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EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF *RHUS MYSORENSIS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR RATS

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ABSTRACT

Background and Objectives: Urinary stone disorder has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. The common component of urinary stone is calcium oxalate (CaOx). In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. In the indigenous system of medicine, the leaves of *Rhus Mysorensis* are reported to be useful in the treatment of urinary stones. Hence, in the present study, the *Rhus Mysorensis have* been selected for their antiurolithiatic activity on experimentally induced urolithiatic rats.

Materials and Methods: The *Rhus Mysorensis* were shade dried at room temperature and coarsely powdered in such a way that the material passed through sieve no. 20 and was retained on sieve no. 40 for desired particle size and then extracted with 80 %v/v ethanol, separately. Acute toxicity study was carried out using "Up and Down" method. Male Wistar albino rats were used for assessment of antiurolithiatic activity. Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce urolithiasis. Thirty animals were randomly divided into five groups containing six animals in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to groups II-V for induction of renal calculi until the 28th day. As well as ethylene glycol, groups 2-5 also received the following treatments: Groups III received standard antiurolithiatic drug, cystone (750 mg/kg body weight).Group IV received methanolic extract of *Rhus Mysorensis* (200 mg/kg body weight) Group V received methanolic extract of *Rhus Mysorensis* (400 mg/kg body weight). Extract was given once daily by oral route. After 28 days, urine 24 h) was analyzed for

oxalate, calcium and phosphate excretion.

KEYWORDS: Rhus Mysorensis, antiurolithiatic, ethylene glycol, hyperoxaluria model, urinary stones.

MATERIALS AND METHODS

Preparation of ethanolic extract

About 500g of dried powder was extracted with 80%v/v ethanol in a soxhlet extractor. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and solvent was distilled off. The extract was concentrated to dry residue. The percentage yield of the extract was calculated with reference to air dried powder.

Qualitative Phytochemical screening

Preliminary phytochemical investigation was carried out on 80% ethanolic extract of Rhusmysorensis (80% v/v ERS) for detection of various phytochemicals by standard methods (Kokate CK, *et al*., 1996). The extracts were subjected to the following chemical test for the identification of various active constituents.

1. Test for alkaloids

a) Dragondroff's test

To 1ml of the extract, add 1ml of Dragondroff's reagent, an orange red precipitate indicates the presence of alkaloids.

b) Mayer's test

To 1ml of the extract, add 2ml of Mayer's reagent, a cream coloured precipitate reveal the presence of alkaloids.

c) Wagner's test

To 1ml of the extract, add2ml of Wagner's reagent, the formation of reddish brown precipitate indicates the presence of alkaloids.

d) Hager's test

To 1ml of the extract, add 3ml of Hager's reagent the formation of yellow precipitate confirms the presence of alkaloids.

2. Test for carbohydrates

a) Molisch test

To 2ml of the extract, add 1ml of α-naphthol solution and then add concentrated sulphuric acid through the sides of the test tube, purple or reddish violet ring at the junction of the two reveals the presence of carbohydrates.

b) Fehling's test

To 1ml of the extract, add an equal quantity of Fehling's solution A and B and heat. The formation of the brick red precipitate indicates the presence of carbohydrates.

c) Benedict's test

To 5ml of Benedict's reagent add 1ml of extract solution and boil for 2minutes and cool. Formation of a red precipitate shows the presence of carbohydrates.

d) Barfoed's test

To 5ml of Barfoed's reagent, add 1ml of the extract solution and heat to boil, a red precipitate of copper oxide was formed and confirms the presence of carbohydrates in the test extract.

3. Test for steroids and sterols

a) LibermannBurchard test

Dissolve the extract in 2ml of chloroform in a dry test tube. Add ten drops of acetic anhydride and two drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green, indicating the presence of steroids.

b) Salkowaski test

Dissolve the extract in chloroform and add volume of concentrate sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and whereas the acid layer assumes marked green florescence, represents the steroid and sterol components in the tested extract.

4. Test for glycosides

a) Legal test

Dissolve the extract in pyridine and add freshly prepared sodium nitroprusside solution to make it alkaline. The formation of pink to red colour shows the presence of glycoside.

b) Baljet test

To 1ml of the test extract add 1ml sodium picrate solution and the yellow to orange colour reveals the presence of glycoside.

c) Borntrager"s test

Add a few ml of diluted sulphuric acid to 1ml of the extract solution. Boil, filter and the filtrate extract with chloroform. Separate the chloroform layer and treat with 1ml ammonia. The formation of red colour shows the presence of anthraquinone glycoside.

d) Keller killiani test

Dissolve the extract in acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually becomes blue, confirms the presence of deoxy sugar attached to the aglycon part of glycoside.

5. Test for saponins

a) Foam test

About 1ml of alcoholic extract, dilute separately with 20ml of distilled water and shake in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponins.

To 1ml of the extract, add alcoholic vanillin solution and a few drops of concentrated sulphuric acid. A deep violet colour confirms the presence of saponins.

6. Test for flavonoids

a) Shinoda test

To 1ml of the extract, add magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of pink or red colour shows the presence of flavonoids.

To 1ml of extract, add 1ml of ferric chloride, the formation of brown colour confirms the presence of flavonoids.

7. Test for triterpenoids

a) Dissolve two or three granules of tin metal in 2ml of thionyl chloride solution. Then add 1ml of the extract into test tube. The formation of a pink colour indicates the presence of triterpenoids.

8. Detection of phenolics and tannins

a) Ferric chloride test

The extract was treated with few drops of neutral ferric chloride solution. The formation of bluish black colour indicates the presence of phenolic nucleus.

b) Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicates the presence of tannins.

c) Lead acetate test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

9. Test for protein and amino acid

a) Biuret test

To 1ml of the extract ad 1ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution. Formation of violet colour indicates the presence of protein.

b) Ninhydrine test

Add two drops of freshly prepared 0.2% Ninhydrine reagent to the extract solution and heat. Development of a purple colour reveals the presence of proteins and amino acids.

c) Xanthoprotein test

To 1ml of the extract add 1ml of concentrated nitric acid. The formation of white precipitate confirms the presence of amino acid.

10. Test for fixed oils

a) Spot test

Press a small quantity of extract between two filter paper. Oil stains on paper indicates the presence of fixed oil.

6.4 Animal selection (Kulkarni SK, *et al*., 1993)

Wistar albino rats of either sex weighing between 150 and 200g were selected for acute toxicity studies and for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions of temperature (22 ± 3^0C) and maintained on 12:12h light: dark cycle. They were provided with regular rat chow and distilled water *ad libitum*. The animal care and

experimental protocols were in accordance with CPCSEA / IAEC.

6.5 Chemicals used (Yasui T, *et al*. 2001)

Ethyleneglycol (ARGrade) was obtained from Merck Laboratories, Mumbai, India., Cystone were used as standard antiurolithiatic drug.

6.6 Extracts used

EE of Rhusmysorensis suspended in distilled water (q.s.) using 2% tween 80 as a suspending agent. The extracts were subjected to acute oral toxicity study and depending upon LD_{50}, the calculated quantity of each extract was given to each animal in corresponding group, once daily, through per oral route.

6.7 Acutetoxicitystudy (Ghosh MN. 1984, Revised Document (Oct-2000) OECD.)

The acute oral toxicity study was carried out as per the guidelines set by organization for economic co-operation and development (OECD) revised draft guide lines 423B ("Up and Down"method) received from committee for the purpose of control and supervision of experiments on animals (CPCSEA),Ministry of Social Justice and Empowerment, Government of India. TheLD_{50} cut-off dose for EE are $1/5^{th}$and$1/10^{th}$ of the LD_{50} dose was taken as a therapeutic dose.

- **Principle of the test**

It is the principle of the test that based on a step wise procedure with the use of a minimum number of animals per step; sufficient in formation is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a step wise procedure, each step using three animals of a single sex (normally females).Absence or presence of compound related mortality of the animals dosed at one step will determine the next step.

- No further testing isrequired.
- Dosing of three additional animals with the same dose.
- Dosing of three additional animals at the next higher or the next lower dose level.

The method enables adjustment with respect to classifying the test substances to one of the series of toxicity classes (i.e. GHS classification) defined by fixed LD_{50}cut-off values.

- **Housing and feeding condition**

The temperature in the experimental animal room was kept 22±3^{0}C. Artificially lighting was maintained for 12:12h light: dark cycle. The animals were provided with regular rat chow and distilled water ad libitum.

- **Preparation of animals**

The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

- **Preparation and administration of doses**

All the doses were prepared in distilled water using 2% tween 80 as suspending agent. In all cases the concentrationswere prepared in1ml/100g of b.w. The test substances were administered in a single dose using a gastric incubation tube after fasting for 3 to 4 h.

- **Number of animals and dose levels**

In each steps three animals were used. Since there was no information on the substance to be tested (i.e. extract), starting dose was selected to be 200 mg/kg b.w. and 400 mg/kg b.w.

- **Observations**

Animals were observed initially after dosing atleast once during the first 30min, periodically during the first 24h. In all cases death was observed with in first 24h. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato motor activity and behavioural pattern were also done. Attention was also given to observations of tremors and convulsions.

6.8 Screening method/model used

- **Ethyleneglycol induced urolithiasis model in albino rats (**Baheti, *et al*., 2013)

Animals were divided in 5 groups containing six in each. All animals had free access to regular rat chow and drinking water *ad libitum* for 28 days. Renal calculi were induced in group II to V by supplementing with 0.75%v/v ethylene Glycol and ammonium chloride in drinking water ad libitum. Group IV to V were treated with plant extracts starting from 1stday to 28thday (**Preventiveregimen**).

TableNo.3: Protocol for Antiurolithiatic activity.

Group	Status	Induction of urolithiasis	Treatment
I	**Normal**	**---**	**Vehicle.**
II	Calculi induced	0.75%v/v Ethyleneglycol in distilled water daily for 28days.	Vehicle.
III	Standard drug treated	0.75%v/v Ethyleneglycol in distilled water daily for 28days.	Cystone (750mg/kgb.w., p.o.) from1stdayto28th
IV	Ethanolic extract treated	0.75%v/v Ethyleneglycol in distilled water daily for 28days.	ethanolic extract (200mg/kg) b.w ., p.o.) from1stdayto28th
V	Ethanolic extract treated	0.75%v/v Ethyleneglycol in distilled water daily for 28days.	ethanolic extract (400mg/kg) b.w ., p.o.) from1stdayto28th

- **Collection and analysis of urine**

Urine samples (24h) were collected on 28thday (Fig.9). A drop of concentrated HCl was added to the urine before being stored at 4^{0}C. Urinary calcium, phosphate and oxalate (Sumathi R, *et al*., 1993) content were determined. Urine was centrifuged to the pool crystals and observed underlight electron microscope at 5X or 10X. Size and shape of crystals were observed and reported.

- **Serum analysis**

After the experimental period, the animals were sacrificed by spinal dislocation under conditions and blood was collected from the retro orbital puncture. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine, uric acid calcium and urea. (Huang HS, *et al*., 2002).

- **Histopathology (**Anand R, *et al*., 1996)

The abdomen was cut open to remove either kidney from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. One of the isolated kidneys was then embedded in paraffin using conventional methods and cut in to 5µm thick sections and stained using hematoxylineosin dye and finally mounted in diphenyl xylene. Then the sections were observed under microscope for histopathological changes in kidney architecture and their photo micrographs were taken.

RESULTS

Percentage (%) Yield

The extract showed maximum yield (9.23%w/w). On successive solvent extraction with solvents like ethanol.

Table No.3: Percent yield of extract of *Rhusmysorensis*.

Extract	Yield(%w/w)
Crudeextract:	
EE	9.23

7.2 Phytochemical Analysis

TableNo. 3: Phytochemical Analysis of ethylene extract of *Rhus Mysorensis.*

S.NO.	TEST	RESULT
1.	**ALKALOIDAL TEST** a.Dragondroffs test b.Mayer's test c.Wagner's test d. Hager's test	 Positive Positive Positive Positive
2.	**CARBOHYDRATES TEST** a. Molish's test b. Fehling's test c. Benedict's test d. Baeford's test	 Positive Positive Positive Positive
3.	**STEROIDS TEST** a. LibermannBuchard test b. Salwoski test	 Negative Negative
4.	**GLYCOSIDES TEST** a. Legal test b. Baljet test c. Killerkilaini test d. Borntagers test	 Positive Positive Positive Positive
5.	**SAPONINS TEST** a. Foam test	 Positive
6.	**FLAVONOIDS TEST** a. Shinoda test	Posittive
7.	**TRITERPINOIDAL TEST**	Negative
8.	**TANNINS TEST** a. Ferric chloride test b. Gelatin test c. Lead acetate test	Positive Positive Positive
9.	**PROTIEN& AMINOACIDS TEST** a. Buret's test b. Ninhydrin test c. Xanthoprotic test	Positive Positive Positive

7.3 Pharmacological investigations

7.3.1Acute toxicity study

Acute toxicity study was carried out according to OECD guidelines. The LD_{50}cut-off doses obtained for various extractsare enlisted in Table.

TableNo.4: Acute oral toxicity study of various extracts of *Rhus mysorensis*.

Extract	GHSClassification	LD_{50}cut-offdose	Therapeuticdose*
EE	Class 4	2000 mg/kg b.w.	200 mg/kg b.w.
EE	Class 4	2000 mg/kg b.w.	400 mg/kg b.w.

*$1/5^{th}$ $1/10^{th}$of this LD_{50} dose were taken as therapeutic dose for subsequent pharmacological screenings viz. antiurolithiatic activity.

Table 9: Effect of rhus mysorensis against ethylene glycol induced urolithiasis in wistar rats urine parameters.

Parameters	Animal number	Group				
		Normal	Control	Standard	T1	T2
CALCIUM (mg/dl)	1	5.1	7.19	4.0	4.26	3.67
	2	4.98	6.56	3.26	4.56	3.27
	3	5.56	5.30	4.56	4.68	4.82
	4	4.43	4.26	3.26	4.39	4.48
	5	5.28	5.21	3.5	4.16	4.66
	6	4.16	4.83	4.83	3.43	4.37
Inorganic phosphate	1	0.17	0.86	0.16	0.58	0.49
	2	0.10	0.88	0.41	0.86	0.46
	3	0.19	0.83	1.75	0.66	0.48
	4	0.26	0.93	0.40	0.58	0.54
	5	0.8	0.74	1.56	0.75	0.40
	6	1.87	0.78	0.76	0.64	0.51
Uric acid	1	10.11	25.56	9.68	20.18	16.33
	2	12.3	25.8	9.75	12.46	15.66
	3	8.07	18.33	9.17	19.46	14.01
	4	7.68	14.08	8.96	18.59	11.35
	5	8.58	12.98	8.19	22.94	11.76
	6	6.08	14.56	7.59	21.38	12.8
Oxalate Mg/dl	1	1.73	2.91	0.81	0.85	0.43
	2	1.56	4.18	0.75	1.08	0.81
	3	0.70	2.92	0.81	0.76	0.76
	4	0.62	6.10	0.82	0.81	0.44
	5	1.61	1.81	0.57	0.62	0.93
	6	2.78	0.78	0.80	0.93	0.89

Table 10: Effect of rhus mysorensis against ethylene glycol induced urolithiasis (serum parameters.

Parameters	Animal number	Group				
		Normal	Control	Standard	T1	T2
CALCIUM (mg/dl)	1	5.34	4.56	4.0	4.26	3.67
	2	6.56	5.63	3.26	4.56	3.47
	3	5.56	4.30	4.56	3.68	4.28
	4	5.34	4.26	4.26	4.39	3.48
	5	5.28	4.21	3.5	3.16	3.66
	6	5.16	4.83	3.83	3.33	3.47
Inorganic phosphate	1	1.29	0.85	0.63	0.88	0.69
	2	0.84	0.98	0.51	0.86	0.66
	3	0.89	0.83	0.75	0.66	0.81
	4	0.54	0.93	0.40	0.58	0.54
	5	1.08	0.54	0.56	0.75	0.40
	6	0.87	0.48	0.76	0.64	0.51
Uric acid	1	2.47	4.16	1.68	2.18	1.33
	2	2.83	2.58	1.75	2.46	1.66
	3	3.07	3.33	1.07	1.46	1.01
	4	2.68	4.08	2.96	2.59	1.35
	5	2.58	2.98	1.19	2.94	1.76
	6	3.08	4.56	1.59	1.38	1.8
Creatinine Mg/dl	1	0.73	0.91	0.81	0.85	0.83
	2	0.85	1.18	0.75	1.08	0.81
	3	0.70	0.92	0.81	0.76	0.76
	4	0.62	0.10	0.82	0.81	0.74
	5	0.61	0.81	0.57	0.62	0.93
	6	0.78	0.78	0.80	0.93	0.89

A) Urine analysis

- **Microscopic examination of Urine**

The microscopic examination (at 5X and 10X), the urine of normal group animals was devoid of any crystal or similar structure (Fig.5a). In calculi induced rats (GroupII), the urine sample showed abundant, large

- crystals of CaOx with characteristic rectangular shape (Fig.5b). The Cystone treated animals showed very less or almost dissolved small crystals (Fig.5c). On preventive treatment, showed better prevention of stone formation along with the dissolution of preformed stones (Fig.5dand5e).

- **Urine biochemistry and Urine excretion**

24h urinary excretion of oxalate, calcium and phosphate and uric acid in normal and

experimental animals. Ethyleneglycol feeding for 28days resulted in significant (P<0.001) hyperoxaluria as compared tocontrol animals (GroupI). On prophylactic treatment, with *R. mysorensis*, a significant (P<0.05) body weights were decreased, volume of urine excreted was increased urine pH was decreased, kidney weights were decreased in treated animals compared with control animalsa significant (P<0.05) lowering of elevated urinary levels of oxalate, calcium and phosphate and uric acid was seen; The results were significantly (P<0.05) comparable to those of cystone treated animals

B) Serum analysis

The serum uricacid, calcium, inorganic phosphate were remarkably increased in calculi-induced animals Serum creatinine was only slightly elevated in-group II. prophylactic treatment, with *Rhusmysorensis* the elevated serum levels of creatinine (P<0.001) uricacid, calcium and phosphate (P<0.05) were significantly r e d u c e d . However, the results were significantly (P<0.05) comparable to those of cystone treated animals.

C) Histopathology

The histopathological study of the kidney sections also supported the above results. In all the stone forming rats there was damage to the last part of the nephron, collecting system and peritubular interstitium as compared to the normal rat kidney architecture (Fig.6a). The tubules appeared focally ecstatic and surrounded by inflammatory in filtration (Fig.6b). Flattened epithelium with focal vacuolar degeneration and single cell necrosis bordered the tubules, which focally contained hyaline casts. Inflammatory infiltration was mainly composed of mature lymphocytes infiltrating tubular epithelium. Irregular crystals were present inside the tubules and in the peritubular interstitium, along the nephron and at papillary level. The treated groups (Fig. 6d, e) showed normal histology of the kidney, and shows normal glomeruli, slight oedema of the tubular cells. However, the renal tubular epithelial recovery was less significant compared to standard drug treated animals (Fig.6c).

TableNo.11: Effect of ethanolic extract of Rhusmysorensis on various physical parameters in ethylene glycol induced urolithiasis preventive study.

PARAMETRS	NORMAL	CONTROL	STANDARD	T1	T2
CHANGE IN BODY WEIGHT (gm)	251.33±4.96	271.33±4.69	215.45±3.59$^{\#}$	243.33±2.02**	250±4.21*
VOLUME OF URINE IN (ml)	18.22±0.21	16.14±0.42$^{\#\#}$	26.11±0.42*	22.42±0.70*	24.25±0.42*
URINE pH	6.3±0.18	8.15±0.21$^{\#}$	7.18±0.21*	7.46±0.16	7.28±0.21**
KIDNEY WEIGHT (gm)	0.82±0.03	1.38±0.08$^{\#}$	0.72±0.02*	0.78±0.02*	0.73±0.01*

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control.

Highly significant difference from normal p ≤ 0.05.

Significant difference from normal p < 0.01.

TableNo.12: Changes in kidney retention of stone forming constituents in control and experimental animals in urine.

PARAMETRS	NORMAL	CONTROL	STANDARD	T1	T2
CALCIUM (mg/dl)	5.1±0.24	7.19±0.53#	4.14±0.37**	5.14±0.23*	4.36±0.22*
OXALATE (mg/dl)	1.56±0.34	6.23±0.17#	0.55±0.05*	2.83±0.30*	2.56±0.20*
INORGANIC PHOSPHATE (IP) (mg/dl)	0.17±0.01	0.86±0.05#	0.16±0.01*	0.58±0.08**	0.48±0.07*
URIC ACID (mg/dl)	10.11±0.51	25.56±0.76#	9.18±0.43* *	20.64±0.84**	16.28±0.90*

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control.

Highly significant difference from normal p ≤ 0.05.

Significant difference from normal p < 0.0.

TableNo.13: Changes in serum parameters in control and experimental animals.

PARAMETES	NORMAL	CONTROL	STANDARD	T1	T2
CALCIUM (mg/dl)	4.26±0.15	5.34±0.21##	3.47±0.13*	4.56±0.36	3.67±0.23**
INORGANIC PHOSPHATE (I.P.) (mg/dl)	0.85±0.01	1.29±0.09##	0.63±0.02*	0.88±0.05	0.69±0.09*
URIC ACID (mg/dl)	2.47±0.19	4.16±0.25#	1.68±0.13*	2.18±0.26*	1.33±0.16*
Creatinine (mg/dl)	0.73±0.01	0.91±0.01	0.81±0.01***	0.85±0.01***	0.83±0.01***

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control.

Highly significant difference from normal p ≤ 0.05.

Significant difference from normal p < 0.01.

CONCLUSION AND DISCUSSION

In the present study, dried powder of *R. mysorensis* was subjected to extraction using 80%v/v ethanol.

The pharmacological screening included evaluation of antiurolithiatic activity using 0.75%

ethyleneglycol and ammonium chloride induced urolithiasis model in male Wistar rats. The kidney stone formation induced in rats,as a result of 28 days chronic administration of 0.75% ethyleneglycol, was significantly inhibited by oral administration of *R. mysorensis*. On prophylactic treatment, *R. mysorensis* extract prevented the urinary stone formation. In conclusion, the presented data indicate that administration of the *R. mysorensis* extract to rats with ethyleneglycol induced lithiasis reduced and prevented the formation of urinary stones. The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. The protective effect against oxalate induced lipid peroxidation may be contributory to the recovery of renal damage. These effects could conclude the antiurolithiatic property of *R. mysorensis*extract.

REFERENCES

1. Abraham PA, Smith CL. Medical evaluation and management of calcium nephrolithiasis: clinical implications of basic science research. J Pediatr, 2013; 68: 281-99.
2. Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with image. J Biophotonics Int, 2004; 11: 36–42.
3. Akerele O, Nichols CG. WHO's traditional medicine programme: progress and prospectives, WHO Chronicle, 1984; 38: 76-81.
4. Akerele O. Nature's Medicinal Bounty: Don't throw it away. World Health Forum, WHO, Geneva, 1993; 14: 390-95.
5. Anand R, Patnaik GK, Kulshrestha DK, Dhawan BN. Activity of certain fractions of Tribulusterrestris fruits against experimentally induced urolithiasis in rats. Indian Exp. Biol., 2014; 32: 548-52.
6. Anderson EE, Rundles RW, Silberman HR, Metz EN. Allopurinol control of hyperuricosuria: a new concept in the prevention of uric acid stones. J.Urol, 2008; 97: 344-7.
7. Anderson KR, Keetch DW, Albala DM, Chandhoke PS, Ciennan BL, Clayman RV. Optimal therapy for the distal ureteral stone: extracorporeal shock wave lithotripsy versus ureteroscopy. J Urol, 2004; 152: 62-65.
8. Ani V, Varadaraj MC, Naidu KA. Antioxidants and antibacterial activities of polyphenolic compounds from bitter cumin (Cumin nigrum L.). European Food Res. Technology, 2006; 224: 109–115.

9. Assimos DG, Boyce WH, Harrison LH, Cullough DL, Kroovand RL, Sweat KR. The role of open stone surgery since extracorporeal shock wave lithotripsy. J UroI, 2010; 142: 263-267.
10. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by Herniariahirsuta on experimentally induced nephrolithiasis in rats. BJU International, 2003; 92: 137- 40.
11. Baerheim SA, Scheffer JJ. Natural products in therapy. Prospects, goals and means in modern research. Pharm. Wkly.[Sci.], 2012; 4: 93-103.