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PREVENTIVE EFFECT OF *Moringa oleifera* Lam. STEM BARK TOWARDS UROLITHIASIS

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ABSTRACT

The plant *Moringa oleifera* Lam. is a small monogeneric family, the Moringaceae. It is called as "Miracle Tree" and the most widely cultivated species allover India and in tropical and subtropical regions. In Moringa all its parts like leaves, stem bark, root bark, flowers, fruits and seeds are used in different systems of medicines and used in food products for their immense neutraceutical potential. This present study evaluated the pharmacognostical features of the stem part and also tried to screen for the antiurolithiatic effect and antimicrobial effect of moringa tree stem bark. The microscopical evaluation of the plant stem has been studied and documented, it has been used for the documentation and future reference purposes. The antimicrobial activity of the test sample extracts were carried out by standard disc diffusion method (Kirby Bauer method). The antiurolithiatic effect of the fresh juice, methanol and water extract of stem bark of the plant was evaluated using ethylene glycol induced hyperoxaluria model in rats. The acute oral toxicity study was carried out as per the guidelines set by (OECD). The LD50 and ED50 of the extracts were calculated. Albino rats were selected and given 1% ethylene glycol in drinking water to induce hyperoxaluria. In six groups the oral administration of the fresh juice, aqueous and methanol extract of the drug, the others are standard and control at the dose of 200mg/kg. After 14th and 28th day of treatment urine was collected and estimated for ionic concentrations. In

vivo antioxidant activity parameters such as Lipid peroxidation, Superoxide dismutase, Glutathione, Catalase were also monitored. The drug treated group animals were resulted in significant reduction in the bladder stones compared to the control and standard cystone treated group and it shows significant enzyme activity for antioxidant like reduction in MDA and increased GSH and antioxidant enzyme likes SOD and CAT in the order of fresh juice > methanol extract > aqueous extracts. The action of herbal drugs exerts their antilithogenic properties by altering the ionic composition of urine. The extracts were showed significant antimicrobial activity towards bacteria and fungi.

Keywords: ***Moringa oleifera*** **Lam, Antiurolithiatic, Neutraceutical, Lipid peroxidation**

INTRODUCTION

Urolithiasis or Nephrolithiasis is consequence after complex physical and metabolic process in human body **[1]**. The major factors for it are the supersaturation of urine with salts and crystalisation in urine, retaining of some salts in the kidney and become nucleus for the formation of stone **[2]**. Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. Kidney stones may contain various combination of chemicals. There are four most common types of kidney stones, they are Calcium, Struvite, Uric acid and Cystine **[3]**. Most calculi in the urinary system arise from a common component of urine, e.g.calcium oxalate (CaOx), representing up to 80% of analyzed stones **[4]**. The sruvite (magnesium ammonium phosphate) is the another type of stone usually with the infection of urease producing bacteria like *Proteus, Klebsiella, Pseudomonas and Enderobacter* by hydrolysis of urea into ammonium and increase the urinary pH.

The struvite type of stone accounts for approximately 70% of these calculi, these are usually large (staghorn calculi). These stones need to be treated surgically and the entire stone removed, including small fragments, as otherwise these residual fragments act as a reservoir for infection and recurrent stone formation **[5]**. Uric acid and cystine are also found as minor components. The prevention of stone formation is good as well as the first step in preventing kidney stones is to understand what is causing the stones to form. Analysing the type of stone and prevent through change diet, nutrition and medication especially herbal medicines. A number of vegetable drugs have been used in

India which claim efficient cure of urinary stones **[6]**.

Moringa oleifera Lam is an important medicinal plant, considered as miracle tree all parts of the plant are useful for human health. It is also known as drum stick tree, horse radish tree, clarifier tree and mother's best friend in different parts of the world, based on their appearance and unique uses **[7]**.The plant is a small tree which is one of the pan-tropical species. It is a most useful plant with highly nutritional value and medicinal properties. It's the native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. All parts of the Moringa tree are edible and have long been consumed by humans **[8, 9]**. They are rich in protein, minerals, beta-carotene, thiamin, riboflavin, and other vitamins, particularly vitamins A and C are considered useful in scurvy and respiratory ailments **[10]**. The Moringa seeds are relatively high yield of 36% Ben oil. The oil contains 65.7% of Oleic acids, 9.3% of Palmitic acid, 7.4% of Stearic acid and 8.6% of Behenic acid. The seeds are good for hypertension, gout, asthma, cancer, and anti-aging. It is also having anti-arthritic activity of anti– inflammatory activity **[11]**. Oil extracted from Moringa flowers can also be helpful for arthritic pains and rheumatic and gouty joints **[12]**. Moringa flower is a rich reservoir of bioactive phytochemicals and crude flower extracts showed promising antibacterial, antifungal, anti-larval, antioxidant, anti-inflammatory and anticancer properties **[13]**. The aqueous extract of root of this plant is having anti-inflammatory action and hepato protective action **[14]**. Its leaves are also used for asthma, gout, backache, rheumatism, kidney stone,skin wounds, hyperlipidemia and hypocholesterolemic effect **[15-18]**.

In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of aqueous, methanol extracts and fresh juice of Moringa stem bark using ethylene glycol induced hyperoxaluria model in rats. The acute oral toxicity study was carried out as per the guidelines set by (OECD) .The antioxidant enzymatic activity and antimicrobial activity of the same has been evaluated to support the antiurolithiatic activity.

MATERIALS AND METHODS

Plant selection and authentication

The plant *Moringa oleifera* Lam. was collected from Coimbatore. The botanical identity has been authenticated by the Director, Botanical survey of India, Coimbatore, No: BSI/SRC/5/23/2012-13/Tech/496. The voucher specimen has

been submitted and preserved in herbarium for future reference.

Processing of plant material

The plant material (stem Bark) was collected, cut into small pieces and shade dried at room temperature. Then the dried material was subjected to size reduction to get course powder of desired particle size. This powdered material was subjected to successive extraction **[19]**. One kilogram powdered drug was extracted with methanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and solvents were evaporated under reduced pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts were calculated and stored in desiccators and used for further experiments. Fresh juice of the plant bark was collected daily as per the daily requirement for urolithiatic study.

Chemicals*:* All the chemicals and reagents were purchased from Merck,Mumbai,India. Solvents and all the reagents used were of analytical grade. The creatinine kit purchased from (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit from (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid level.

Animal selection

For acute toxicity study, Wistar albino mice of either sex weighing between 25 and 30 g were selected and for the antiurolithiatic activity healthy adult male Wistar albino rats weighing between 150 and 200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature: 25±2 ◦C) and maintained on 12-h light: 12-h dark cycle **[20]**. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC).**Approval for the project*:*** Approval for the animal experiment was obtained from the Institutional Animal Ethical Committee (IAEC), K.M. College of Pharmacy, Madurai. vide letter No. KMCP /IAEC/Ph.D/60.

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose **[21]**.

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model **[18-21]** was used to assess the antilithiatic activity in albino rats. Animals were divided into six groups containing six animals in

each. The grouped animal's received the treatment as follows. The Group I animals received normal diet and served as controls. The Group II animals are the *Lithiatic control*, here the animals were given normal diet and 1% Ethylene glycol in drinking water. Group III animals received 1% ethylene glycol in drinking water and then treated with methanol extract (ME) of the drug at a Dose of 200 mg/kg orally. Group-IV animals received 1% Ethylene glycol in drinking water and then treated with aqueous extracts (AE) of the drug at a dose of 200mg/kg orally. Group-V animals **r**eceived 1% Ethylene glycol in drinking water and then treated with Fresh Juice (FJ) of drug at a dose of 200mg/kg orally. Group- VI animals received 1% Ethylene glycol in drinking water and then treated with Standard drug Cistone 100mg / kg orally. All extracts were given once daily by oral route for 28 days **[22-26]**.

2.4.3 Collection and analysis of urine

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

Serum analysis

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

In-vivo Antioxidant activity: Enzyme assays

A portion of kidney was taken from all the groups and a 30% w/v homogenate was prepared in 0.9% buffererd KCL (pH 7.4) for the estimation of glutathione (GSH), Super oxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) **[31-34]**.

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

Evaluation of Antimicrobial Activity

The antimicrobial activity of the test sample (stem bark) extracts were carried out by standard disc diffusion method (Kirby Bauer

method). The bacterial strains used were *Staphylococcus aureus* (+ve) (NCIM 2079), *Bacillus subtilis* (+ve) (NCIM2063), *Pseudomonas aeruginosa* (-ve) *(NCIM 2036), Klebsiella aerogenes* (-ve) (NCIM 2098) and fungi *Candida albicans* (NCIM 3102*)* and *Aspergillus flavus* (NCIM105) were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on Nutrient agar and Sabourad dextrose agar medium for bacteria and fungi respectively. From the culture obtained, using sterilized Pasteur loop, one loop full each of the microorganisms were transferred into the test tubes containing sterile nutrient broth for screening studies. The pH of the above media were maintained at 7.2, it is then sterilized by autoclaving at 121^{o}C at 15lbs pressure for 15 minutes, in which the nutrient broth was used for sub culturing and MHA (Mueller Hinton Media) media was used for screening studies **[35]**.

Nutrient broth with standard modification was prepared and sterilized by autoclaving at 121 ^{o} C (151b/in^{2}) about 30ml of nutrient agar medium was transferred aseptically into every sterilized petriplates to get thickness of 5 to 6 nm. The plate were allowed to solidify and upturned to prevent the condensate declining on the agar surface. The plates were dried at 37^{o} C sooner than organisms were inoculated in the plates prepared prior, by dipping sterilize swab in the previously standardized inoculums and spread the organism by shacking the swab all over the surface of the medium. The plates were left at room temperature. Reference standard disc (6 mm diameter) was used as positive antibacterial and antifungal (Ciprofloxacin 5µg/disc for bacteria; Nystatin 100µg/disc for fungi) as control. Each extracts were reconstituted with solvents and tested at the concentration of 200µg /ml. The paper discs were impregnated appropriately labeled and evenly spaced sides over the inoculated plates. On incubation the bacteria grow on area of the plate excluding those approximately the inhibitory compound of the plant, which they are sensitive. In the duration of overnight the phytocompound present in the plant extract prevents the development of visible growth which indicates the extract is having antibacterial action[36,37].

The inhibition was measuring the diameter of the inhibition zone after prior incubation and the experiment was executed two fold and the average determination was recorded. The effect produced by the sample was compared with the effect produced by the positive control (Reference Standard Ciprofloxacin 5µg/disc for bacteria; Nystatin

100µg/disc for fungi).The antimicrobial action was evaluated by measuring the width of inhibition zone **[38]**.

RESULTS AND DISCUSSION

In the present work, studied the antilithiatic activity, after chronic administration of 1% (v/v) ethylene glycol in aqueous solution to wistar rats resulted in hyperoxaluria. The urine volume in the GP_2 reduced on14^{th} and 28^{th} day, where as in drug treated group the urine volume increased at the same level of standard animals in the GP_6 **[39] [Table 1]**. Urinary concentration of the various ions investigated varied drastically, following ethylene glycol treatment. The results are discussed below.

Effect of Drug Extract on Urinary Parameters on Day 14 & 28

The oxalate excretion was increased significantly on day 14^{th} & 28^{th} day in GP_2 following ethylene glycol treatment. Treatment with drug extract of (GP_3 to GP_5) reduced the oxalate excretion significantly on 14^{th} day treatment. Likewise on 28^{th} day, treatment with this extracts reduced the oxalate excretion significantly. The urinary calcium excretion was increased significantly on day 14^{th} & 28^{th} day in GP_2 following ethylene glycol treatment. The calcium excretion was significantly reduced to drug treated at a dose of 200mg/kg (GP_3 to GP_5) reduce the calcium excretion significantly on 14^{th} day treatment likewise on 28^{th} day calcium excretion was significantly reduced. Likewise phosphate and creatinine excretion values gradually increased in GP_2 on the 14^{th} & 28th day. However in (GP_3 to GP_5) grouped treated animals these elevated values were brought down on 14^{th} day and on 28^{th} day respectively comparing with the standard group (GP_6).

Likewise urinary protein and uric acid concentration increased following ethylene glycol treatment in GP_2 and it reached maximum on the 14^{th} & 28^{th} day. On treatment with plant extracts (GP_3 to GP_5) the protein and uric acid excretion was restored to near normal limits on 14^{th} day and on 28^{th} day **[Table 2, 3]**. In GP_1 normal rats the magnesium excretion was estimated as 4.20±0.52 mg/dl/24hr, 4.42±0.58 mg/dl/24hr on 14^{th} & 28^{th} day. Contrary to this, in GP_2 lithiatic control rats, the magnesium level in urine gradually decreased to 0.98±0.14 mg/dl/24hr 1.35± 0.11 mg/dl/24hr following ethylene glycol treatment on the 14^{th} & 28^{th} day **[Figure 2]** . Subsequent administration of the extract enhanced the magnesium excretion significantly increased nearer to the normal respectively on 14^{th} day & 28^{th}day [40-42] . The drug extracts showed significant antiurolithiatic effect when comparing with

that of the standard drug treated group GP_6. The results are given in **[Table 2 & 3]**.

Effect of Drug on Serum Parameters on Day 28

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in GP_2 (Lithiatic control) following ethylene glycol treatment, Treatment with Methanol, Aqueous extract and fresh juice of the selected drug at a dose of 200mg/kg (GP_3 to GP_5) reduce the all above mentioned parameters significantly. On the contrary the magnesium levels were decreased significantly in GP_2 (Lithiatic control) following ethylene glycol treatment **[Table 4]**. After treatment with drug at a dose of 200mg/kg the magnesium level was restored near to normal levels **[Figure 2]**. The drug extracts showed significant antiurolithiatic effect when comparing with that of the standard drug treated group GP_6 **[43, 44]**.

In-vivo Antioxidant activity - Enzyme assay:

This study also revealed the increased lipid per oxidation and lessened levels of antioxidant potential in kidneys of rats supplemented with ethylene glycol. Oxalate, the chief stone forming constituent, has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes **[44]**. Phenolic compounds present in the extracts may prevent the lipidperoxidation induced renal damage caused by calcium oxalate crystal deposition in the kidney. Hence these extracts can prevent calcium oxalate crystal attachment as well as stone formation **[45, 46]**. The extracts treatment produced noteworthy decrease in MDA and increased GSH, SOD, and CAT these consequences indicate the protective effects of drug extracts aligned with the oxidative changes induced by ethylene glycol **(Table 5)**. Thus, the consequences revealed that the extracts posses a potent antiurolilithiatic and antioxidant activity.

For in vivo antioxidant activity ethylene glycol treatment increased MDA ($P<0.01$) and decreased GSH ($p<0.01$) SOD ($p<0.01$) and CAT (0.01) levels in control animals. Extracts of the selected drug at a dose of 200mg/kg produced significant ($p<0.001$) reduction in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone **[Figure 4]**.

Table 1: Effect of drug extracts on urinary output in urolithiasis induced rats.

Days	GP1	GP2	GP3	GP4	GP5	GP6
0	7.25±0.52	7.30± 0.60	8.35± 0.75	8.52±0.68	8.60±0.90	8.35±0.76
14	7.89±0.60	5.35±1.36**a	8.49±1.42**b	8.60±1.32**b	8.45±1.50**b	9.30±1.32**b
28	7.56±0.76	4.95±1.60**a	8.86±1.50**b	9.72±1.60**b	9.90±1.65**b	10.10±1.60**b

Table 2: Effect of drug extracts on urinary Biochemical parameters on the 14th day

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	70.90±3.76	4.42±0.58	6.15±0.70	3.30±0.62	0.90±0.08	16.30±1.50	33.60±2.26
GP_2	158.40±7.30**(a)	1.35 ±0.11**(a)	22.15±1.60**(a)	13.60±1.32**(a)	1.86 ±0.24**(a)	48.20±4.45**(a)	78.6 ±4.26**(a)
GP_3	80.36±4.36**(b)	3.26 ±0.42**(b)	9.15±0.28**(b)	7.88±0.80**(b)	1.10 ±0.12**(b)	18.26±1.80**(b)	36.22±2.55**(b)
GP_4	82.40±4.45**(b)	3.05 ±0.36**(b)	9.20±0.33**(b)	7.59±0.62**(b)	0.95 ±0.08**(b)	18.88±1.92**(b)	34.30±2.34**(b)
GP_5	83.40±4.60**(b)	3.12 ±0.40**(b)	9.10±0.30**(b)	7.55±0.56**(b)	0.92 ±0.06**(b)	18.50±1.72**(b)	32.30±2.22**(b)
GP_6	81.42±3.60**(b)	3.30 ±0.48**(b)	9.50±0.42**(b)	7.33±0.60**(b)	0.91 ±0.07**(b)	18.70±1.83**(b)	36.18±2.60**(b)

- Values are expressed as Mean± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a)values were significantly different from normal control (GP_1)at P< 0.01
- **(b) values were significantly different from Lithiatic control (GP_2)at P<0.01

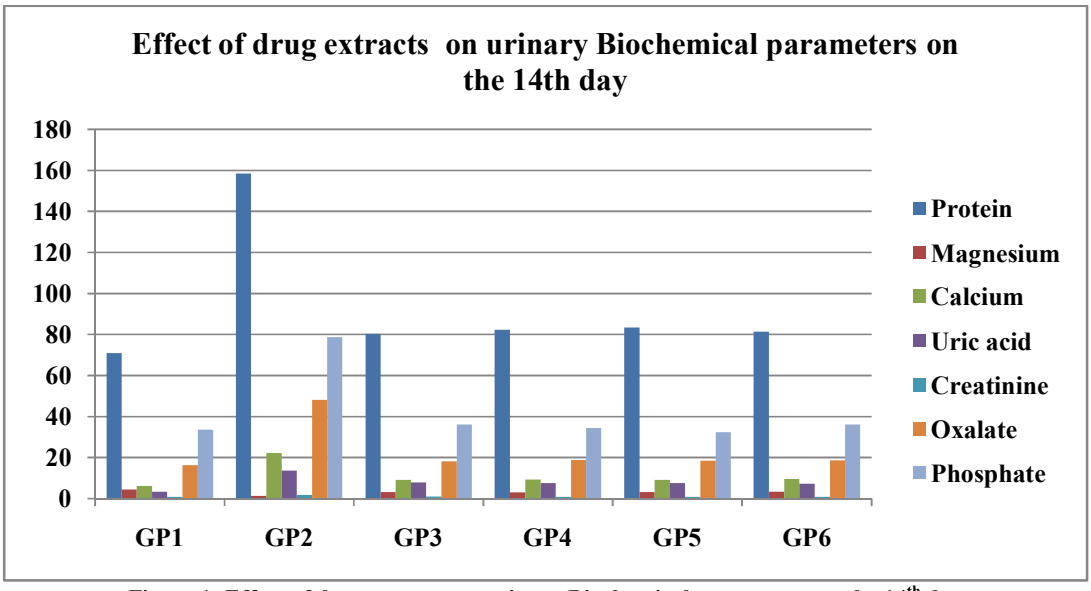

Figure 1: Effect of drug extracts on urinary Biochemical parameters on the 14th day

Table 3: The effect of Drug Extracts on Urinary Biochemical parameters on 28th day

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	65.96±2.86	4.20± 0.52	5.63± 0.54	3.22± 0.65	0.80± 0.08	15.80±1.83	32.90±2.20
GP_2	152.22 ±6.30**(a)	0.98 ±0.14**(a)	20.15±1.98**(a)	12.56 ±1.62**(a)	1.56 ±0.14**(a)	32.65 ±3.42**(a)	73.60 ±4.26**(a)
GP_3	82.66 ±3.55**(b)	2.88 ±0.40**(b)	8.90 ±0.92**(b)	5.20 ±0.85**(b)	0.86 ±0.11**(b)	21.30 ±2.32**(b)	37.80 ±3.15**(b)
GP_4	76.30±2.28**(b)	3.15 ±0.55**(b)	7.75 ±0.65**(b)	4.90 ±0.80**(b)	0.84 ±0.10**(b)	20.06 ±1.90**(b)	33.30 ±2.28**(b)
GP_5	74.55±2.40**(b)	2.95 ±0.48**(b)	7.90 ±0.58**(b)	4.85 ±0.78**(b)	0.90 ±0.15**(b)	21.78 ±1.75**(b)	35.40 ±2.35**(b)
GP_6	67.85±1.62**(b)	3.25 ±0.58**(b)	6.85 ±0.63**(b)	5.76 ±0.68**(b)	0.74 ±0.06**(b)	18.55 ±1.54**(b)	30.22 ±1.85**(b)

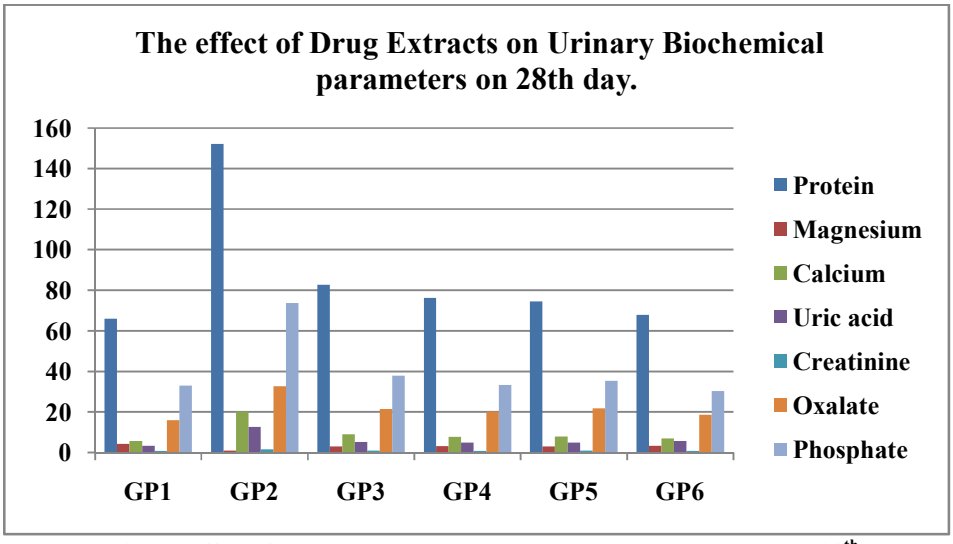

Figure 2: The effect of Drug Extracts on Urinary Biochemical parameters on 28th day

Table 4: Effect of drug extracts on serum Biochemical parameters on 28day

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	4.80 ±0.86	9.40 ±1.32	3.45 ±0.40	0.56 ±0.03	6.6 ±0.57	12.06 ±1.43
GP_2	1.38 ±0.25**(a)	18.30 ±2.34**(a)	9.7 ±1.10**(a)	1.01 ±0.13**(a)	12.60 ±1.61**(a)	26.01 ±3.25**(a)
GP_3	3.67 ±0.52**(b)	11.22 ±1.60*(b)	4.10 ±0.46**(b)	0.80 ±0.07**(b)	8.12 ±0.78**(b)	19.85 ±2.05**(b)
GP_4	3.86 ±0.65**(b)	10.68 ±1.52**(b)	3.90 ±0.40**(b)	0.72 ±0.04**(b)	7.75 ±0.65**(b)	18.23 ±1.75**(b)
GP_5	3.40 ±0.52**(b)	10.75 ±1.60**(b)	3.88 ±0.36**(b)	0.82 ±0.08**(b)	7.86 ±0.72**(b)	18.78 ±1.83**(b)
GP_6	3.90 ±0.70**(b)	10.65 ±1.48**(b)	3.95 ±0.45**(b)	0.82 ±0.06**(b)	8.06 ±0.70**(b)	18.58 ±1.60**(b)
GP_7	3.50 ±0.56**(b)	10.50 ±1.35**(b)	3.85 ±0.36**(b)	0.80 ±0.05**(b)	7.92 ±0.45**(b)	18.26 ±1.40**(b)

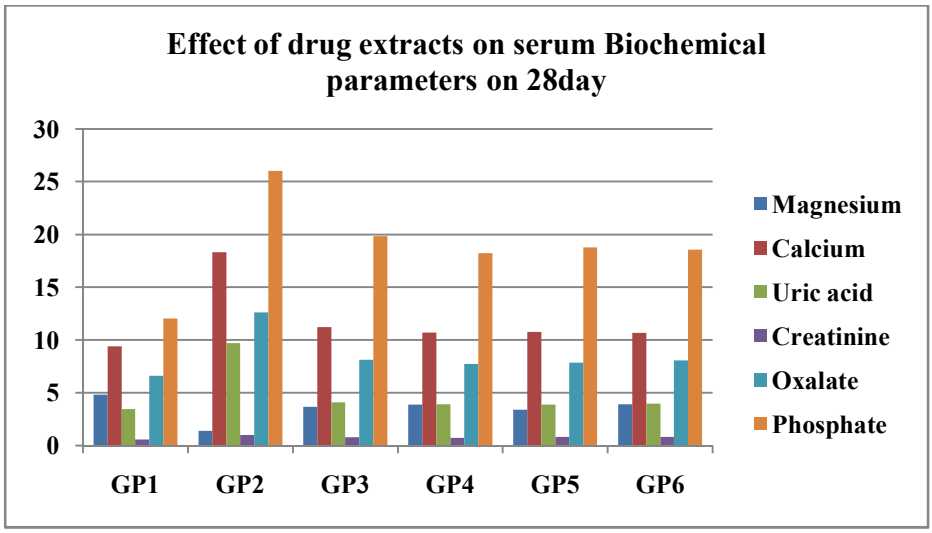

Figure 3: Effect of drug extracts on serum Biochemical parameters on 28day
GP_1- Normal; GP_2- Lithiatic Control; GP_3- ME (200mg/kg); GP_4- AE(200mg/kg); GP_5- FJ(200mg/kg); GP_6- Cystone herbal tablets(100mg/kg)

- **Values are expressed in ml/24 h urine sample as mean ± SEM**
- **Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.**
- ****(a) Values were significantly different from normal control (GP_1) at P< 0.01**
- ****(b) Values were significantly different from Lithiatic control (GP_2) at P<0.01**

Table 5: Effect of drug extracts on antioxidant activity towards enzymes in renal tissue

Treatment	Catalase A/protein	SOD B/mg protein	GSH nmoles/mg protein	MDA nmoles/mg protein
Normal control	3.20±0.18	9.20±0.18	3.72±0.16	3.88±0.28
Ethylene glycol	0.98±0.02	3.68±0.07	0.52±0.06	10.05±0.46
Cystone	2.80±0.16	7.05±0.11	2.68±0.14	4.20±0.30
ME 200mg/kg	2.28±0.08	6.60±0.12	2.20±0.11	5.76±0.45
AE 200mg/kg	2.36±0.10	6.85±0.14	2.24±0.12	5.89±0.50
FJ 200mg/kg	2.22±0.07	6.62±0.13	2.05±0.10	5.55±0.28

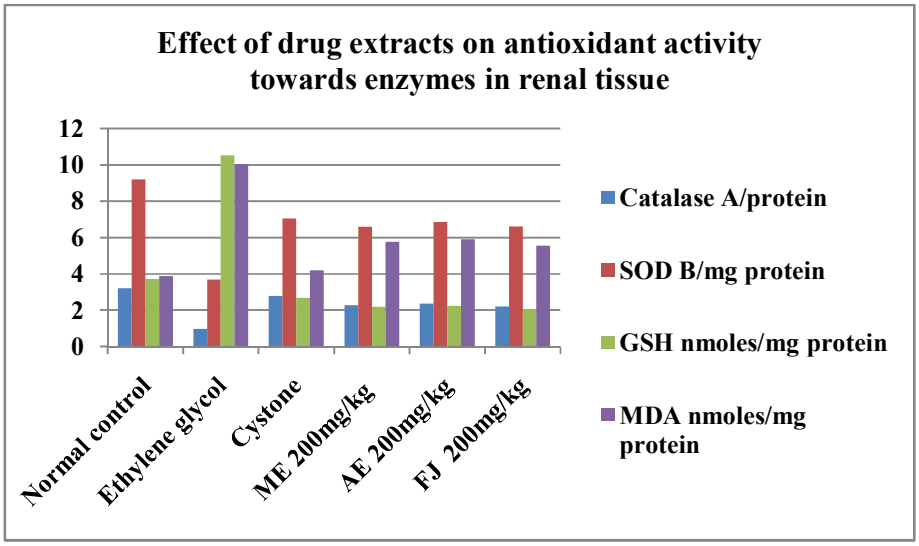

Figure 4: Effect of drug extracts on antioxidant activity towards enzymes in renal tissue

Statistical analysis

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

Table 6: The antimicrobial activity of the extracts

S. No.	Name of the Microorganisms	ZONE OF INHIBITION nm				
		Extracts 200µg /ml			Solvent control	Standard
		Aqueous extract	Methanol Extract	Fresh juice		
1	*Staphylococcus aureus*	16	20	21	-	24
2	*Bacillus subtilis*	14	16	18	-	23
3.	*Klebsiella aerogenes*	12	14	17	-	23
4.	*Pseudomonas aeruginosa*	17	19	20	-	26
5.	*Aspergillus niger*	16	17	19	-	24
6.	*Candida albicans*	15	18	20	-	26

Standards- Ciprofloxacin 5µg /disc for bacteria; Nystatin 100 units / disc for fungi

The aqueous, methanol extracts and fresh juice of the plant was screened for their antibacterial and antifungal activity. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi). The microbial growth inhibition was measuring the diameter of the zone of inhibition after prior incubation and the experiment was done twice and the average determination was recorded. The methanol

extract shows better antibacterial activity than the aqueous extract. The antibacterial activity of the drug extracts towards the bacterial organisms in the given order like *Staphylococcus aureus*> *Pseudomonas aeruginosa* > *Bascillus subtilus* > *Klebsiella aerogenes*. The extract shows significant antifungal effect towards the fungal organisms in the order of *Aspergillus niger* < *Candida albicans i*n the dose of 200mg itself comparatively related to that of the standard drug. As per the literature different part of this selected plant is having more pharmacological activities anti-inflammatory, analgesic and antipyretic activities **[48]**. This study also reveals that the stem bark of *Moringa oleifera* plant is having significant antimicrobial activity.

CONCLUSION

The results reveal that the stem bark of *Moringa oleifera* plant extracts posses potent antiurolilithiatic and antioxidant activity. The presented data indicate that administration of the extracts to rats with ethylene glycol induced urolithiasis reduced the formation of urinary stones. The mechanism underlying this effect is apparently related to diuretic effect, lowering of urinary concentrations of stone forming constituents and altering the ionic concentration it prevents the formation of Lithiasis. The extracts shows antimicrobial activity but the fresh juice shows better activity than the methanol and aqueous extract. From the antimicrobial activity screening confirms this plant extract is more useful for struvit type stone also.

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