EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 1
192-199
Year: 2024

EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF WHOLE PLANT OF OCIMUM BASILICUM AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS

S. Dada Khalandar*, Dr. Shainaz Begum and Dr. P. Dharani

[1]Assistant Professor Department of Pharmacology Dr. Gurachar Nargund College of Pharmacy, Muradi.
[2]Assistant Professor Department of Swasthavritta TMAE'S AMC Hospete.
[3]Associate Professor Department of Pharmacology Krishna Teja College of Pharmacy, Tirupati.

***Corresponding Author: Irshad Ahmad Mohd. Salim**
HOD of Pharmaceutics New Montfort Institute of Pharmacy Ashti, Wardha.

Article Received on 10/11/2023 **Article Revised on 01/12/2023** **Article Accepted on 22/12/2023**

ABSTRACT
The Urolithiatic activity may induced by chemical induced model: Ethylene glycol (EG) (0.75% v/v), and ammonium chloride(1% w/v) may leads to hyperoxaluria in male wister rats. Several parameters were used including urinary volume, urine pH, serum calcium, uric acid, urea and creatinine to assess lithiatic activity. Oral administration of OBEE 200 and 400mg/kg could significantly inhibited Ca oxalate crystal disposition in renal tubules and protected against changes in polyurea and kidney weight loss. Stone forming promoters like oxalate, calcium, phosphate, uric acid and urea were increased in disease control group compare to normal control group rats. Deposition of crystalline components like calcium, phosphate and uric acid were increased in renal tissue compare to normal control group rats. Treatment with OBEE SOD, CAT levels were increased compare to disease control group.

KEYWORDS: Ocimum basilicum, Urolithiasis, Ethylene glycol, Ammonium chloride, Antiurolithiatic, Ethanolic extracts.

INRODUCTION
Urolithiasis or nephrolithiasis are the oldest and wide spread painful urological disorders. It is the third most prevalent disorder in urinary system.

Urinary stones affect 10–12% of the population in industrialized countries. It has a recurrence rate of ~ 50%, hence it has an important effect on the healthcare system.[1]

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (WHO, Document No.A44/20, 1991).

Plants have the ability to synthesize a wide variety of effectively to treat human diseases.[2,3] India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. Of these, about 15000-20000 plants have good medicinal value.[4]

Herbal medicine is still the mainstay of about 75–80% of the world's population, for primary health care. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethno medical" plant sources, 80% of these have an ethno medical use identical or related to the current use of the active elements of the plant.[5]

Allopathic drugs for the prevention of stone formation have not been very effective and their adverse effects have put certain limits on their use. However, recent studies have shed light on the fact that apart from the high cost of lithotripsy, exposure to shockwaves even in therapeutic doses is associated with several adverse effects, such as renal injury and decrease in renal function. The chances of stone recurrence tend to increase as a direct consequence chemical compounds that are used to perform important biological functions, and can be used of such a form of treatment. Hence the search of new herbal medicines is still going on.[6]

All plants have been used in the treatment of urolithiasis and they have been rearge number of Indian medicinal ported to be effective with fewer side effects. Even

today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological in vitro and in vivo investigations on the medicinal plants used in traditional antiurolithiatic therapy revealed their therapeutic potential.[7]

The standardized, natural, safe, effective and inexpensive herbal preparations for the treatment of urolithiasis would certainly provide a ray of hope for the people suffering from this life-threatening disease.[8] Now, I choose the drug of cystone 750 mg/kg b.w.

MATERIAL AND METHODS

Plant material and extraction procedure

The whole plant of *Ocimumbasilicumlinn.* Collected from local areas of Tirupati in Chittoor district of AP, India, during the month of January. The whole plant of *OcimumbasilicumL*. (Lamiaceae) was authentified by DR. K. Madhavachetty, Asst professor. Department of Botany, Sri Venkateswara University, Tirupati. About 300 g of powder was subjected to solvent extraction using 70% hydro alcoholic solvent (ethanol and distilled water) in the ratio of 1:10 (drug: solvent) in Round bottom flask at room temperature for 3 days and the solvent was removed at 40°C usinga rotary evaporator.

Phytochemical analysis[9]

The extract was screened for various constituent such as alkaloids, glycosides, carbohydrates, proteins & amino acids, flavonoids, steroids & triterpenoids, tannins, phenols, saponins.

Animals

Healthy male adult Wistar rats (150-200) were procured from Ragavendra Enterprises, Bangalore, India. The animals were housed in clean and transparent polypropylene cages and maintained at 25^{o} c with 12/12 hr light-dark cycle, after 7 days of acclimatization period, they were randomized in different experimental groups. They were fed standard rat chow and water ad libitum. All the experimental procedure was carried out in accordance with committee, (KTPC/PG/IAEC/2018/07) for the purpose of control and supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Studies (OECD, 2002)[10]

The procedure was followed by using OECD 423(Acute Oral Toxic Class Method).The acute toxic class method is as step wise procedure with two rats of a single sex per step. Depending on the mortality or morbidity status of the rats and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substances. This procedure results in the use number of rats while allowing for acceptable data based scientific conclusion. The method used to defined doses (5,50, 300, 2000 mg/kg body weight) the results allow a substance to be ranked and classified according to the GHS for the classification of chemicals which cause acute toxicity.

Grouping of Animals

Healthy Male Wistar rats (150-200 gms) were selected and divided into five groups having six animals in each. Group-I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) & Ammonium chloride (1%) in drinking water was fed in Group-II to Group-V for induction of renal calculi for 28 days. Group-III received standard antiurolithiatic drug Cystone (750 mg/kg b.wt).Group-VI and Group-V received ethanolic extract of *ocimumbasilicum*200 mg/kg b.wt and 400 mg/kg b.wt for 28 days. Extracts were given once daily by oral route.

Table no. 1: Grouping of Animals.

Group	Status	Induction of Urolithiasis	Treatment
I	Vehicle control	Vehicle(1%CMC)	To serve as normal animal
II	Disease control	0.75% ethylene glycol and 1% ammonium chloride in drinking water for 28 days	To serve as control
III	Standard Control	0.75% ethylene glycol and 1% ammonium chloride in drinking water for 28 days	Cystone (750mg/kg body weight) up to 28 days
IV	Test extract – I (OBEE)	0.75% ethylene glycol and 1% ammonium chloride in drinking water for 28 days	OBEE 200 mg/kg b.wt (P.O)
V	Test extract – II (OBEE)	0.75% ethylene glycol and 1% ammonium chloride in drinking water for 28 days	OBEE 400 mg/kg b.wt (P.O)

Assessment of Antiurolithiatic Activity[11]

Collection and analysis of urine (Lee *et al.*, 2000)

Urine samples (24 h) were collected on 28th day by keeping the animals in metabolic cages. Animals had free access to drinking water during urine collection period. The volume of urine from each group of animal was measured. A drop of concentrated Hydrochloric acid was added to the collected urine before being stored at 4^{0}C.Urine was analysed for Urinary volume, Calcium, Magnesium, Phosphate, Oxalate.

Serum Analysis

After the experimental period, blood was collected from the retro orbital puncture under ether anaesthesia and

Serum was separated by centrifugation at 1000 rpm for 10 min and analysed for calcium, creatinine, uric acid, and urea nitrogen **(**Lee *et al*., 2000).

Kidney Homogenate for Antioxidant Estimations (Chow *et al*., 1975)[12]

The abdomen is cut open to remove both kidneys from each animal. Isolated kidneys are cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys are dried at 80^0c in hot air oven. A sample of 100mg of the dried kidney was boiled in 10ml of 1N hydrochloric acid for 30min and homogenized. The homogenate is centrifuged at 2000rpm for 10mins and supernatant liquid is collected and estimated for antioxidant parameters.

Histopathological studies

Kidney samples were weighed and fixed rapidly with 10% neutralized formalin (pH 7.4) section of the kidney fixed in paraffin was prepared and stained with haematoxylin & eosin and observed for pathological changes.

Statistical analysis

The statistical significance was assessed by using one way analysis of variance (ANOVA) and followed by dunnet`s comparison test. *p* value <0.05 was considered statistically significant.

RESULTS

Extract

About 300 gms of powder subjected to Maceration extraction. 6.5%yield of ethanolic extract of the whole plant of *Ocimumbasilicum L*is optioned.

Preliminary Phytochemical Screening

Phytochemical screening of ethanolic extract of whole plant of *Ocimumbasilicum.L* reveals the presence of alkaloids, flavonoids, phenolic compound, saponins, tannins and amino acids.

Acute Toxicity Studies

There were no signs of toxicity observed up to a dose level of 4000 mg/kg b.wt for *Ocimumbasilicum L*. The mortality rate was found nil and the herbal extract was found safe up to these dose levels. The effective dose was calculated based on the acute toxicity results, i.e. 1/10 and 1/20 of the LD_{50} value i.e. 200 mg/kg and 400 mg/kg.

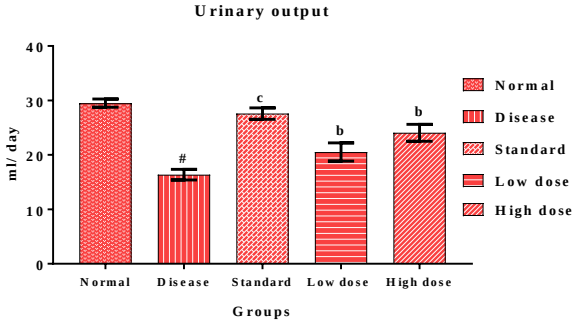
Urinary Parameters in EG&AC Induced Urolithiasis

It shows a significant decrease ($P < 0.001$) in urinary volume and magnesium with an increase in calcium, oxalate, phosphate in Disease control. Treatment with ethanolic extract of *Ocimumbasilicum.* L. (200 & 400 mg/kg b.wt) altered the urinary parameters compared to Disease control group were tabulated in the following **table no.2 and Graph1 & 2.**

Table no. 2: Effect of OBEE on Urinary Parameters in EG&AC Induced Urolithiasis.

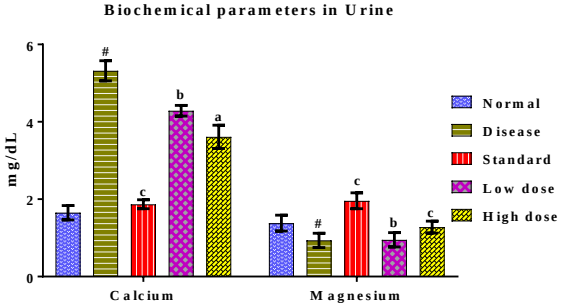
S.No	Parameters	Vehicle Control	Disease Control (EG/AC)	Standard Control (Cystone 750 mg/kg)	OBEE	
					Test I 200 mg/kg	Test II 400 mg/kg
1	Urinary output	29.51± 0.74	16.37±0.97$^{\#}$	27.59±1.06^{c}	20.53±1.67^{b}	24.06±1.55^{b}
2	Calcium	1.651± 0.185	5.3180±0.2610$^{\#}$	1.869±0.116^{c}	4.2880±0.1380^{b}	3.612±0.300^{a}
3	Magnesium	1.382± 0.205	0.9355±0.1840$^{\#}$	1.958±0.206^{c}	0.9502±0.1850^{b}	1.277±0.155^{c}
4	Phosphate	3.773±0.305	8.484±0.285$^{\#}$	5.189±0.376^{c}	8.474±0.378^{b}	6.806±0.257^{c}
5	Oxalate	2.859±0.297	5.851±0.398$^{\#}$	3.294±0.206^{c}	5.502±0.376^{b}	3.574±0.266^{c}

Data represents the Mean ± SEM values (n=6).Statisticalsignificance:^{a}P<0.05, ^{b} P<0.01, ^{c} P<0.001 with respect to Disease control on 28th day (One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control



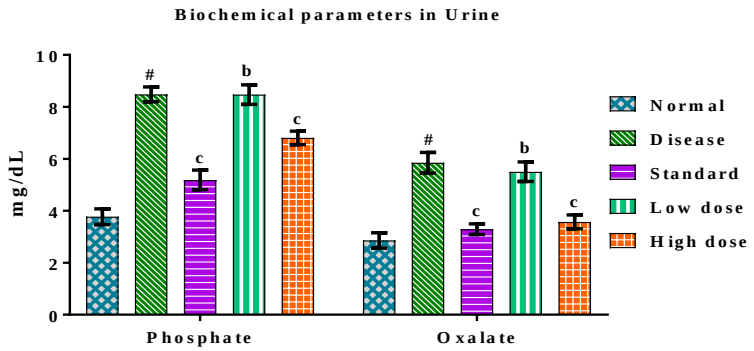
Graph 1: Effect of OBEE on urinary output in EG&AC Induced Urolithiasis.

Data represents the Mean ± SEM values (n=6). Statistical significance is done by one way ANOVA followed by Dunnets: compare all columns vs. Disease control.



Graph 2: Effect of OBEE on Urinary parameters (calcium and magnesium) in EG&AC Induced Urolithiasis.

Data represents the Mean ± SEM values (n=6). Statistical significance is done by one way ANOVA followed by Dunnets: compare all columns vs. Disease control.



Graph 3: Effect of OBEE on Urinary parameters (oxalate and phosphate) in EG&AC Induced Urolithiasis.

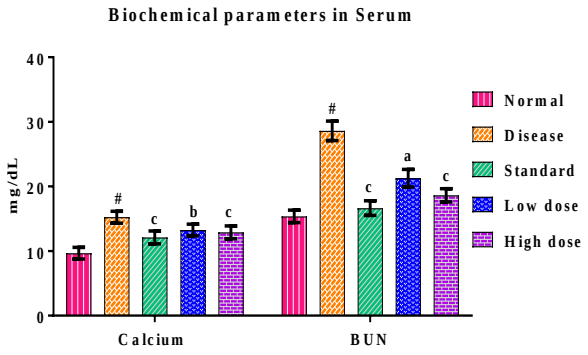
Data represents the Mean ± SEM values (n=6). Statistical significance is done by one way ANOVA followed by Dunnets: compare all columns vs. Disease control.

Table no. 3: Effect of OBEE on Serum Parameters in EG & AC Induced Urolithiasis.

S.No	Parameters	Vehicle Control	Disease Control (EG/AC)	Standard Control (Cystone 750 mg/kg)	OBEE	
					Test I 200 mg/kg	Test II 400 mg/kg
1	Calcium	9.669± 0.903	15.24± 0.92$^{\#}$	12.40± 1.00^{c}	12.74± 0.91^{b}	12.90± 0.99^{c}
2	Creatinine	1.598± 0.359	7.404± 0.302$^{\#}$	2.095± 0.371^{c}	6.313± 0.386^{b}	3.905± 0.375^{b}
3	Uric Acid	3.410± 0.487	7.378± 0.307$^{\#}$	4.583± 0.481^{b}	6.313± 0.308^{c}	5.039± 0.484^{b}
4	BUN	15.370± 0.960	28.61± 1.50$^{\#}$	16.65± 1.12^{c}	21.28± 1.35^{a}	18.62± 1.02^{c}

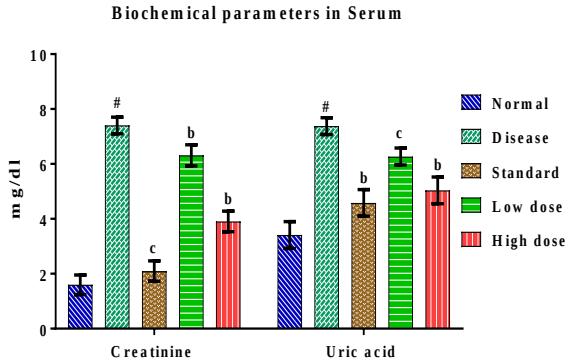
Data represents the Mean ± SEM values (n=6). Statisticalsignificance:^{a}P<0.05, ^{b} P<0.01, ^{c} P<0.001 with respect to Disease control on 28th day One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control.

It shows a significant increase (P <0.001) in serum parameters i.e. Calcium, creatinine, uric acid, BUN in Disease control Treatment with ethanolic extract of *Ocimumbasilicum L.* altered the serum parameters compared to Disease control group were tabulated in the following **table no.3 and Graph 4 & 5.**



Graph 4: Effect of OBEE on Serum parameters i.e. calcium and BUN in EG&AC Induced Urolithiasis.

Data represents the Mean ± SEM values (n=6). Statistical significance is done by one way ANOVA followed by Dunnets: compare all columns vs. Disease control.



Graph 5: Effect of OBEE on Serum parameters i.e., uric acid and creatinine in EG&AC Induced Urolithiasis.

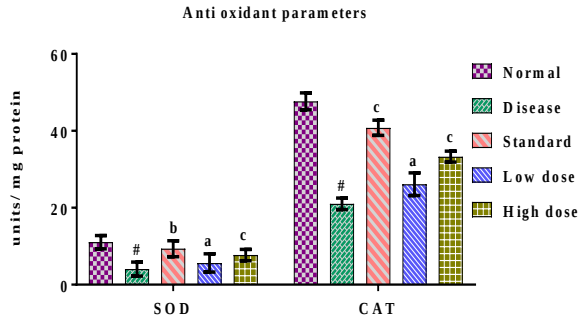
Data represents the Mean ± SEM values (n=6). Statistical significance is done by one way ANOVA followed by Dunnets: compare all columns vs. Disease control.

Table no. 4: Effect of OBEE on Tissue Parameters (SOD, CAT) in EG&AC Induced Urolithiasis.

S.No	Parameters	Vehicle Control	Disease Control (EG/AC)	Standard Control (Cystone 750mg/kg)	OBEE	
					Test I 200 mg/kg	Test II 400 mg/kg
1	SOD	11.052± 1.720	4.063± 1.829$^{\#}$	9.356± 2.075^{b}	5.636± 2.380^{a}	7.710± 1.482^{c}
2	CAT	47.660± 2.206	21.040± 1.502$^{\#}$	40.790± 1.977^{c}	26.120± 2.956^{a}	33.300± 1.418^{c}

Data represents the Mean ± SEM values (n=6).Statisticalsignificance:^{a}P<0.05, ^{b} P<0.01, ^{c} P<0.001 with respect to Disease control on 28th day (One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control.

It shows significant decrease (P < 0.001) in antioxidant enzymes SOD and CAT in Disease control. The pretreatment with OBEE showed increased levels of SOD and CAT were tabulated in the following **Table no. 4 Graph 6.**



Graph 6: Effect of OBEE on Tissue parameters SOD, CAT levels in EG&AC Induced Urolithiasis.

SOD- units/mg protein; CAT- µ moles of H_2O_2 utilized/mg protein/min.

Data represents the Mean ± SEM values (n=6). Statistical significance is done by one way ANOVA followed by Dunnets: compare all columns vs. Disease control.

HISTOPATHOLOGICAL STUDIES

Histopathology of wistar rat kidney section.

(A) Control Group

The kidney tissue found to be normal cyto-architecture which indicates the regeneration of kidney tissue with lower magnification (10X); And Higher magnification (40X).

(B) Model Group (EG)

The degenerative changes in Kidney tissue takes place and it shows necrosis and destructive bowman's capsule of tissue with lower magnification (10X) and higher magnification (40X).

(C) EG+STD

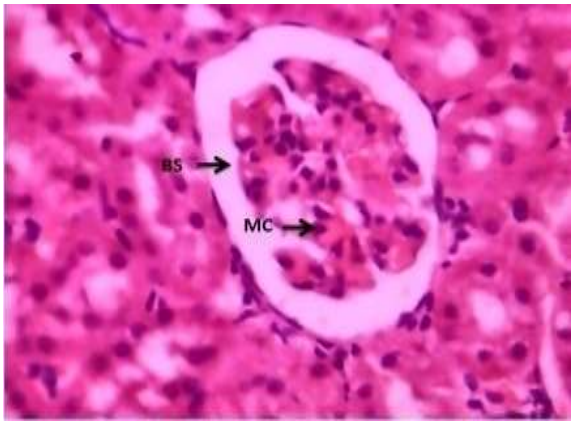
The regenerative changes in kidney tissue takes place and tissue shows similar to normal cytoarchitecture of kidney with lower magnification (10X); And Higher magnification (40X).

(D) EG+OBEE- 1

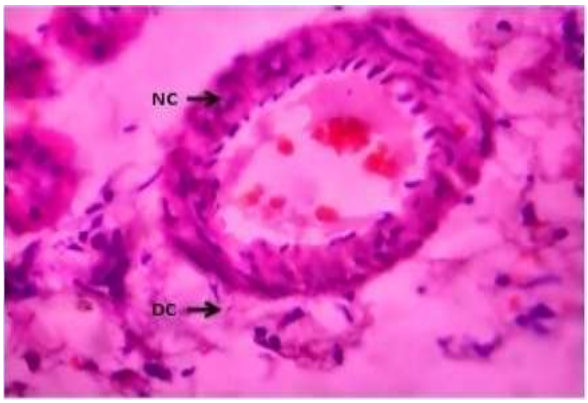
The normal cyto-architecture of kidney tissue indicates the regenerative changes with lower magnification (10X); And Higher magnification (40X).

(E) EG+OBEE - 2

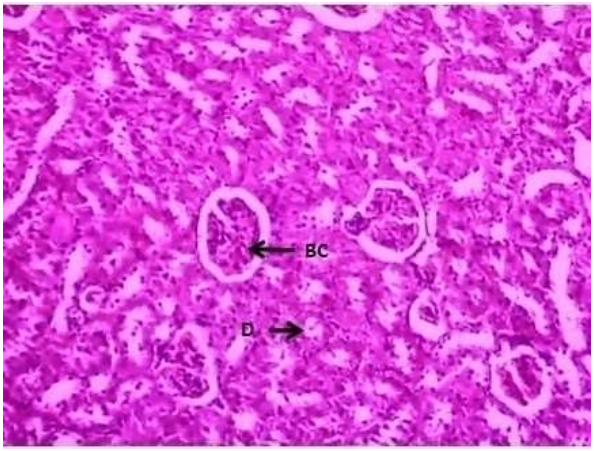
The regenerative changes in the kidney tissue indicates the similar to normal cyto-architecture with lower magnification (10X); And Higher magnification (40X). Same as control group (haematoxylin and eosin, original magnification(X400).



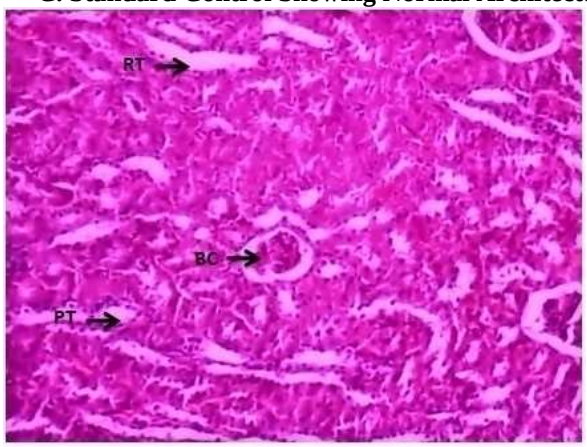
A. Kidney with normal glomeruli and tubules.



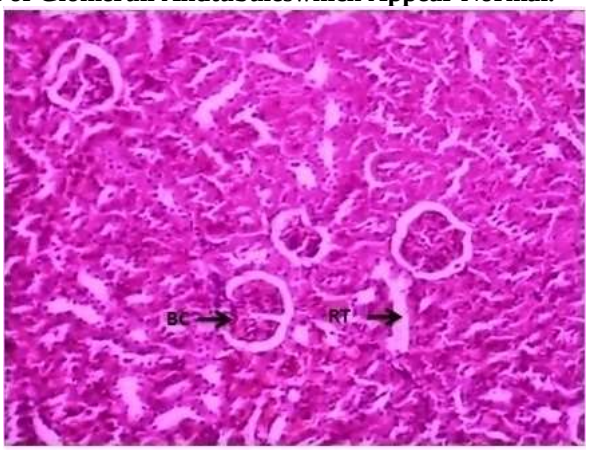
B. Disease Control Showing Degeneration of Epithelial Cells Iglomeruli And Tubules.



C. Standard Control Showing Normal Architecture of Glomeruli Andtubuleswhich Appear Normal.



(D).OBEE (200 mg/kg b.wt) shows regenerative changes in glomeruli and renal tubule



(E).OBEE (400 mg/kg b.wt) shows regenerative changes in glomeruli and renal tubule

DISCUSSION

Urolithiasis (kidney stones) is the third most common disorder, with a reported life time prevalence of 10-12% in men & 5-6 % in women. Kidney stones results from crystals in the urine aggregating together when the urine becomes highly concentrated. It is a complex multi factorial disorder, which results from the combined influence of dietary, geographical, biochemical and genetic risk factors and sometimes due to imbalance between promoters and inhibitors of stone forming constituents. Treatment of urolithiasis involves either conventional therapy or interventional procedure. Several natural dietary and non-dietary constituents and plant parts of several species of edible plants are having anti urolithiatic activity.

In the present study OBEE was used to evaluate the antiurolithiatic effect. About 300 g of plant powder (*ocimumbasilicum*) were subjected to maceration extraction, and the percentage yields obtained were 8.5%. The phytochemical screenings of OBEE reveal the presence of alkaloids, phenolic groups, amino acids, flavonoids, saponins and tannins. The extracts were found safe up to a dose of 4000 mg/kg body weight. The dried extract was suspended in 1% CMC at dose levels of 200 mg/kg and 400 mg/kg b.wtp.o.

Different chemicals used to induce lithiasis in experimental animals include ethylene glycol (EG), glycolic acid (Sathya and Kokilavani, 2012).[13] and ammonium oxalate. To achieve a uniformity high rate of kidney crystal deposition, other drugs such as ammonium chloride, vitamin D and gentamycin (Sujatha Dodoala*et al*., 2010),[14] or a magnesium deficient diet have been used in conjunction with EG. The most commonly employed method is EG and AC induced urolithiasis model. The mechanism of this process may be due to an increase in the urinary concentration of oxalates. Stone formation in EG is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. In the present study, ammonium chloride is used to promote urinary acidification and corresponding decrease in urinary citrate excretion, which is a potent inhibitor in stone formation (Fan *et al*., 1999),[15] so in the present study, ammonium chloride has been used to accelerate lithiasis.

In the present study male wistar rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans (Vermeulen, 1962)[16] whereas earlier studies have shown that the amount of stone deposition in female rats was significantly less due to inhibitory effect of female sex hormone on kidney stone formation (Iguchi *et al.* 1999 and Lee *et al.*,

1996).[17,18] Therefore in the present study ethylene glycol (0.75%) and Ammonium chloride (1%) for 28 days was given to the male wistar rats to induce calcium oxalate stones in the kidney.

Cystone (750 mg/kg b.wt) was used as a standard drug in inhibiting urinary stones, due to its potent diuretic, spasmolytic and saluretic effect, which is beneficial in relieving crystalluria (Phukan, Choudhury, 1973).[19]

The pathology of urolithiasis was assessed by measuring the urinary parameters include urinary volume, calcium. magnesium, phosphate, oxalates and serum parameters include calcium, BUN, creatinine, and uric acid.

In the present study, the urinary volume was significantly decreased in ethylene glycol treated animals due to obstruction of stones in the bladder (Ghodkar).[20] Cystone treated animals showed significant increase in urinary volume due to its potent diuretic activity (Shrivastava, 1971).[21] Pretreatment with OBEE (Group IV-V) shows dose dependent increase in urinary volume, compared to disease group.

EG & AC administration in the control rats, enhanced excretion and deposition of calcium, oxalate and phosphates levels indicate supersaturation of urine with CaOx. Increase in urinary phosphate excretion along with oxalates provide an environment appropriate for stone formation by forming calcium phosphate crystals (Prie*et al*., 2001)[22] in disease control animals. Cystone treatment significantly lowered the calcium, oxalate values probably by its inhibitory action on glycolate oxidase (Singh *et al*., 1983).[23] Pretreatment with OBEE (200 and 400 mg/kg), shows prominent decrease in these elevated levels, when compared with disease group.

Magnesium in urine plays an inhibitory role in the growth and nucleation rates of calcium oxalate crystals by forming complexes with calcium and oxalates and decreases its excretion in urine (Schwille*et al*., 1999).[24] Decreased levels of magnesium was observed in EG&AC disease rats. Pretreatment with OBEE at both dose levels, increase the magnesium levels.

In EG& AC induced rats, there was a significant raise in calcium, uric acid, creatinine and BUN in serum, because of decreased glomerular filtration rate due to obstruction in the urine flow in urinary system with the deposition of calcium oxalate in renal tubule (Ghodkar). Cystone treatment showed significant reduction of these elevated levels. Pretreatment with OBEE grouped animals showed significant reduction in such nitrogenous substances in serum.

SOD and CAT were the most important enzymes in the enzymatic anti-oxidant defense system. SOD scavenges the superoxide anion to form hydrogen peroxide and diminishes the effect caused by the free radical and catalase (CAT), which decomposes hydrogen peroxide and protects the tissue from the highly reactive hydroxyl radical. Depletion in the activities of these enzymes with EG&AC induced rats was observed in disease control rats. Cystone treated animals showed significant increase in the enzyme activities of SOD and catalase. Administration of OBEE showed dose dependent increase in enzymatic activity. The antioxidant activity may be due to the presence of poly phenols such as flavonoids, tannins and saponins, which contribute to induce antioxidant potential activity.

Histopathological observation of the kidney sections of EG&AC induced disease rats showed the presence of polymorphic irregular calcium oxalate crystals in Lumina of tubules accompanied by edema and cast formation which causes dilation of proximal tubules; this might be attributed to oxalate formation. On administration of OBEE at two different doses (200 and 400 mg/kg.wt) showed moderate to few crystals along the mild appearance of edema dilation in tubules and crystals are present focally indicating the ability of text extracts to dissolve the preformed stones to some extent.

CONCLUSION

In the present study, administration of EG&AC in male wistar rats for 28 days period bring altered urinary, serum and antioxidant enzymatic activity. The pretreatment with EETA showed a significant restoration of altered levels near to normal control. It was concluded that the antiurolithiatic activity of EETA may be due the diuretic property and presence of phytochemicals like alkaloids, phenols, flavonoids, saponins, terpenes. Further studies were required to isolate the chemical moiety which showing potent anti urolithiatic activity.

REFERENCE

1. Thenmozhi. P, S. Prasanna Saravana Guru, M. Kannan, P. Sathiyarajeswaran. Overview on selected medicinal plants used in the management of urolithiasis, WJPPS, 2016; 5(10): 280-294.
2. Tapsell LC, Hemphill I, Cobiac L. Health benefits of herbs and spices: the past, the present, the future. Med. J., Aust., 2006; 185(4): S4–24.
3. Lai PK, Roy J. Antimicrobial and chemo preventive properties of herbs and spices. Curr. Med. Chem., 2004; 11(11): 1451–60.
4. Thomas J, Medicinal and aromatic plants research in India, 1997 In UNDP.
5. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery". Environ Health Perspect, 2001; 109(1): 69–75.
6. Arafat OM, Tham SY, Sadikum A, Zhari I, Haughton PJ, Asmawi MZ. Studies on diuretic and hypouricemic effects of Orthosiphon stamineus methanol extract in rats. Journal of Ethnopharmacology, 2008; 118: 354-360.
7. Bashir S, Gilani AH. Antiurolithiatic effect of Berenia ligulata rhizomes: an explation of the underlying mechanisms. Journal of Ethnopharmacology, 2009; 122: 106-116.

8. Anwar M, Waqar and Fauzia Sarwar. Herbal treatment for urolithiasis. Sci-tech world, 2006.
9. Kokate CK, Purohit AP, Gokhale SB. Text Book of Pharmacognosy. Nirali publication, 1994; 4th edition, 123-24.
10. Acute oral toxic class method guideline 423 adopted in: Eleventh Addendum to the OECD, guideline for the testing of chemicals Organization for Economic Co-operation and Development, 2002; 12: 245-255.
11. Lee YH, Tsaj JY and Huang JK. Combined use of 30% Lactose Rich Diet and 1% ethylene Glycol: A New Animal Model for Study of Urolithiasis. J urol R.o.c., 2000; 11(4): 149-154.
12. Chow FC, Dysent IM, Hamer DW, Udall HR. Control of oxalate urolithiasis by DLalanine. Investigative Urology, 1975; 13: 113.
13. M. Sathya and R. Kokilavani. Acute and sub acute toxicological assessment of the ethanolic root extract of Saccharum spontaneum Linn. (Poaceae) in male wistar albino rats. Journal of Chemical and Pharmaceutical Research, 2012; 4(8): 3894-3899.
14. Sujatha Dodoala, Ranganayakulu. D, Bharathi koganti and K V S R G Prasad. Effect of ethanolic extract of phyla nodiflora(Linn.) greene against calculi producing diet induced uroliyhiasis. Indian journal of natural products and resources, 2010; 1(3): 314-321.
15. Fan J, Shen SJ. The role of Tamm-Horsfall mucoprotein in calcium oxalate crystalluria. Nacetylcysteine - A new therapy for calcium oxalate urolithiasis. Br J Urol, 1994; 74: 288-293.
16. Vermeulen CW. Experiments on causation of urinary calculi, in, Essays in Experimental Biology. University of Chicago Press (Chicago), 1962; 253-269.
17. Iguchi M, Takamura C, Umekawa T, Kurita T, Kohri K. Inhibitory effects of female sex hormones on urinary stone formation in rats. Kidney International, 1999; 56: 479–485.
18. Lee YH, Huang WC, Huang JK, Chang LS. Testosterone enhances whereas oestrogen inhibits calcium oxalate stone formation in ethylene glycol treated rats. Journal of Urology, 1996; 156: 502–505.
19. Phukan DP, Choudhury AK. Pharmacological and Clinical Study on Cystone. Journal of the Gauhati Medical College, 1973; 9: 12.
20. Ghodkar PB. Chemical tests in kidney disease. In: Textbook of Medical Laboratory Technology, First ed. Bhalani Publishing House, 1994; 118–132.
21. Shrivastava JG. Herbal plants used in stone. Quart J Crude Drug Res., 1971; 11: 1683–9.
22. Prie D, Ravery V, Boccon-Gibod and Friedlander G. Frequency of renal phosphate leak among patients with calcium nephrolithiasis. Kidney Int., 2001; 60: 272-276.
23. Singh PP, Singh NB, Singh LBK. Effect of cystone on urinary excretion of calcium, oxalic acid and uric acid in stone formers. The Antiseptic, 1983; 5(30): 234.
24. Schwille PO, Schimiedi A, et al: Urol. Res., 1999; 27: 117–126.