kg body weight, p.o. were almost comparable to those of cystone treated animals (Group III) (Graph No. 5,6,7,8).

Histopathological Studies

The histopathological study of the kidney sections also supported the above results. Normal group showed intact architecture of medullary and papillary tubules. Sharp demarcation between cortex and medulla was seen. However, the disease control group which was treated with ethylene glycol and ammonium chloride showed oedema of tubular epithelium which resulted in distortion of the tubules. There was evidence of patchy area showing distortion and dilatation of tubules and deposition of few crystals. Few areas showed aggregates of non-specific inflammatory cells and haemmorhage. Standard drug cystone reversed the degenerative changes to normal. Group VI showed near normal histology except one area showing haemmorhage. Group IV and V also reversed the degenerative changes to a lesser extent (Fig. No: 1-6).

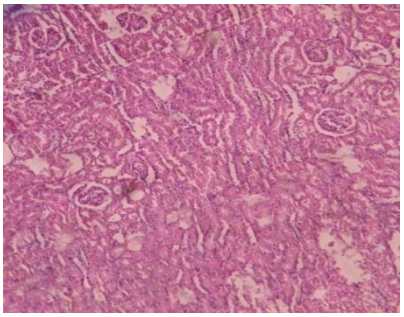


Fig. 1

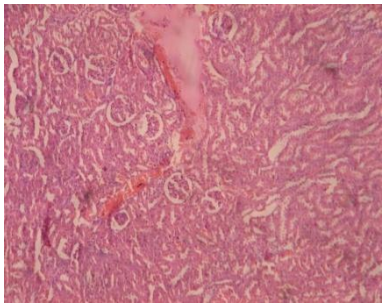


Fig. 2

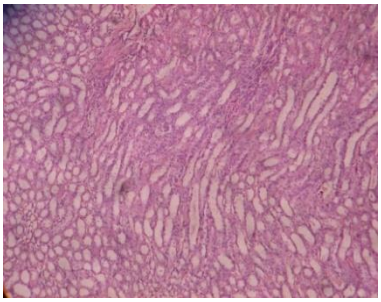


Fig. 3

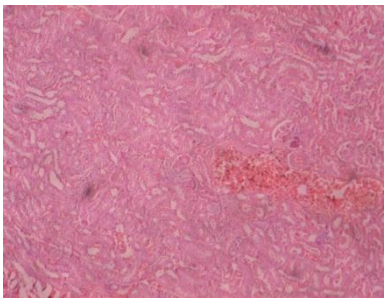


Fig. 4

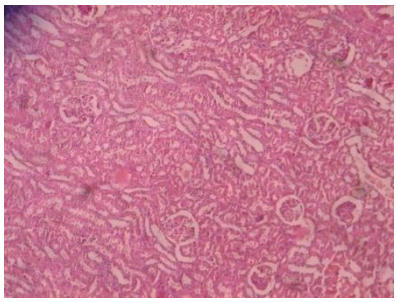


Fig. 5

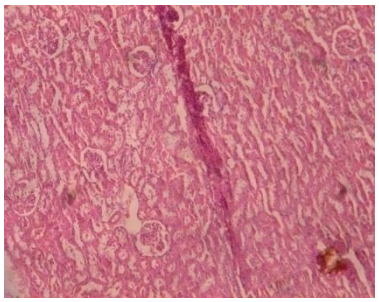


Fig. 6

Photomicrograph of Kidney Section of Group I (Normal Group-CMC), Group II (Disease Control), Group III (Standard Drug Cystone-750mg/kg), Group IV (Test Drug-100 mg/kg), Group V (Test Drug-200 mg/kg), Group VI (Test Drug-400 mg/kg) (Fig.1-6)

DISCUSSION

In the present study, hyperoxaluria was induced in rats by employing ethylene glycol (EG) and ammonium chloride (AC) in drinking water orally for 15 days. Studies indicate that oral administration of EG (a metabolic precursor of oxalate), induce oxalate lithiasis in rats by being converted to endogenous oxalic acid in the liver and AC when ingested, induce urinary acidification, thus favouring adhesion and retention of CaOx crystals within the renal tubules. Supersaturation of urine with CaOx, the most common component of kidney stones is an important factor in crystallization and enhanced urinary creatinine levels are indicators of renal impairment. In the present study, on EG and AC administration in the control rats, enhanced excretion and deposition of calcium oxalate indicated supersaturation of urine with CaOx as reported earlier**[13]**. Treatment with the ethanolic extract of *Portulaca oleracea* caused a significant reduction in the urinary excretion of calcium oxalate crystals (Groups IV, V, VI), thus reducing supersaturation of urine. The extract significantly lowered the calcium oxalate crystals in urine at higher dose i.e. (Group V and VI, Table No.1). This might be responsible for preventing the calcium oxalate type of stones.

Urinary excretion of calcium and creatinine was significantly (P<0.05) increased in disease control animals (Group II). On treatment with ethanolic extract for 15 days, a significant decrease in the urinary excretion of calcium and creatinine was observed in Group IV, V, and VI when compared to the disease control i.e. (Group II, Table 3). These results of the urinary excretion data clearly support that the ethanolic extract of the plant can reduce supersaturation of urine with calculogenic ions such as calcium and oxalate. Enhanced urinary creatinine indicates hyperoxaluria promoted renal impairment. The decrease in the elevated urinary creatinine in test groups (Group IV, V, and VI) reflects improved renal function.

The type of stone formed in human subjects can be predicted from the pH of the fasting urine. Crystalluria is pH dependent, thus by changing urinary pH, dissolution of calculi can be attained. Urinary pH of 5.0-6.5 promotes mostly CaOx type of stones **[16][14]**. In the present study, the decrease in urine pH from 8.0 - 8.5(Group I) to 6.0 - 6.2(Group II), supports the formation of CaOx type of stones. In the ethanolic extract administered rats, restored urinary pH (8.4 - 9.1), indicates prevention of CaOx stone formation which was evident through the marked decrease in urinary excretion of calcium and oxalate.

Diuretic action is also needed to increase the amount of fluid going through the kidneys and flush out the deposits. Increase in urine volume decreases the saturation of the salts and prevents the precipitation of the crystals at physiological pH **[3]**. In the treatment of kidney stones, plants are used as antilithics either to dissolve the stones or to aid their passing to guard against further retention. There are reports in the literature attributing the antilithiatic activity to the diuretic property of the plants. **[16]**

In the present study, urine volumes were significantly increased (P<0.05) by the ethanolic extract of the plant when compared to the disease control (Group II, Table 2). Thus, the antiurolithiatic activity of *Portulaca oleracea* may be due to its diuretic activity which is attributed to the presence of high percentage of potassium salts**[19]** and to the presence of flavonoids.**[20]**

Studies indicate that mucoproteins exhibit significant affinity for CaOx surface, thus promote the growth of crystals and cement them**[21]**. Saponins disintegrate the mucoproteins thereby prevent CaOx retention and deposition**[22]**. Saponins were reported to decrease CaOx crystal adhesion to renal epithelial cells by pre-coating the crystals **[23]**. In the present study, the antiurolithiatic effect of ETP may be attributed to its saponin principles as the extract was found to contain saponins in preliminary phytochemical screening.

In the present study, the saponins of *Portulaca oleracea* might have prevented the crystal adhesion to renal epithelial cells by pre-coating the crystals and by disintegrating mucoproteins. Polyphenols and flavonoids may have attenuated hyperoxaluria induced oxidative stress and subsequent CaOx stone formation by scavenging reactive oxygen species and metal chelation.**[13]**

In urolithiasis, the glomerular filtration rate (GFR) decreases, due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and BUN get accumulated in blood. In addition, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane. In calculi-induced rats (Group II, Table 4), the elevated serum levels of creatinine, urea, and BUN indicate marked renal damage **[11]** . On treatment with ethanolic extract of *Portulaca oleracea* the elevated serum levels of calcium, creatinine, urea, and BUN were significantly (P<0.05) reduced and comparable to those of cystone treated animals (Group II, Table 4).

The markedly elevated serum levels of BUN, creatinine, calcium and urea in stone-forming animals are indicative of prominent necrosis of renal epithelia. Elevated levels of oxalate in urine and even its retention in kidney may be one of the causative factors for the peroxidative degeneration of renal epithelia. 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 significant 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, reduced glutathione) leads to the development of Oxidative Stress (OS) followed by renal cell injury and inflammation due to lipid peroxidation. Loss of membrane integrity subsequently facilitates the retention of calcium oxalate crystals and growth of stones in renal tubules. Recent studies evidenced that treatment with anti-oxidants and free radical scavengers reduced CaOx crystal induced renal injuries**[6]**. Pre-treatment with vitamin E along with mannitol abolished the deposition of CaOx crystals in the kidneys of rats injected with sodium oxalate**[24]**. Alanine-induced deposition of CaOx crystals in rat kidneys was blocked by dietary supplementation with vitamin E plus selenium**[25]**. These antioxidant therapies restore the activity of antioxidant enzymes and free radical scavengers. Therefore, treatments with natural antioxidants and free radical scavengers, seems to be possible therapeutic strategy for ameliorating hyperoxaluria induced oxidative stress and renal cell injury in urolithiasis**[6]**.

The protective role of glutathione, as an antioxidant and detoxifying agent, has been demonstrated in various clinical studies. It is a ubiquitous compound that is synthesized rapidly in the liver, kidney and other tissues, including the gastrointestinal tract. In animal cells, glutathione acts as a substrate for glutathione peroxidase, which reduces lipid peroxides that are formed from polyunsaturated fatty acids (PUFA) in the diet and as a substrate for glutathione-S transferase, which conjugates electrophilic compounds. Many evidences showed that glutathione obtained from the diet is directly absorbed by the gastrointestinal tract and thus dietary glutathione can readily increase the antioxidant status in humans. However, treatment with the ethanolic extract of aerial parts of *Portulaca oleracea* prevents oxalate induced lipid peroxidation and causes regeneration of renal epithelium owing to its antioxidant potential. Decreased levels of urea, BUN and creatinine may be attributed to its antioxidant potential **[26]**. Purslane is also reported as an excellent source of the antioxidant vitamins α-tocopherol (vitamin E), ascorbic acid and β- carotene, as well as glutathione.

It has also been reported that *P.oleracea* exhibits analgesic and anti-inflammatory activity**[25]** and also antispasmodic activity**[27]** and hence can be considered for symptomatic treatment of renal stones.

The histopathological study supported these results. The kidney sections from disease control group which was treated with ethylene glycol and ammonium chloride showed extensive hypertrophy and large crystalline deposits in most parts of the kidney but those from treated rats were apparently of near normal architecture with very few crystals and deposits. The effect was more pronounced in higher dose group (Group VI).

The preliminary qualitative phytochemical study of *Portulaca oleracea* showed presence of phytochemical constituents viz. alkaloids, flavonoids, glycosides, saponins, tannins and phenolic compounds, steroids and also proteins and free amino acids. A detailed and quantitative estimation of its phytochemical constituents will be helpful in establishing some co-relation with the pharmacological activity observed in the present study.

CONCLUSION

The Present study was conducted to evaluate the antiurolithiatic activity of ethanolic extract of aerial parts of ***Portulaca oleracea* Linn.** The extract prevented the formation of stones supporting folklore information. The antiurolithiatic activity of the plant may probably be due to synergism of diuretic activity, crystallization inhibition, improved renal function along with antioxidant activity (reported earlier). Histological findings also supported the study. The antiurolithiatic activity may be apparently due to flavonoids, saponins and phenolic compounds present in aerial part of plant. Further work is necessary to isolate the active constituents responsible for the antiurolithiatic activity and studies on larger animal models and on humans is warranted to draw final conclusion.

REFERENCES

1. Deepak Verma, Pendse A.K., Singh P.P. Hyperoxaluria in Urolithiasis and Cystone Therapy, The Antiseptic (1989):86(5), 257.
2. Veronika Butterweck, Saeed R. Khan. Herbal Medicines in the Management of Urolithiasis: Alternative or Complementary? Planta Med 2009; 75:1095-1103.
3. Surendra K. Pareta, Kartik C. Patra, Papiya M. Mazumder and Dinakar Sasmal. Establishing the Principle of Herbal Therapy for Antiurolithiatic Activity: A Review. Journal of Pharmacology and Toxicology. 6:321-332.
4. Rahul Deo Yadav, S. K. Jain, Shashi Alok, Alok Mahor, Jay Prakash Bharti and Manoj Jaiswal. Herbal Plants Used in the Treatment of Urolithiasis: A Review. IJPSR, 2011; Vol. 2(6): 1412-1420.
5. D Sudhakar, R Krishna Kishore and P R Parthasarthy. *Portulaca oleracea* L. extract ameliorates the Cisplatin-induced toxicity in chick embryonic liver. Indian Journal of Biochemistry & Biophysics. Vol. 47, June 2010, pp.185 – 189.
6. Anthony C. Dweck FLS FRSC FRSH. Purslane (*Portulaca oleracea*) - the global panacea, Consultant, Dweck Data, Personal Care Magazine 2, 4, p.7-15. (2001).
7. Gholamreza Karimi, Alireza Khoei, Abbas Omidi, Mahmudreza Kalantari, Javad Babaei, Elahe Taghiabadi, Bibi Marjan Razavi. Protective Effect of Aqueous and Ethanolic Extracts of

Portulaca oleracea against Cisplatin induced Nephrotoxicity. Iranian Journal of Basic Medical Sciences, Vol. 13, No. 2, Spring 2010, 31-35 Received: Sep 6, 2009; Accepted: Dec 6, 2009.

8. Nadkarni, K.M., Nadkarni, A.K. Indian Materia Medica- With Ayurvedic, Unani, Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies. Vol 1, 1999.Popular Prakashan Private Ltd., Bombay, India.
9. Gholamreza Karimi, Hossein Hosseinzadeh, Negin Ettehad.Evaluation of the gastric Antiulcerogenic Effects of *Portulaca oleracea* L. Extracts in Mice. Phytotherapy Research, Volume 18, Issue 6, pages 484–487, June 2004.
10. Vunta Prabhakaran, Bagepalli Srinivas Ashok Kumar, Devangam Sheshadri Shekar, Rudrappa Nandeesh, Peta Subramanyam, Divati Ranganayakulu. Evaluation of the Hepatoprotective Activity of *Portulaca oleracea* L. on D-galactosamine-induced Hepatic Injury in Rats. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas, 9 (3), 2010, pp. 199-205.
11. Yogendr Bahuguna, Mohan Singh Maniyari Rawat, Vijay Juyal, Vikas Gupta. Antilithiatic Effect of Flowers of *Jasminum auriculatum* Vahl. International Journal of Green Pharmacy 2009; 3:155-8.
12. N. I. Khan, J. S. Shinge, N. S. Naikwade. Antilithiatic Effect of *Helianthus Annuus* Linn. Leaf Extract in Ethylene Glycol and Ammonium Chloride Induced Nephrolithiasis. International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 2, Suppl. 4, 2010.
13. B. Sailaja, K. Bharathi and KVSRG Prasad. Protective Effect of *Tridax procumbens* L. on Calcium Oxalate Urolithiasis and Oxidative Stress. Pharmanest - An International Journal of Advances in Pharmaceutical Sciences, Vol. 2 (1) January - February 2011.
14. Gilhotra Umesh Kr., Christina A.J.M. Effect of *Rotula aquatica* Lour. on Ethylene Glycol induced Urolithiasis in Rats. International Journal of Drug Development & Research Jan-March 2011, Vol. 3, Issue 1.
15. Elias Edwin Jarald, Pankaj Kushwa, Sheeja Edwin, Suhail Asghar, Shoukat Ahmad Patni. Effect of *Unex* on Ethylene Glycol induced Urolithiasis in Rats. Indian Journal of Pharmacology, August 2011, Vol. 43, Issue 4.
16. Sujatha Dodoala, Ranganayakulu Diviti, Bharathi Koganti and K V S R G Prasad. Effect of Ethanolic Extract of *Phyla nodiflora* (Linn.) Greene against Calculi producing diet induced Urolithiasis. Indian Journal of Natural Products and Resources, Vol. 1(3), September 2010; pp.314-321.
17. Touhami M, Laroubi A, Elhabazi K, Loubna F, Zrara I, Eljahiri Y, Oussama A, Grases F, Chait A. Effect of Oral Lemon Juice Administration on Calcium Oxalate Urolithiasis. BMC Urol. 2007 Oct 5:7(1): 18.
18. Purnima Ashok, Basavaraj C Koti, A.H.M. Vishwanathswamy. Antiurolithiatic and Antioxidant Activity of *Mimosups elengi* on Ethylene Glycol Induced Urolithiasis in Rats. Indian Journal of Pharmacology 2010; 42:380-3.
19. Alok Sharma, G.D. Reddy, M.Vijayakumar, M.K. Unnikrishnan and Ch.V. Rao. Action of *Portulaca oleracea* against Streptozocin-induced Oxidative Stress in Experimental Diabetic Rats. Continental J. Pharmacology and Toxicology Research 2: 12 - 18, 2008.
20. K.Y. Musa, A. Ahmed, G. Ibrahim, O.E. Ojonugwa, M. Bisalla, H. Musa, U.H. Danmalam. Toxicity Studies on the Methanolic Extract of *Portulaca oleracea* Linn. (Fam. Portulacaceae). Journal of Biological Sciences 7(7):1293-1295, 2007.
21. Leal JJ and Finlayson B. Adsorption of naturally occurring polymer on to calcium oxalate crystal surfaces. Investigational Urology. 1977; 14:278.
22. Grases F, March JG, Ramis M, Costa-Bauza A. The influence of *Zea mays* on urinary risk factors for kidney stones in rats. Phytotherapy Research. 1993;7:146-
23. Atmani F, Farell G, Lieske JC. Extract from *Hernaria hirsuta* coats calcium oxalate monohydrate crystals and blocks their adhesion to renal epithelial cells. Journal of Urology. 2004; 172:1510-514.
24. Mohamed A. Dkhil, Ahmed E. Abdel Moniem, Saleh Al-Quraishy and Reda Awadallah Saleh. Antioxidant Effect of Purslane (*Portulaca oleracea*) and its Mechanism of Action. Journal of Medicinal Plants Research Vol. 5(9), pp. 1589-1563, 4 May, 2011.
25. Chan, K. Islam, M. W., Kamil, M. Radhakrishnan, R. Zakaria, M.N.M. Habibullah, M. Attas, A. The Analgesic and Anti-inflammatory Effects of *Portulaca oleracea* L. Celak. Journal of Ethnopharmacology. 73, 445-451(2000).
26. Ivo Oliveira, Patrícia Valentão, Rosario Lopes, Paula B. Andrade, Albino Bento, José Alberto Pereira. Phytochemical Characterization and Radical Scavenging Activity of *Portulaca oleracea* L. Leaves and Stems. Micro chemical Journal 92 (2009) 129–134.
27. Kumar S. and R. Selvam, 2003. Supplementation of Vitamin E and Selenium Prevents Hyperoxaluria in Experimental Urolithic Rats. Journal of Nutrition and Biochemistry; 14:306-313.