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Research Article

ANTIUROLITHIATIC ACTIVITY OF SOME TRADITIONAL MEDICINAL PLANTS AGAINST CALCIUM OXALATE INDUCED UROLITHIASIS IN RATS

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ABSTRACT

The present study was undertaken to investigate the effects of some traditional medicinal plants i.e *Plectranthus mollis Spreng, Didymocarpus pedicellata, Teraxacum officinale, Dendrophthoe elastic desr* on experimentally-induced kidney stones. Oxalate urolithiasis in male rats was induced experimentally by administration of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water for three days followed by only 0.75% v/v ethylene glycol for 25 days. The petroleum ether extract of *Plectranthus mollis Spreng* (PM), hydroalcoholic extract of *Didymocarpus pedicellata* (DP), ethyl acetate extract of *Taraxacum officinale* (EA), methanolic extract of *Dendrophthoe elastic desr* (DE) and, hydroalcoholic extract of *Citrus medica Linn* (CM) were administered to urolithiasis induced test group rats at two doses i.e. 100 and 200 mg/kg respectively for 28 days. After 28 days, highly significant deposition of calcium oxalate in the kidneys was noticed along with increase in the urine volume, urinary oxalate, calcium, levels and magnesium levels in urolithiasis control group rats as compared to normal group rats. The serum analysis showed significant increase in the serum uric acid, serum creatinine, and blood urea in urolithiasis control group rats. In addition, vehicle treated induction control group rats showed significant increase in the biochemical parameters such as ACP, ALP, AST, ALT levels and decrease in LDH levels in the kidney homogenate which indicated the induction of urolithiasis. Daily oral treatment with all most all extracts not significantly decreased the quantity of calcium oxalate deposited in the kidneys but also reverted all the biochemical changes induced by calcium oxalate urolithiasis thus supporting its traditional claim. Petroleum ether extract of *Plectranthus mollis Spreng* however, was found to be insignificant in these regards.

Keywords: Calcium oxalate, ethylene glycol, urolithiasis, traditional medicine

INTRODUCTION

Urolithiasis is the formation of stones in the urinary tract that prominently cause variable degree of pain, bleeding, and further may lead to secondary infection. It is one of the third most common afflictions found in humans[1]. The size and nature of crystals governs overall clinical manifestations of this complaint whereas urinary chemistry is one of the important factors in determining the type of crystals formed and the nature of macromolecules included on the surface of the crystals. Calcium oxalate stones make up the majority as they account for 70-80% of all kidney stones. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common[2].

Various therapies including thiazide diuretics and alkali-citrate are being used in attempt to prevent recurrence but scientific evidence for their efficacy is less convincing 3. In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful

though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects[3].

As per the indigenous system of medicine, the plants *Plectranthus mollis Spreng* (PM), *Didymocarpus pedicellata* (DP), *Taraxacum officinale* (EA), *Dendrophthoe elastic desr* (DE) and *Citrus medica Linn* have been taditionaly claimed as well as scientifically documented for their various pharmacological activities revealing their usefulness various diseases and disorders.

Out of those *Plectranthus mollis* (Lamiaceae) is used in India as a respiratory stimulant, vasoconstrictor and a cardiac depressant[4]. It is also recognised as a febrifuge and useful in rheumatism[4,5]. *Plectranthus is* used to treat various blood conditions include *Plectranthus mollis* as a cure for haemorrahage[4]. In Asia *Plectranthus mollis* is used for the treatment of mental retardation[6]. It is also claimed to be useful in snakebites and mental retardation. It is widely accepted as a general tonic in major part of Indian subcontinent[7]. The leaves of *Plectranthus mollis* are cooked as a vegetable[8]. The seeds of *Plectranthus mollis* are fried in mustard oil and then massaged all over the body as an insect repellant[7]. *Plectranthus mollis* has cytotoxic and anti-tumour promoting activity and can be used in the treatment of cancer. *Plectranthus mollis* is also reported to relax smooth as well as skeletal muscles[4].

Didymocarpus pedicellata (Gesneriaceae) is widely used in variety of renal afflictions. It is considered to be of great value in the management of kidney and bladder stones[9]. According to a hypothesis this effect is due to regulation of calcium absorption in the body coupled with its diuretic effect and in maintaining healthy urinary tract. Used for centuries for natural kidney and bladder support, the leaves of DM contain an essential oil whose chief constituent, didymocarpene, is used in indigenous healthcare systems for its well-rounded urinary tract support. It has also been documented for protective activity on ferric nitrilotriacetate (Fe-NTA) induced renal oxidative stress and hyperproliferative response[10].

Taraxacum officinale (Asteraceae), the Common Dandelion flowers are used to make honey substitute syrup with added lemon (so-called May-honey). This "honey" is believed to have a medicinal value, in particular against liver complaints. A hepatoprotective effect of chemicals extracted from dandelion root has been reported. Drunk before meals, dandelion root coffee is claimed to stimulate digestive functions and function as a liver tonic. "Dandelion and Burdock" is a soft drink that has long been popular in the United Kingdom with authentic recipes sold by health food shops. The milky latex has been used as a mosquito repellent; the milk has also been used to treat warts, as a folk remedy[11].

The hemi-parasitic plant *Dendrophthoe elastic desr* Ettingsh (Loranthaceae) of the order Santalales, is used ethnomedicinally for treating ulcers, asthma, impotence, paralysis, skin diseases, and wounds. The aerial parts are also used in menstrual troubles, psychic disorders, pulmonary tuberculosis, consumption and mania by the tribal of India. Leaf paste is used in skin diseases. Its paste is applied on boils, setting dislocated bones and extracting pus. The plant has been scientifically proved to have antilithiatic, diuretic, cytotoxic and immunomodulatory activities. The whole plant is used in indigenous system of medicine as cooling agent, astringent, aphrodisiac, narcotic and diuretic[12] and is useful in treating pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesical calculi and vitiated conditions of kapha and pitta[13-15]. The decoction of plant used by women as an anti-fertility agent has been evidenced to possess anticancer activity[16]. The leaf ethanolic extract significantly and dose dependently inhibits the acetic acid induced writhing in mice[17] and has indicated a low level toxicity in the brine shrimp lethality assays. Besides, a more recent work by Pattanayak et al. (2008)[15] shows significant tumor reduction in induced mammary carcinogenesis in Wistar female rats when fed with hydroalcoholic extracts of *Dendrophthoe elastic desr*. It possesses remarkable potentials as a medicinal plant evident from the wound healing, anti-microbial, anti-oxidant, antinociceptive properties of its ethanolic extracts[15,17]. Medicinal properties of this hemiparasite may vary in effects respective to different hosts it establishes a relation with. Its ethanolic extract has shown potent antioxidant activity by inhibiting lipid peroxidation, reduced glutathione, superoxide dismutase levels and increased the catalase activity along with significant wound healing properties[18].

Citrus medica Linn (Rutaceae) possess properties like astringent, narcotic, bitter, diuretic and used in the treatment of tuberculosis, asthma, mania. It is used in menstrual disorder, wounds, in prevention of stone in kidney & bladder, hemorrhage,

miscarriage and abortion during pregnancy[19]. Used as an anti-ulcer, anthelmintic, blood purifier. It is also used in fetus development and blood pressure management[20,21]. It is used as substitute for betel-nut. Plant grown on *Ficus fistul*a host is used for foetus development in Ayurveda. It is used in Vatta, Kapha and Pitta. This plant is used for avoiding abortion occurs during 3rd month of pregnancy[19]. Its flowers are used medicinally by the Chinese. The candied peel is sold in China as stomachic, stimulant, expectorant and a tonic. In West Tropical Africa, the citron is used only as a medicine, particularly against rheumatism. Emmanuel *et al.* reported that *C. medica* essential oil showed fungitoxicity against some fungi. *C. medica* is relevant to treatment of diabetes and alzheimer's disease[22].

Even though these plants have been documented for different pharmacological activities, their effect on urolithiasis has yet not been explored. In light of this the present study was carried out to evaluate their effects of these extracts on calcium oxalate induced urolithiasis.

MATERIALS AND METHODS

Plant material

The plants *Plectranthus mollis Spreng, Didymocarpus pedicellata, Teraxacum officinale, Dendrophthoe elastic desr* and *Citrus medica* were purchased from local vendors and were identified and authenticated from National institute of science communication and information sources (NISCAIR). Certification No: NISCAIR/RHMD/Consult/08-09/1052/83/06.

Preparation of Extracts

The petroleum ether extract of *Plectranthus mollis Spreng* (PM), hydroalcoholic extract of *Didymocarpus pedicellata* (DP), ethyl acetate extract of *Taraxacum officinale* (EA), methanolic extract of *Dendrophthoe elastic desr* (DE) and, hydroalcoholic extract of *Citrus medica Linn* (CM) were prepared using standard procedures. All the extracts were prepared from whole plants.

Chemicals and apparatus

Ethylene glycol was obtained from Merck Ltd., Mumbai, India. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers. Apparatus such as the metabolic cages (Tecniplast, Italy), semiautoanalyzer (Metrolab, 1600-DR), cold centrifuge (Remi Instruments, C-30BL), UV-spectrometer (Shimadzu Scientific Instruments, UV-3600) were used in the study.

Animals

Male Wistar albino rats (120-150gm) were used fo this experiment. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 hr. light 12 hr. dark cycle). The animals had free access to food (Chakan Oil Mills, Pune, India) and water ad libitum. Institutional Animal Ethical Committee (IAEC) approved the protocol. All experiments were carried out between 12:00- 16:00 hour.

Antiurolithiatic activity of extracts

Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce calcium oxalate urolithiasis[23]. Seventy two animals were divided into twelve groups with six animals in each group. Group I served as normal group and received 1ml/kg distilled water.

All the remaining groups received calculi-inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water *ad libitum* for three days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for 25 days. Group II served as induction control group and received distilled water 1ml/kg. Groups III to XII served as test groups received the extracts at doses 100 and 300 mg/kg respectively from first day to 28th day of calculi induction.

Collection and analysis of urine

On the 28th day of calculi induction treatment, all animals were kept in individual metabolic cages and urine samples of 24 h were collected. The collected urine samples were measured for following parameters.

Urine volume

Animals were placed in separate metabolic cages for 24 h and total urinary volume was measured using the measuring cylinder and reported in ml.

Urine pH

Uric acid crystals were found to deposit most frequently in the concentrated acid urine. Thus, the acidity of the urine was tested using the pH meter.

Urinary oxalate

The 1 ml of urine was acidified beforehand by concentrated HNO3 to solubilize crystals and then adjusted to pH 7 by NaOH in the presence of color indicator, the bromothymol blue. About 2ml of saturated CaSO4 and 14 ml of pure ethanol were added to precipitate oxalate overnight. The samples were centrifuged at 450

× *g* for 10 min and then filtered on filter paper. The precipitate obtained was solubilized in 10 ml of water acidified by 2ml of concentrated sulfuric acid. The samples were titrated by a solution of KMnO4.

Urine calcium
It was estimated by using commercially available standard kit of Biolab diagnostics Pvt. Ltd. Tarapur (India) as per o-cresolphthalein complexone method. Determination of urine calcium was done by using CHARIOT prince autoanalyser.

Urine magnesium
It was estimated by using commercially available standard kit supplied by Biolab diagnostics Pvt. Ltd. Tarapur (India) as per Calmagite method. Determination of urine magnesium was done by using CHARIOT prince autoanalyser.

Collection and Serum analysis
After urine collection period, blood was obtained from the retro-orbital under anaesthetic condition and animals were sacrificed by cervical decapitation. Serum were separated by centrifugation and analyzed.

Serum uric acid
It was estimated by using commercially available standard kit of Biolab diagnostics Pvt. Ltd. Tarapur (India) as per Colorimetric enzymatic method and analysed by CHARIOT prince autoanalyser.

Serum creatinine
It was estimated by using commercially available standard kit as per Urease/salisylate method. Determination of serum creatinine was done by using CHARIOT prince biochemistry autoanalyser.

Blood urea
It was estimated by using commercially available standard diagnostic kit of Biolab Diagnostics-India using diacetymonoxime colorimetric end-point method. Determination of Blood urea was done by using CHARIOT prince biochemistry autoanalyzer.

Kidney homogenate analysis
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 left kidney was finely minced and 10 % homogenate was prepared in Tris-Hcl buffer (0.02 mol/l, pH 7.4). The homogenate was used for measurement of various biochemical parameters.

Estimation of biochemical markers
The homogenate was used to assay the marker enzymes in serum, urine and tissue constituents like ACP, Alkaline phosphate (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) were estimated respectively using different types of enzyme marker kits.

STATISTICAL ANALYSIS
Data expressed as Mean ± S.E.M. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test and $p<0.05$ considered as statistical significant.

RESULTS
Calcium oxalate induced urolithiasis
Collection and analysis of urine
The oxalate induced urolithiasis produced severe alterations in the urinary parameters as compared to normal control group. Increase in the urinary volume, urinary oxalate and calcium levels and decrease in the urinary magnesium levels were observed. These alterations were significantly attenuated in the extract treated groups. All most all the extracts were significant in reverting these urinary alterations and more significant at higher doses than the lower ones. Group III was found to be insignificant in this concern.

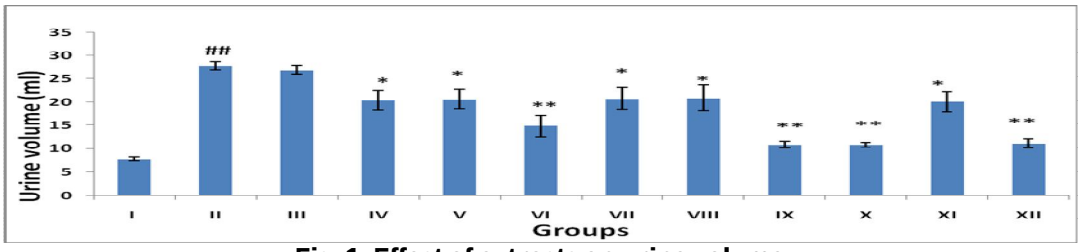

Fig. 1: Effect of extracts on urine volume

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

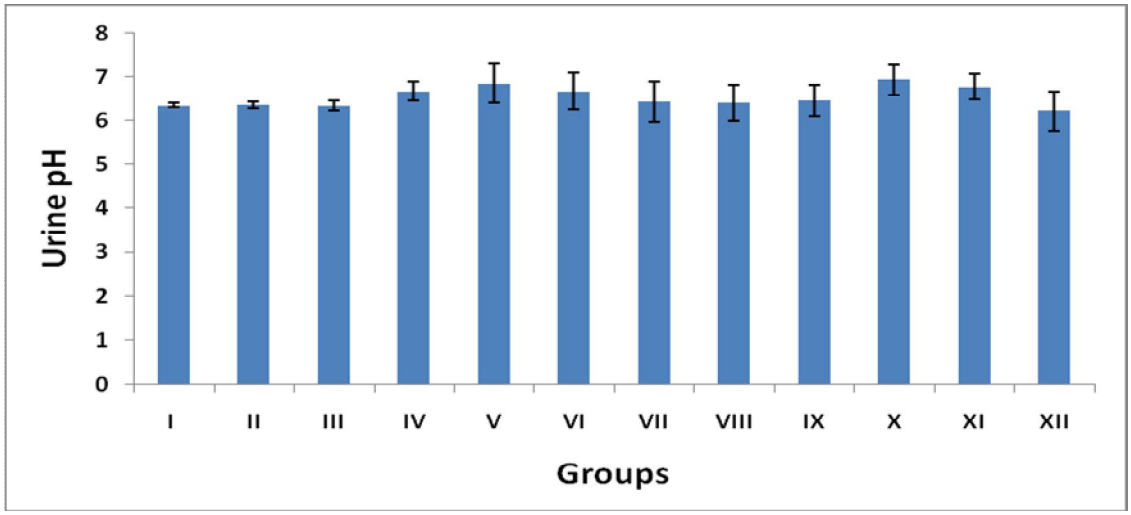

Fig. 2: Effect of extracts on urine pH

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

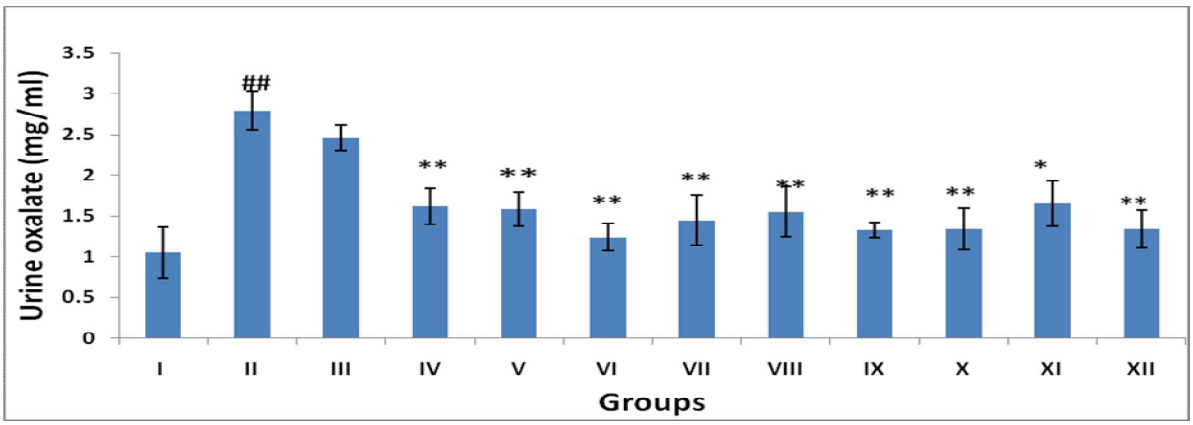

Fig. 3: Effect of extracts on urine oxalate

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

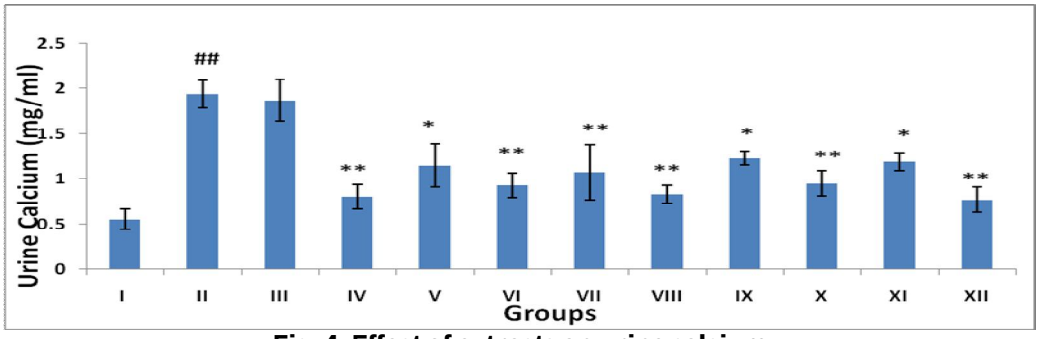

Fig. 4: Effect of extracts on urine calcium

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

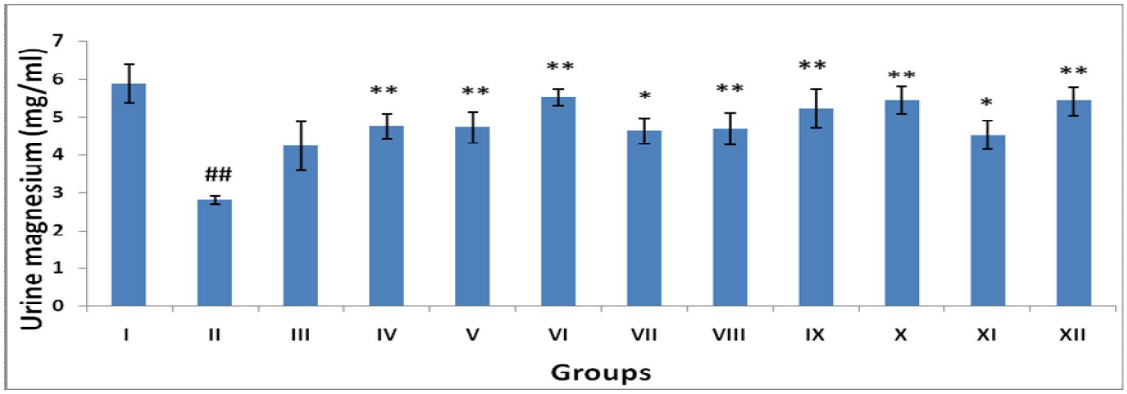

Fig. 5: Effect of extracts on urine magnesium

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

Collection and Serum analysis

The induction control group showed significant increase in the serum uric acid, serum creatinine and blood urea levels as compared to normal control group which were significantly and dose dependently decreased by all most all the extracts.

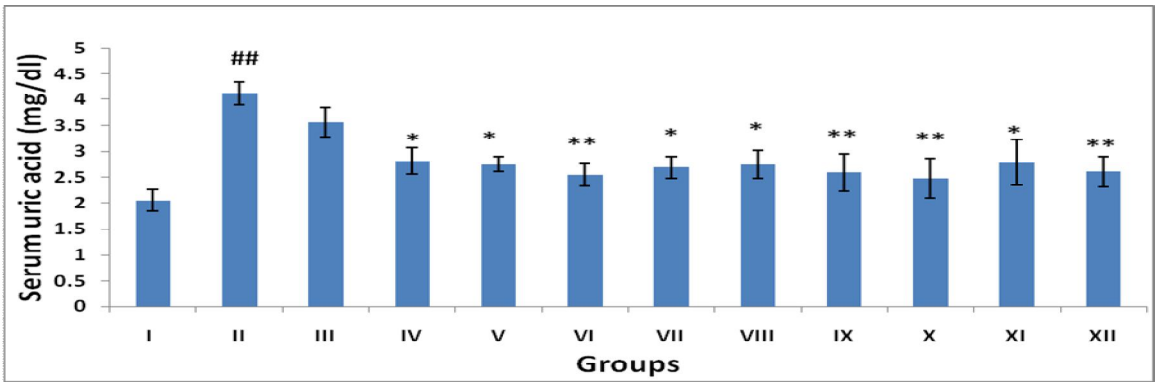

Fig. 6: Effect of extracts on serum uric acid

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

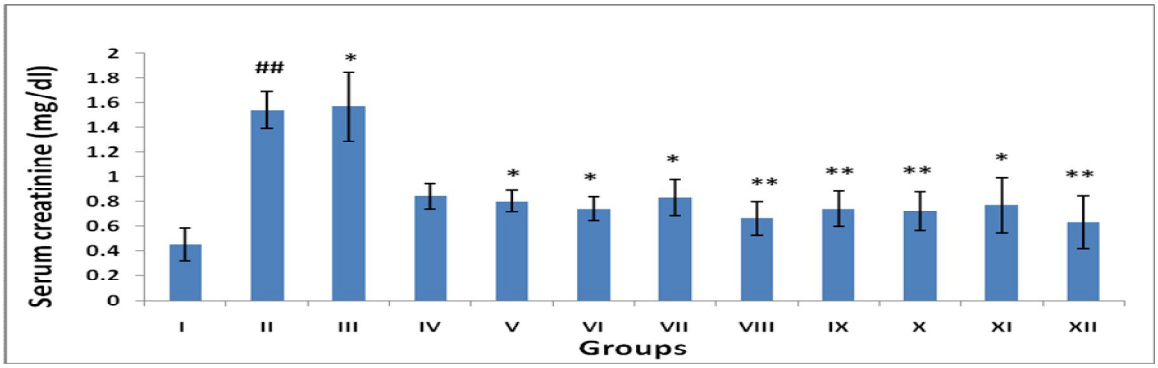

Fig. 7: Effect of extracts on serum creatinine

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

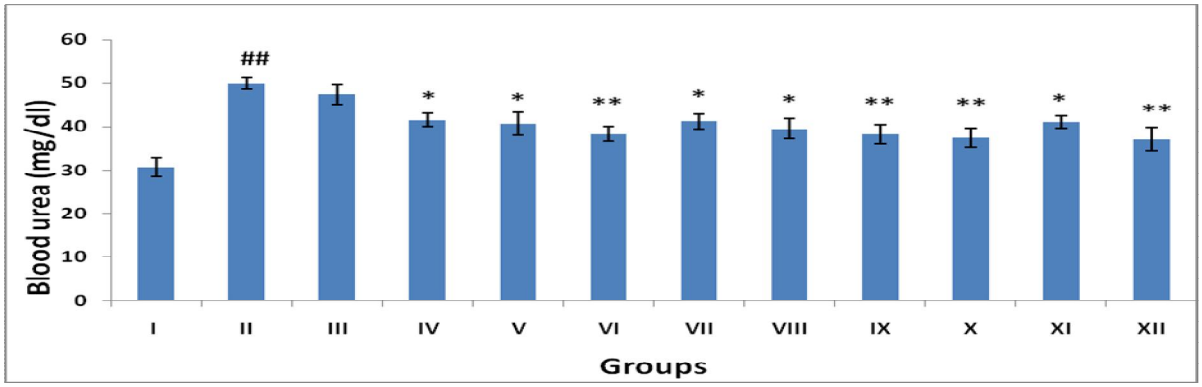

Fig. 8: Effect of extracts on blood urea

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

Kidney homogenate analysis

The oxalate induced urolithiasis showed significant increase in the biochemical parameters such as ACP, ALP, AST, ALT levels and decrease in LDH levels in the kidney homogenate of the induction control group as compared to normal control group. These alterations were significantly and dose dependanty reversed in the extract treated group animals. In overall study group III was found to be insignificant in reverting the alterations.

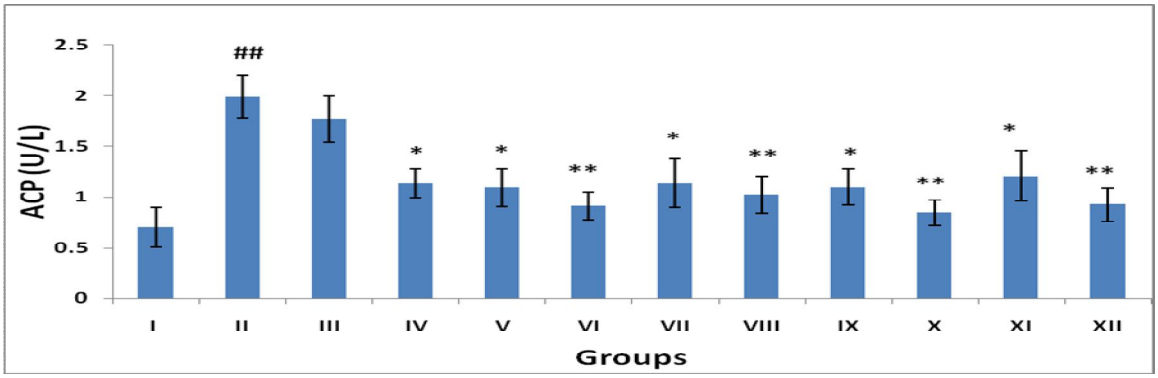

Fig. 9: Effect of extracts on ACP levels of kidney

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

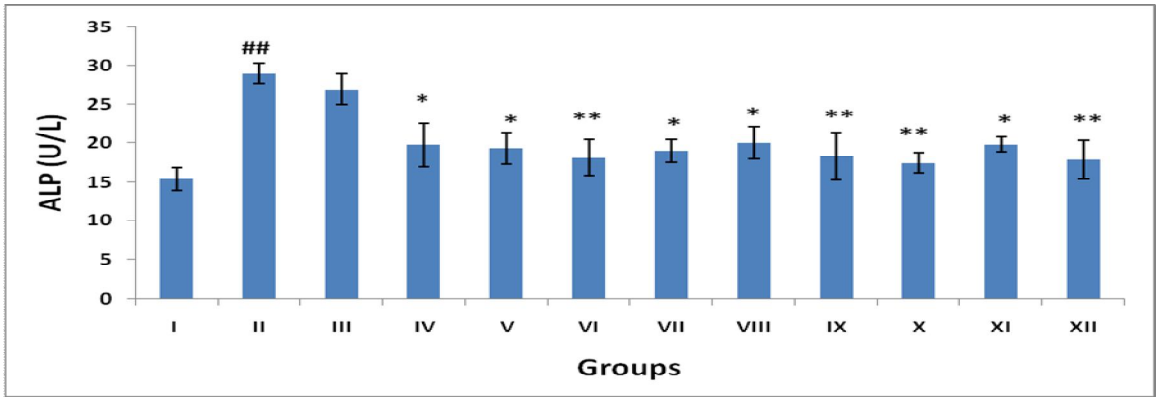

Fig. 10: Effect of extracts on ALP levels of kidney

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

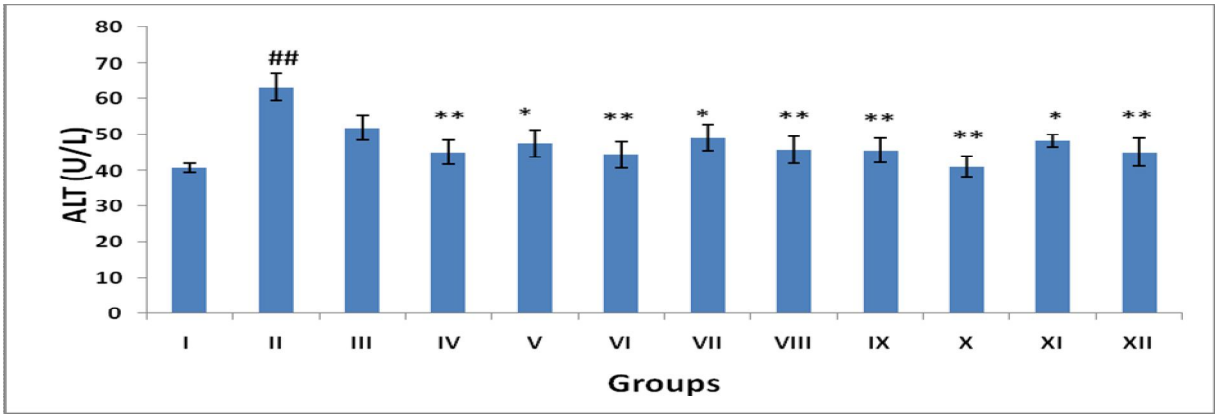

Fig. 11: Effect of extracts on ALT levels of kidney

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

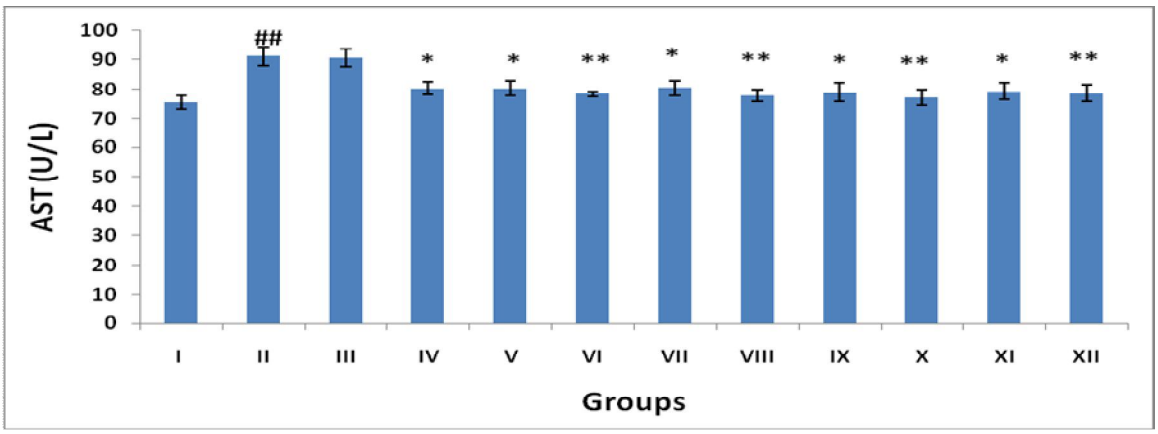

Fig. 12: Effect of extracts on AST levels of kidney

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

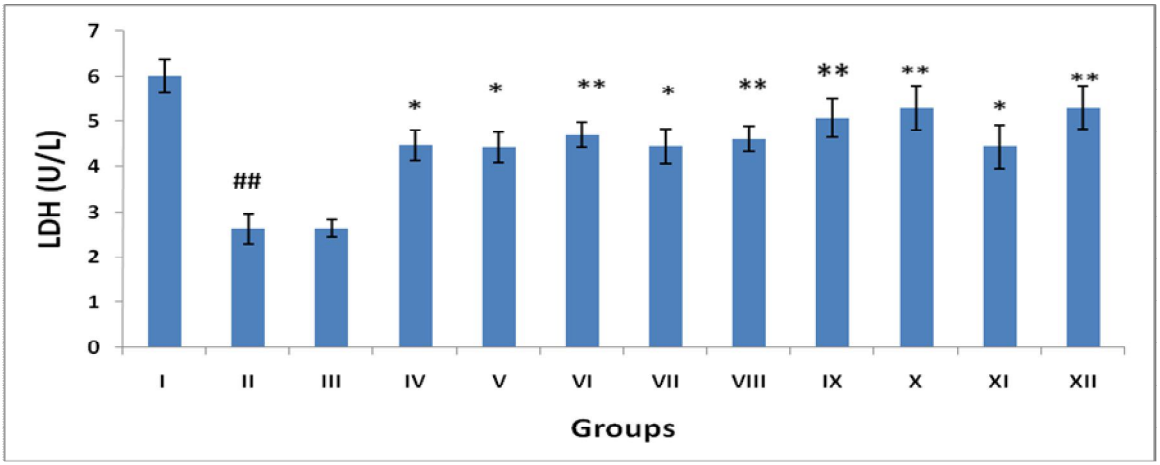

Fig. 13: Effect of extracts on LDH levels of kidney

Results are expressed as mean ± SEM. (n = 6). Data was analysed by comparing Group I with Group II using Student T test and comparing Group II with remaining groups using one way analysis of variance (ANOVA) followed by Dunnetts test. ##P<0.01, *P<0.05, **P<0.01.

DISCUSSION

Epidemiological data have shown that calcium oxalate (CaOx) is the predominant mineral in the majority of cases of urolithiasis and accounts for greater than 80% cases[24-26]. Hence it is advisable to screen initially, kidney stones often do not cause any symptoms and usually, the first symptom of a kidney stone is renal colic, which occurs when a stone acutely blocks the flow of urine. The pain often begins suddenly when a stone moves in the urinary tract, causing irritation or blockage. If the stone is too large and then moves, blood may appear in the urine[27-29].

Hence in the present study, all the extracts were screened using calcium oxalate (CaOx) induced urolithiasis using experimental models of calcium oxalate urolithiasis, ethylene glycol (EG) induced urolithiasis which are highly recommended models[30-33]. Administration of ethylene glycol, a metabolic precursor of oxalate to rats results in hyperoxaluria, CaOx crystalluria followed by deposition of CaOx crystals in the kidney. However, variable crystal deposition rates have been reported with EG[34-38].

In this study, oxalate induced urolithiasis produced severe alterations in the urinary parameters. Increase in the urine volume, urinary oxalate, calcium, levels and magnesium levels were observed. These alterations were significantly attenuated with the extract treatments. All the extracts were significant except *Plectranthus mollis* in reverting these urinary alterations and this effect was dose dependant suggesting potential antiurolithiatic activity. In serum analysis, the vehicle treated induction control group showed significant increase in the serum uric acid, serum creatinine and blood urea levels as which were in accordance with the previous reports which were significantly and dose dependently decreased by all the extracts except *Plectranthus mollis* extract. In addition, vehicle treated induction control group showed significant increase in the biochemical parameters such as ACP, ALP, AST, ALT levels and decrease in LDH levels in the kidney homogenate which indicated the induction of urolithiasis. These changes were significantly and dose dependently reversed in all the extract treated animals indicating significant antiurolithiatic activity. *Plectranthus mollis* extract was found to be insignificant in reverting the alterations.

CONCLUSION

In the present study all the plants except *Plectranthus mollis* exhibited significant antiurolithiactic activity. Further analysis and fractionation of these extracts is needed to predict the phytochemical constituents responsible for the antiurolithiatic activity.

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