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Original Article

A comparative study of antilithiatic effect of three traditional plants and their antioxidant activity

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ABSTRACT

Achyranthus aspera Linn (AA)**,** *Moringa oleifea* Lam (MO) and *Scopario dulcis* Linn (SD) are the traditional plants which have many applications in pharmaceuticals, alternative medicines and natural therapies. AA the whole plant is characterized by its uterine stimulant and many more activities.SD is traditionally used in the treatment of diabeties, jaundice and stomachache etc.MO is an important medicinal herb and is considered as *miracle tree.* All parts of the plant are useful for human health. In the indigenous system of medicine, AA, MO and SD is reported to be useful in the treatment of urinary disorders. In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of aqueous and alcoholic extract of leaves of AA, MO and SD 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 drugs 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 aqueous and alcoholic extract of AA, MO and SD, the others are standard and control. After 14th and 28th day of treatment urine was collected and estimated for ionic concentrations. After 28 days the histopathological study has been done. In vivo antioxidant activity parameters such as Lipid per oxidation, Superoxide dismutase, Glutathione, Catalase were also monitored. It shows decrease in MDA and increase in GSH, CAT, and SOD. The drug treated group animals were resulted in significant reduction in the bladder stones compared to the control and standard cystone treated group and enzyme activity for antioxidant property and more in methanolic extract. Among these *Scoparia dulcis* is having better effect than the others. The action of herbal drugs exerts their antilithogenic properties by altering the ionic composition of urine viz.; decreasing the calcium and oxalate ion concentration or increasing magnesium and citrate excretion. The extracts of AA, MO and SD at a dose of 200mg/kg produced significant reduction in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone. When comparing these three plants the *Scoparia dulcis* is having better activity than the others.

Key Words: Hyperoxaluria, Antiurolithiatic, Alternative, Cystone.

INTRODUCTION

Urolithiasis is a consequence of complex physical processes. The major factors are super saturation of urine with the offending salt and crystallization. Crystals retained in kidney can become nucleus for stone formation. This process is known as Urolithiasis or Nephrolithiasis. Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing upto 80% of analyzed stones [1]. Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of extracorporeal shockwave lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones. However, in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence [2,3]. A number of vegetable drugs have been used in India and elsewhere which claim efficient cure of urinary

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stones [4]. In the indigenous system of medicine, AA, MO and SD is reported to be useful in the treatment of urinary disorders. However, so far no systematic study has been reported regarding the antiurolithiatic property of aqueous and alcoholic extract AA, MO and SD. ***Moringa oleifera*** lam is an important medicinal herb, belonging to the family Moringaceae. It is considered as ***miracle tree*** as all the parts of the plant are useful for human health. It is also known as drum stick tree, horseradish tree, clarifier tree and mother's best friend in different parts of the world, based on their appearance and unique uses[5]. The various parts of the plant used include flower, pod, leaves, bark and roots. The ***flowers***, after cooking, are eaten either mixed with other foods or fried in butter and have been shown to be rich in potassium and calcium [6,7].

The ***leaves*** are eaten as greens, in salads in vegetable curries, as pickles and for seasoning. ***Achyranthus aspera*** *Linn* is a common weed growing in all tropical places. It is an herbaceous plant about 1m height [8,9]. The whole plant is used for their anti-inflammatory and uterine stimulant activity. The drug is used on its own or in combination with some other plants. The plant contains alkaloids, saponins, terpenes and sterols. ***Scoparia dulcis Linn*** is available in most tropical country. In Ecuador the indigenous tribes brew a tea of the entire plant to reduce swelling, aches and pains and the leaf decoction is used as an antiseptic wash for wounds, as an anti-nausea. In Brazil the leaf juice is used to wash infected wounds. One particular important chemical compound is isolated That has been named Scopadulcic acid B, which is used to inhibit cancerous growth in laboratory animals and in test tubes[10,11] .

MATERIALS AND METHODS

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**

Plant material: The leaf of *Moringa oleifera* Lam.and *Achyranthus aspera L* are available locally were collected in and around Coimbatore and *Scopario dulcis L* is available and collected from Palakad district in Kerala. 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 materials were collected and shade dried at room temperature and was subjected to size reduction to get course powder of desired particle size. This powdered material was subjected to successive extraction. Each (1kg) powdered three drugs were extracted with methanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and solvent 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.

Pharmacological screening for antiurolithiatic activity

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: Inbred Albino rats of *Wistar* strain, of either sex, aged around 2 to 3 months and weighing 150-200 g were used. They were housed in standard conditions of temperature (25 ± 2°C), relative humidity of 45-55%, and maintained on 12–hour light: 12–hour dark cycle in animal house of K.M. College of Pharmacy, Madurai. They were fed with and standard pellet diet (Hindustan Lever rat pellets) and water *ad libitum*.

Acute toxicity studies: The acute oral toxicity study was carried out as per the OECD guidelines (No: 423) using female Wistar albino rats weighing between 180 and 220 g were selected and employing the up and down method prior to evaluating all the extracts for antiurolithiatic activity. One-tenth of the median lethal dose (LD50) was taken as an effective dose [(12).]

Ethylene glycol induced urolithiasis model: Ethylene glycol induced hyperoxaluria model[13] was used to assess the antilithiatic activity in albino rats. Animals were divided into eight groups containing six animals in each.

TREATMENT PROTOCOL

The grouped animal's received the treatment as follows

Group I - Received normal diet and served as controls. Group II – Group IX All the animals were given normal diet and received 1% ethylene glycol in drinking water. **Group II**- ***Lithiatic control***: No treatment for 28 days. **Group III** - treated with Methanolic extract of AA. **Group IV**- treated with aqueous extracts of AA. **Group V**- treated with Methanolic extracts of MO. **Group VI** - treated

with Aqueous extracts of MO. **Group VII** - treated with Methanolic extracts of SD. **Group VIII** - treated with Aqueous extracts of SD. All extracts were given once daily by oral route at a dose of 200mg/kg for 28 days. **Group IX**-Received1% Ethylene glycol in drinking water and then treated with STD drug Cystone at the dose of 100mg/kg orally for 28days.

Collection and analysis of urine: All animals were kept in individual metabolic cages and 24 h urine samples were collected on 14th, and 28th day of calculi induction treatment. The volume of urine were measuredand Calcium content in urine was estimated using kit by COBAS MIRA PLUS" auto analyzer. Urine was analyzed for oxalate[15], magnesium [16,17], phosphate[18], uric acid [19], citrate[20] and total protein[21].

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

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

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)[22] Super oxide dismutase (SOD)[23] catalase (CAT)[24] and malondialdehyde (MDA)[25]

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.

RESULTS

Table -1: **Effect of** AA, MO and SD **on urinary output in urolithiasis induced rats**.

Days	GP1	GP2	GP3	GP4	GP5	GP6	GP7	GP8	GP9
0	7.25± 0.52	7.30± 0.60	7.35± 0.66	7.90± 0.70	8.35± 0.75	8.52± 0.68	8.60± 0.90	8.35± 0.76	8.22± 0.70
14	7.89± 0.60	5.35±1. 36**a	8.45± 1.50**b	8.20± 1.20**b	8.30± 1.42**b	8.60± 1.32**b	8.45± 1.50**b	8.30± 1.32**b	8.24± 1.22**b
28	8.06± 0.76	4.95±1. 60**a	8.65± 1.86**b	8.25± 1.32**b	8.66± 1.50**b	9.72± 1.60**b	9.90± 1.65**b	10.10± 1.60**b	10.25± 1.46**b

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- MEAA (200mg/kg); **GP_4**- AEAA(200mg/kg);
GP_5- MEMO(200mg/kg); **GP_6**- AEMO(200mg/kg); **GP_7**- MESD(200mg/kg); **GP_8**- AESD(200mg/kg)
GP_9- 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 2: The effect of AA,MO and SD 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	86.20 ±4.20 **(b)	2.50 ± 0.36**(b)	9.45 ± 1.10**(b)	5.56 ± 0.94**(b)	0.92 ± 0.09**(b)	23.22 ± 2.76**(b)	43.5 ±3.73**(b)
GP_4	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_5	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_6	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_7	69.22±2.15**(b)	2.60 ± 0.32**(b)	7.05 ± 0.76**(b)	4.62 ± 0.65**(b)	0.78 ± 0.12**(b)	19.20 ± 1.62**(b)	34.28±2.30**(b)
GP_8	70.35±1.90**(b)	2.72 ± 0.40**(b)	7.60 ± 0.72**(b)	4.55 ± 0.52**(b)	0.80 ± 0.07**(b)	20.22 ± 1.96**(b)	32.15±2.05**(b)
GP_9	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)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- MEAA (200mg/kg); **GP_4**- AEAA(200mg/kg);
GP_5- MEMO(200mg/kg); **GP_6**- AEMO(200mg/kg); **GP_7**- MESD(200mg/kg); **GP_8**- AESD(200mg/kg)
GP_9- 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 3:Effect of AA, MO and SD 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)	78.66±4.26**(a)
GP_3	93.12 ±5.78**(b)	2.95 ±0.38**(b)	12.65 ±0.95**(b)	8.40 ±0.86**(b)	1.22 ±0.16**(b)	24.38 ±2.68**(b)	44.82 ±3.32**(b)
GP_4	88.56 ±5.26**(b)	3.26 ±0.45**(b)	10.66 ±0.76**(b)	8.05 ±0.76**(b)	1.08 ±0.10**(b)	20.10 ±2.50**(b)	40.55 ±3.06**(b)
GP_5	84.25 ±4.65**(b)	3.45 ±0.50**(b)	9.45 ±0.40**(b)	7.62± 0.56**(b)	1.05 ±0.09**(b)	19.12 ±2.20**(b)	38.30 ±2.80**(b)
GP_6	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_7	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_8	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_9	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)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- MEAA (200mg/kg); **GP_4**- AEAA(200mg/kg);
GP_5- MEMO(200mg/kg); **GP_6**- AEMO(200mg/kg); **GP_7**- MESD(200mg/kg); **GP_8**- AESD(200mg/kg)
GP_9- 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 4 : Effect of AA, MO and SD 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	3.28 ±0.46**(b)	11.85 ±1.88**(b)	4.55 ±0.55**(b)	0.91 ±0.09**(b)	8.45 ±0.88**(b)	20.10 ±2.65**(b)
GP_4	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_5	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_6	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_7	3.52 ±0.60**(b)	10.30 ±1.10**(b)	3.60 ±0.26**(b)	0.86 ±0.07**(b)	8.10 ±0.80**(b)	18.25 ±1.90**(b)
GP_8	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_9	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)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- MEAA (200mg/kg); **GP_4**- AEAA(200mg/kg); **GP_5**- MEMO(200mg/kg); **GP_6**- AEMO(200mg/kg); **GP_7**- MESD(200mg/kg); **GP_8**- AESD(200mg/kg); **GP_9**- 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

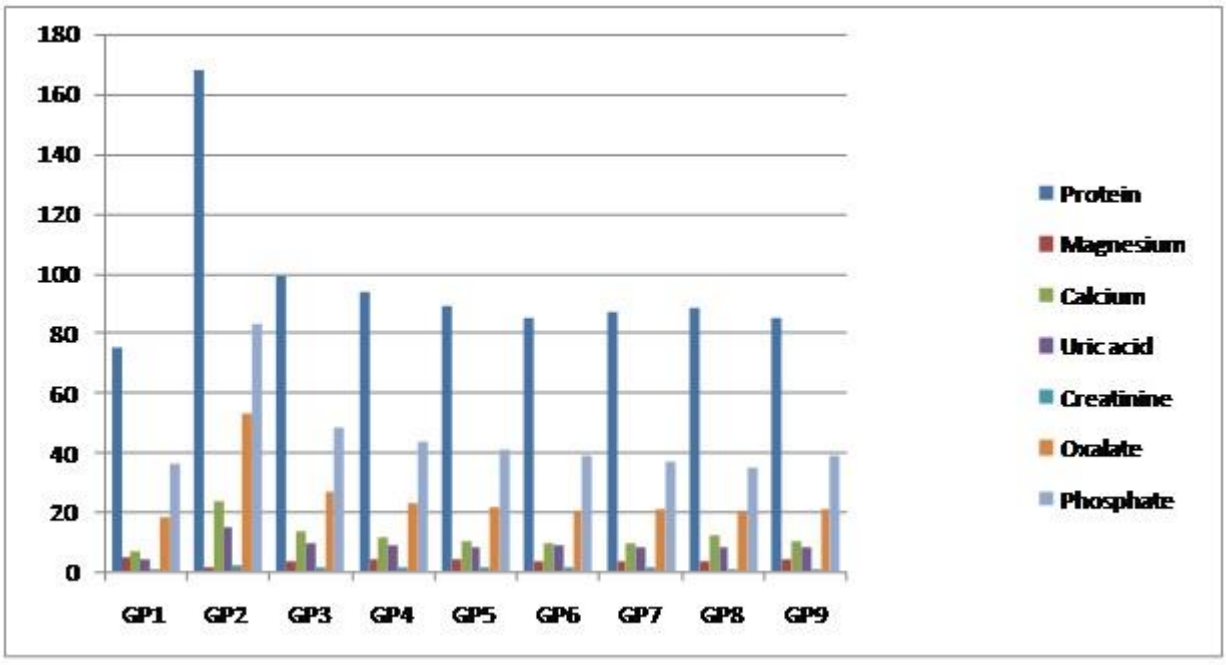

Figure 1 : Effect of AA,MO and SD on Urinary biochemical parameters on the 28th day

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Table 5:Effect of Aqueous and Mehanolic extracts of AA, MO and SD on antioxidant enzymes in renal tissue

Treatment	Catalase A/protein	SODB/mg protein	GSH n moles/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**
AEAA 200mg/kg	2.32±0.09**	6.55±0.10**	2.12±0.10**	5.68±0.35**
MEAA 200mg/kg	2.28±0.08**	6.60±0.12**	2.20±0.11**	5.76±0.45**
AEMO 200mg/kg	2.36±0.10**	6.85±0.14**	2.24±0.12**	5.89±0.50**
MEMO 200mg/kg	2.22±0.07**	6.62±0.13**	2.05±0.10**	5.55±0.28**
AESD200mg/kg	2.30±0.09**	6.67±0.10**	2.18±0.12**	4.73±0.44**
MESD200mg/kg	2.24±0.08**	6.59±0.09**	2.26±0.13**	4.60±0.42**

- 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

DISCUSSION

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to the wistar rats resulted in hyperoxaluria. Urinary concentration of the various ions investigated varied drastically, following ethylene glycol treatment in the lithiatic control.The oxalate, Calcium, Uric acid, Creatinine and Phosphate excretion were significantly increased on day 14^{th} & 28^{th} respectively for GP2 following ethylene glycol treatment. Treatment with Methanolic and Aqueous extract of AA,MO and SD (GP_3 to GP_8) reduced the excretions significantly on 14^{th} day of treatment and more reduced on 28^{th} day, like standard (GP_9) The results are shown in the table 2 and in chart 1.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 for ethylene glycol treatment on the 14^{th} & 28^{th} day . Subsequent administration of the extract enhanced the magnesium excretion significantly on 14^{th} day & 28^{th} day.The results are given in the table 3. 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 methanolic and aqueous extract of AA,MO and SD at a dose of 200mg/kg (GP_3 to GP_8) 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**.** After treatment with methanolic and aqueous extract of AA,MO and SD at a dose of 200mg/kg the magnesium level was restored near to normal and standard levels. The results are tabulated in table no 4. In stone induced models, the following changes were noted, damaged epithelial cells at the inner layer of the tubules, Dilatation of the tubules and Presence of crystals in the tubules. Scores were given according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol (GP_2 in Fig.No:2) showed large quantity of microcrystal deposition and severe dilation of most tubules and mass tubule interstitial inflammatory infiltration with lesion area > 40% (score3). However, kidney sections of animals treated with extract shows obvious dilation of many tubules and tubule interstitial inflammatory infiltration with lesion area < 40% (score 2) in Fig.No:3to8. As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of antilithiatic effect of the methanolic and aqueous extract of three drugs. In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and also earlier studies have shown that the amount of stone deposition in female rats was significantly less.[26]. Urinary super saturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate[27,28]. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate. Therefore, this model was used to evaluate the

antilithiatic effect of Methanolic and Aqueous extract of AA,MO and SD at a dose of 200mg/kg against urolithiasis[29,30]. In the present study oxalate and calcium excretion progressively increased in calculi- induced animals (GP_2), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria,[31] and the changes in urinary oxalate levels are relatively much more important than those of calcium[32]. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth.[33] However Methanolic and Aqueous extract of AA, MO and SD at a dose of 200mg/kg lowered the levels of oxalate as well as calcium excretion. An increase in urinary phosphate is observed in calculi induced rats (GP_2). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which is epitaxially induces calcium oxalate deposition[34]. Treatment with methanolic and aqueous extract of AA,MO and SD at a dose of 200mg/kg restored phosphate level, thus reducing the risk of stone formation.The increases in urinary uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment with methanolic and aqueous extract of AA, MO and SD at a dose of 200mg/kg lowered the excretion of uric acid and reduces the risk of stone formation. Super saturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found in the high concentration in urine, when urine volume decreases, and when urinary concentration of chemicals that inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate; nephrocalcin etc[35,36]. Low urinary magnesium content is a common feature in stone formers [37]. A similar condition was observed in the (GP_2) rats. Treatment with Methanolic and Aqueous extract of AA,MO and SD at a dose of 200mg/kg elevated the urinary magnesium level, and thus, reduced the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers[38].A high urinary colloidal concentration favours crystal growth[39). Such a condition was observed with ethylene glycol treated rats, in this study. Administration of the Methanolic and Aqueous extract of AA,MO and SD reduced the urinary protein excretion in the treated group rats, and hence minimizes the conditions favorable for crystal growth.

In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated [(40)].Also increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi- producing diet (CPD)[41,42]. Elevated oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane[43]. In calculi- induced rats (GP_2), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the prophylactic treatment with Methanolic and Aqueous extract causes diuresis and has tens the process of dissolving the preformed stones and prevention of new stone formation in the urinary system. Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Prophylactic treatment with Methanolic and Aqueous extract of AA,MO and SD suppresses this increase in intracellular calcium. Several studies reported that Flavonoids, polyphones and triterpenes have anti-inflammatory and antioxidant effects[44,45].It can be expected that antilithiatic activity might be through an antioxidant activity and free radical scavenging principle[46].

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats showed polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate[47]. Co-treatment with Methanolic and Aqueous extract of AA,MO and SD decreased the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes. It is shown in the fig.no.1 to fig.no.9.

This study also revealed the increased lipid per oxidation and decreased levels of antioxidant potential in kidneys of rats supplemented with ethylene glycol. Oxalate, the major stone forming constituent, has been reported to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes[48]. 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. The extracts treatment produced significant decrease in MDA and increased GSH,SOD,and CAT these results indicate the protective effects of AA,MO and SD extracts against the oxidative changes induced by ethylene glycol[49]. These properties have been attributed to the triterpenes. Lupeol and polyphenolic compounds like quercetin[50] present in AA,MO and SD extracts. Thus, the results reveal that the three extracts posses a potent antiurolilithiatic and antioxidant activity[51]. 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. Aqueous and methanolic extracts of AA,MO and SD produced significant ($p<0.001$) reduction in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone (table 5).

CONCLUSION

The results reveal that the AA, MO and SD extracts posses potent antiurolilithiatic and antioxidant activity. The presented data indicate that administration of the three extracts to rats with ethylene glycol induced urolithiasis reduced the formation of urinary stones . The mechanism underlying this effect is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. These drugs can be used to prepare a polyherbal formulation for urolithiasis treatment.

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FIGURE 1

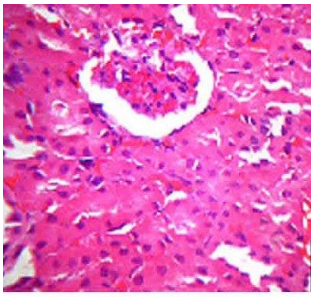

FIGURE 2

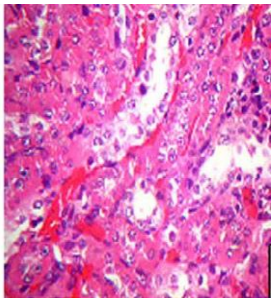

FIGURE 3

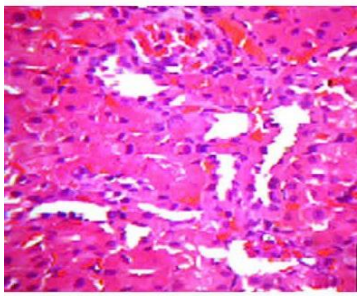

FIGURE 4

FIGURE 5

FIGURE 6

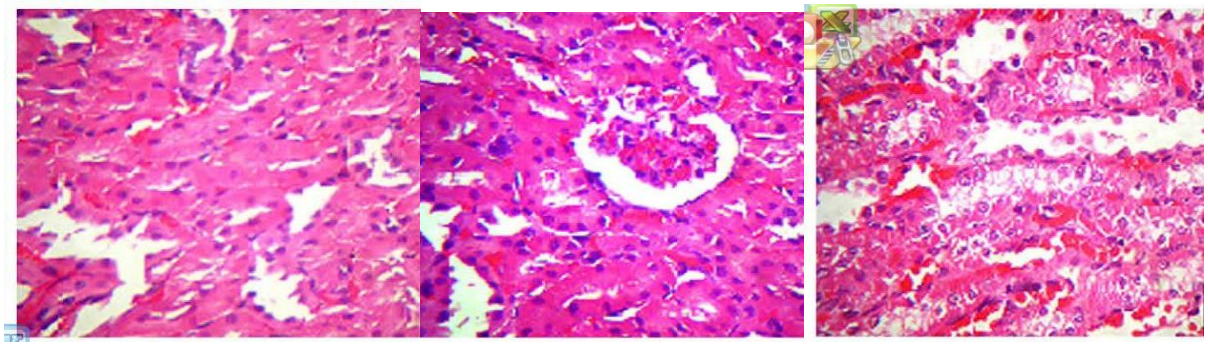

FIGURE : 7 :

FIGURE : 8

FIGURE: 9

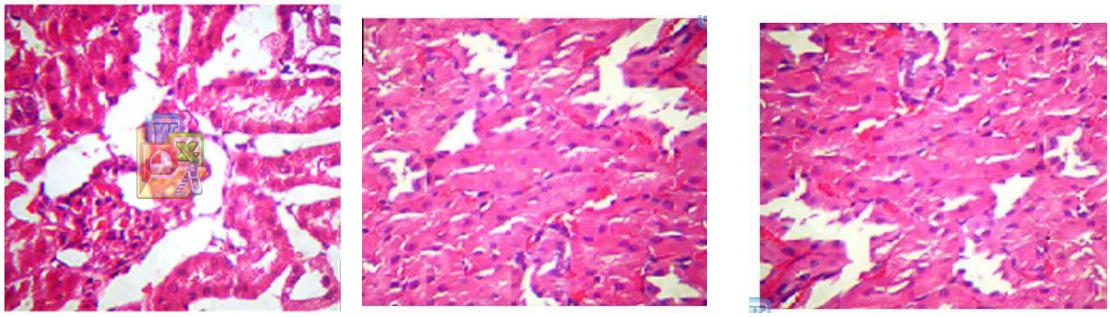

Figure 1: Kidney Section of GP-1, Normal control rats: Section Show structure of kidney with glomeruli and tubules which appear normal

Figure 2: Kidney section of GP-2 (Lithiatic Control) Rat: Section Show structure of kidney with glomeruli and tubules with inflammation. Tubules show crystals in the lumen indicating stone formation.

Figure 3: Kidney Section of GP- 3 Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen.

Figure 4: Kidney Section of GP- 4: Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen.

Figure 5: Kidney Section of GP- 5: Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen.

Figure 6: Kidney Section of GP- 6: Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen.

Figure 7: Kidney Section of GP- 7: Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen.

Figure 8: Kidney Section of GP- 8: Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen.

Figure 9: Kidney Section of GP- 9: Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen

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