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Full Length Original Research Paper

Effect of *Coccinia grandis* on ammonium chloride and ethylene glycol induced urolithiasis in rats

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Abstract:

The hydroalcoholic leaf extract of coccinia grandis was evaluated for its antiurolithiatic property against ammonium chloride and ethylene glycol induced urolithiasis in rats. Albino rats were induced lithiasis by administered with 1% ethylene glycol and ammonium chloride in drinking water for 28 days .Lithiatic animals showed high urinary calcium,phosphate ,oxalate, ,uric acid and creatinine. In other groups after the initial 15 days administration of ethylene glycol with ammonium chloride, the hydroalcoholic leaf extract of coccinia grandis (200mg or 400mg/kg body weight per oral) was administered with ethylene glycol and ammonium chloride from 15 days to 28 days. Plant extracts reduced urinary calcium ,phosphate, oxalate ,uric acid and creatinine .Plant extract by increasing the urine volume, reduce the tendency of crystallization. Histopathological study showed the lithiatic confirmation of micro crystals deposition in kidney section of ethylene glycol treated animals. Plant extract treatment reduced this crystals deposition .These findings confirmed the antiurolithiatic activity of hydroalcoholic leaf extract of coccinia grandis against ethylene glycol and ammonium chloride induced urolithiasis.

Keywords: Antiurolithiatic, coccinia grandis, ethylene glycol with ammonium chloride)

Introduction:

Herbal drugs [1]are the oldest sources for the prevention, medication, treatment and even diagnostic tools known to mankind. Most of the drugs commonly used today are of herbal origin. Herbal drugs are inexpensive and effective with fewer side effects.

A Kidney stone,[2] also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Kidney stones[3] typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size (usually at least 3 millimeters), they can cause obstruction of the ureter. Ureteral obstruction causes postrenal azotemia and hydronephrosis (distension and dilation of the renal pelvis and calyces) and spasm of the ureter. This leads to pain which is felt in the flank, lower abdomen, and groin. Renal colic can be associated with nausea, vomiting, fever, blood in the urine, pus in the urine, and painful urination.

Coccinia grandis (L) J.Voigt plays[4] a major role in the medicinal properties,belongs to the family , Cucurbitaceae. The plant *coccinia grandis* contains active constituents like alkaloids ,flavonoids,phenolic compounds,aminoacids ,lupeol and proteins,which are responsible for

antilithic activity. Mainly the leaf part is used for the diuretic and antilithic activities. In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of *coccinia grandis* leaf extract using ammonium chloride and ethylene glycol induced hyperoxaluria model in rats.

METERIALS AND METHODS

The leaves of *coccinia grandis* were collected from the hills of western ghats of theni district, Tamilnadu. The plant was identified and authenticated. by Mr. Dr. V. PONNUSWAMI, Ph.D., PDF(Taiwan) Dean at Tamilnadu Agricultural University Horticultural College and Research Institute, Periyakulam-625604. Tamilnadu. The voucher specimen was submitted at C.L Baid Metha College of pharmacy, Chennai.

PREPARATION OF PLANT EXTRACT

Aqueous ethanolic extract:

The leaves of *coccinia grandis* were collected, dried, coarsely powdered and used for the extraction purpose. 1 kg of powdered material was evenly packed and thimble was prepared and extraction was carried out in a soxhlet apparatus with a aqueous ethanolic extract that is 50% of alcohol mixed with 50% of water. And was heated until the extraction completes and the extract was removed. They were boiled and the extract was concentrated by vacuum distillation. The concentrated extract was evaporated using rotary evaporator until solvents were removed. The extract was stored in dessicator to avoid contact with atmospheric moisture.

Animal selection:

Healthy adult albino rats of either sex weighing between 150-200gms were selected for the acute oral toxicity study and Anti urolithiatic study. The animals were acclimatized to standard laboratory conditions (temperature: 25±2˚C) and maintained on 12-h light/ dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum. Our Institutional Animal Ethical Committee (IAEC) APPROVAL NO: IAEC/I/05/CLBMCP/2012 dated 28.8.12.

ANIMAL GROUPINGS

Urolithiasis was induced by administration of ethylene glycol and ammonium chloride. Induced lithiatic animals were used to study antilithiatic activity. The animals were divided into five groups, each with 6 rats. Group I act as control group. Group II, act as lithiatic control where animals received 1% ethylene glycol with 1% ammonium chloride in drinking water for 28 days. Group III, animals received 1% ethylene glycol and ammonium chloride in drinking water along with cystone (500 mg/kg body weight) orally for 28 days. Group IV animals received 1% ethylene glycol and ammonium chloride in drinking water along with hydroalcoholic leaf extract of coccinia grandis (400mg/kg body weight) and group V animals received 1% ethylene glycol and ammonium chloride in drinking water along with hydroalcoholic leaf extract of coccinia grandis (200mg/kg body weight) orally for 28 days.

Acute toxicity study(Echobichon DJ 1997)**;-** The acute oral toxicity study was carried out by using OECD 423 guidelines received from Committee for the purpose of control and supervision of Experiments on Animals(CPCSEA). Healthy adult wistar albino rats of either sex, starved over night fasted rats were divided into 5 groups (n=6) and were orally fed with the herbal form. The starting dose level of herbal formulation was 2000 mg/kg

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body weight per oral as most of crude extracts possess LD $_{50}$ value more than 2000 mg/kg per oral. Dose volume was administered 1ml/100 mg body weight to over night fasted rats with water adlibitum. Food with held for further 3-4 hours after administration of herbal formulation and observed continuously for signs of toxicity.. The animals were kept under observation for 14 days.

Collection and analysis of urine.

All animals were kept in individual metabolic cages and urine samples of 24 hr were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4˚ C. Urine was analyzed for calcium, phosphate, oxalate, protein, creatinine and uric acid content..

Serum analysis:-

Blood was collected from retro-orbital under anaesthesia and animals were sacrificed. Separated serum was analysed for creatinine, uric acid and blood urea nitrogen.

Histopathological studies

Weighed and fixed the kidney samples with 10% neutralized formalin. Kidney sections were prepared by fixing in paraffin and stained with hematoxylin and eosin and histopathological changes were observed.

Statistical analysis:-

Results were given as Mean ± SEM from six animals in each group. Comparisons were made by using one way analysis of variance (ANOVA), followed by dunnets t test. P (probability value)< 0.05 was considered as significant.

RESULTS:

Acute oral toxicity study:

Acute oral toxicity was carried out according to OECD guidelines. Plant extracts were safe up to 2000mg/kg.

Urinary analysis:-

The excretion of calcium was increased significantly on 28th day in negative control rats (Group-II) compared with the control group. This calcium excretion was reduced in both cystone treated group and plant extract treated groups. (table 1 and figure 1)

Similarly increase in urinary excretion of oxalate was increased significantly on 28th day in negative control rats (Group-II) compared with the control group. This oxalate excretion was reduced in both cystone treated group and plant extract treated groups. (Table 2 and figure 2)

Similarly increase in urinary excretion of phosphate was increased significantly on 28th day in negative control rats (Group-II) compared with the control group. This phosphate excretion was reduced in both cystone treated group and plant extract treated groups. (Table 3 and figure 3)

Serum analysis:-

The level of creatinine was increased significantly on 28th day in negative control rats (Group-II) compared with the control group. This creatinine level was reduced in both cystonetreated group and plant extract treated groups. (table 4 and figure 4)

The level of serum uric acid was increased significantly on 28th day in negative control rats (Group-II) compared with the control group. This uric acid level was reduced in both cystone treated group and plant extract treated groups. (Table 5 and figure 5)

The level of blood urea nitrogen was increased significantly on 28th day in negative control rats (Group-II) compared with the control group. This blood urea nitrogen level was reduced in both

cystone treated group and plant extract treated groups. (table 6 and figure 6).

Histopathological study

Histopathological observations of hyperoxaluric renal sections showed dilated collecting systems with deposition of crystals. Treatment with hydroalcoholic leaf extracts of coccinia grandis and cystone treatment decreased the number and size of calcium oxalate deposits in different parts of the renal tubules.

Discussion

Urinary supersaturation is one of the main reason responsible for the stone forming constituents.With the first 14 days period of ethylene glycol(0.75%v/v) and ammonium chloride administration, young male albino rats form renal calculi composed mainly of calcium oxalate. And it results in increase in urine concenteration of phosphate and oxalate stones. The main reason for feeding ammonium chloride is to increase the acidosis process which results in the precipitation of salt formation, causes hyperoxaluria, increased renal retention and excretion of oxalate.

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.

Table 1: EFFECT OF HAECG (HydroAlcoholic Extract of Coccinia Grandis) On Urinary Calcium

	GROUP	28TH DAY
Urine Calcium mg/dl	I-Control	2.273 ± 0.580
	II-Negative control	5.700 ±0.603***a
	III-Standard drug(cystone)	2.5133 ±0.588**b
	IV-Lower dose(200mg/kg)	2.466 ±0.317*b
	V-Higher dose(400mg/kg)	2.776 ±0.548*b

Values are expressed as Mean± SEM, n=6Comparison: a-Group I vs. Group II,b-Group II vs Group III, IV, & V;NS Non Significant; *P<0.05, **P<0.01; ***P<0.001.One way ANOVA followed by Dunnet's "t" Test.

Figure No. 1

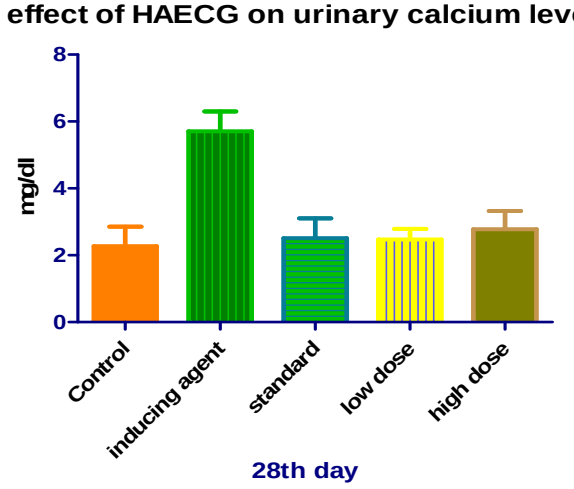


Table 2: Effect of HAECG on Urinary Oxalate

	GROUP	On 28th day
Urinary oxalate mg/dl	I-Control	0.470 ±0.057
	II-Negative control	3.750±0.577**a
	III-Standard drug(cystone)	0.630±0.0577*b
	IV-Lower dose(200mg/kg)	2.293 ± 0.550*b
	V-Higher dose(400mg/kg)	2.213 ± 0.626**b

Figure No 2

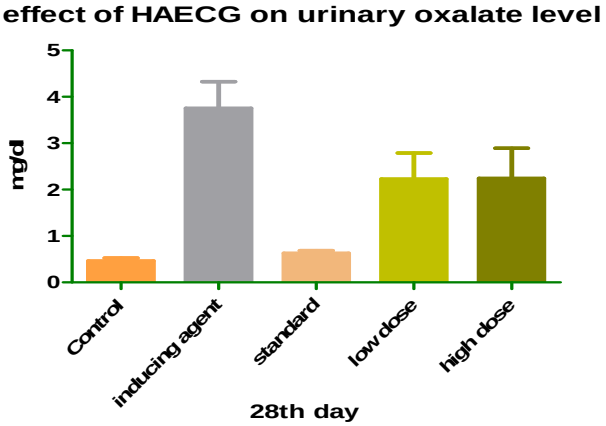


TABLE 3: Effect of HAECG on Urine Phosphates

	GROUP	BEFORE TREATMENT
Urinary Phosphates mg/dl	I-Control	4.706 ±0.607
	II-Negative control	8.250 ± 0.592***a
	III-Standard drug(cystone)	4.706 ± 0.553**b
	IV-Lower dose(200mg/kg)	5.366 ± 0.534***b
	V-Higher dose(400mg/kg)	5.166 ± 0.548***b

Figure No 3

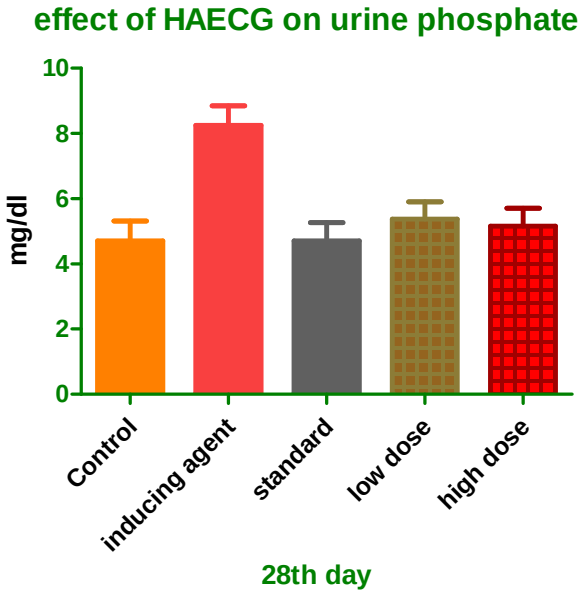


Table 4: Effect of HAECG on Serum Creatinine Level

Group	I-Control	II-Negative control	III-Standard drug (Cystone)	IV-LOWER DOSE (200 mg / kg)	V-HIGHER DOSE (400 mg / kg)
Serum Creatinine IU/100 ml	0.850 ± 0.115	1.040 ± 0.011*a	0.810 ± 0.0057**b	1.020±0.115*b	0.960±0.011*b

Figure 4: Serum Creatinine

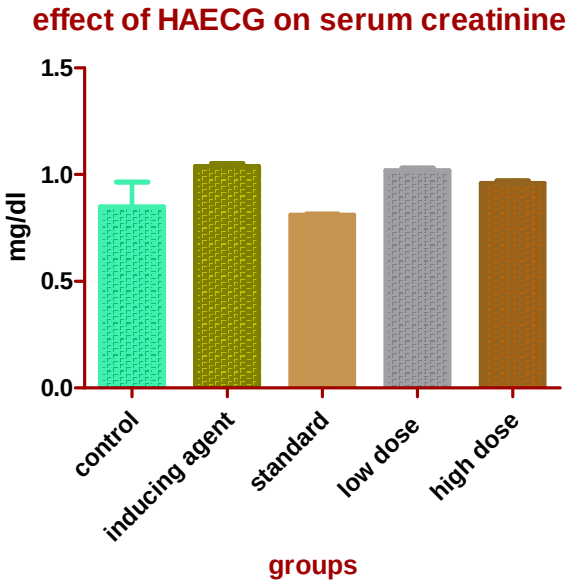


TABLE 5: Effect of HAECG on Serum Uric Acid Level

Group	I-Control	II-Negative control	III-Standard drug(Cystone)	IV-LOWER DOSE(200mg/kg)	V-HIGHER DOSE(400 mg/kg)
Serum Uric acid mg/dl	2.400 ± 0.115	4.556 ± 0.109***a	2.753 ± 0.063*b	3.233±0.088**b	2.930±0.049***b

FIGURE 5: SERUM URIC ACID

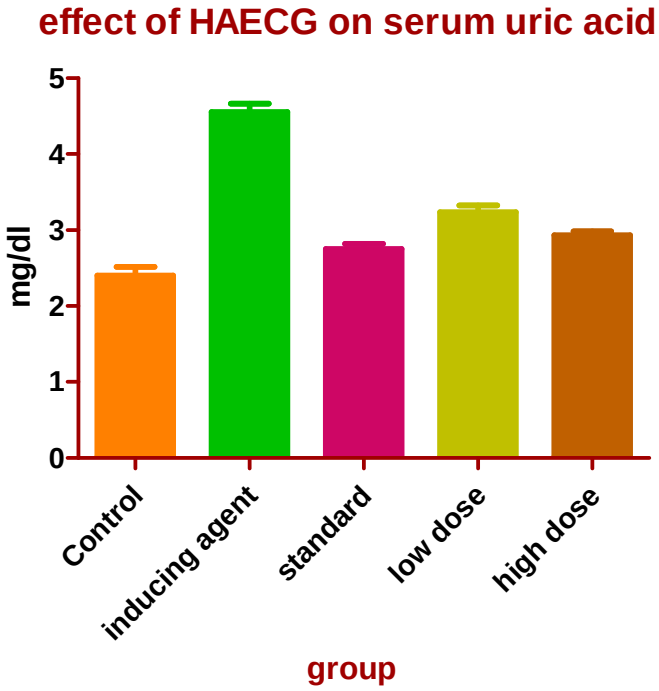


Table 6: EFFECT OF HAECG ON SERUM BUN LEVEL

Group	I-Control	II-Negative control	III-Standard drug (Cystone)	IV-LOWER DOSE (200mg/kg)	V-HIGHER DOSE (400 mg/kg)
Serum BUN mg/dl	39.500 ± 0.472	51.366 ± 0.617***a	41.453 ± 0.647***b	42.523 ± 0.664***b	44.533 ± 0.696***b

Figure 6: SERUM BUN LEVEL

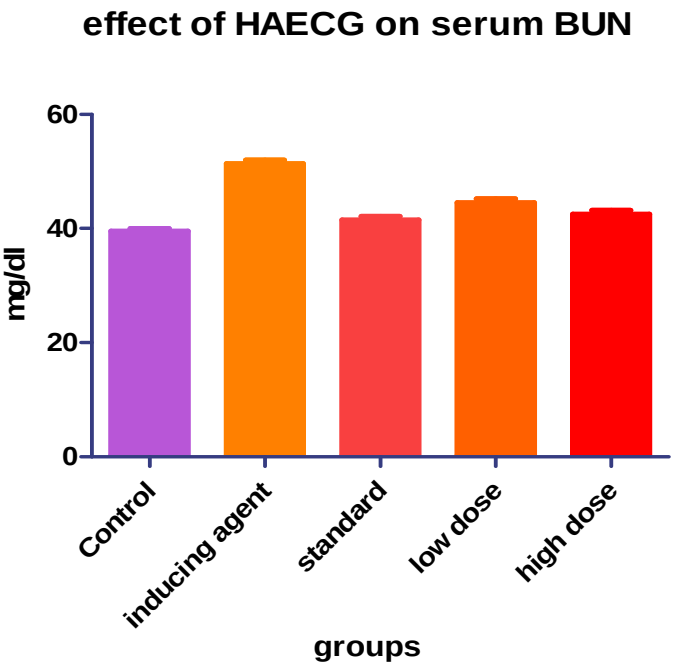
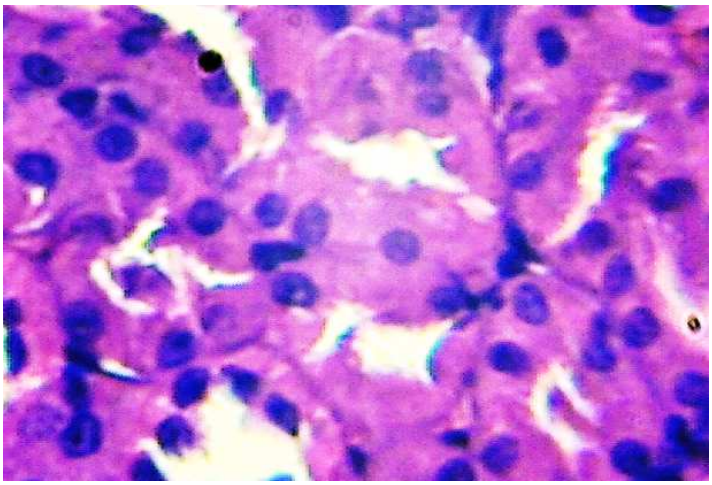
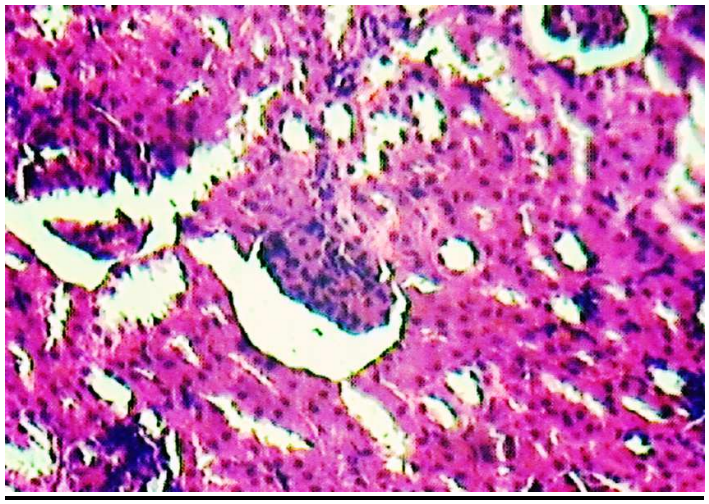


Figure 7: Kidney section-group1-Control animals



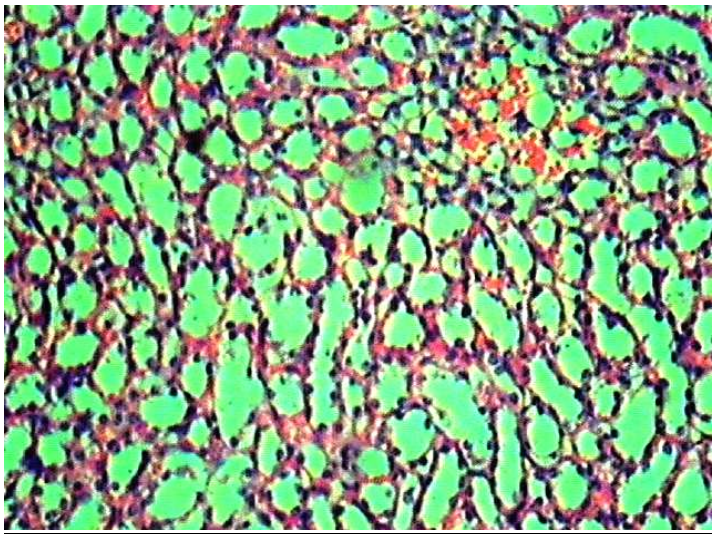
Section show kidney structure with normal glomeruli and tubules

Kidney section of Group 2 -- Negative control animals(showing crystals)

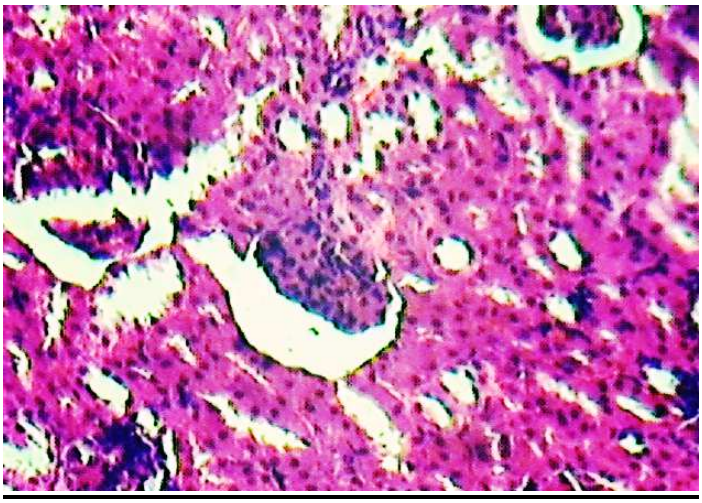


Group 3 - kidney section of animals (STANDARD DRUG TREATED)

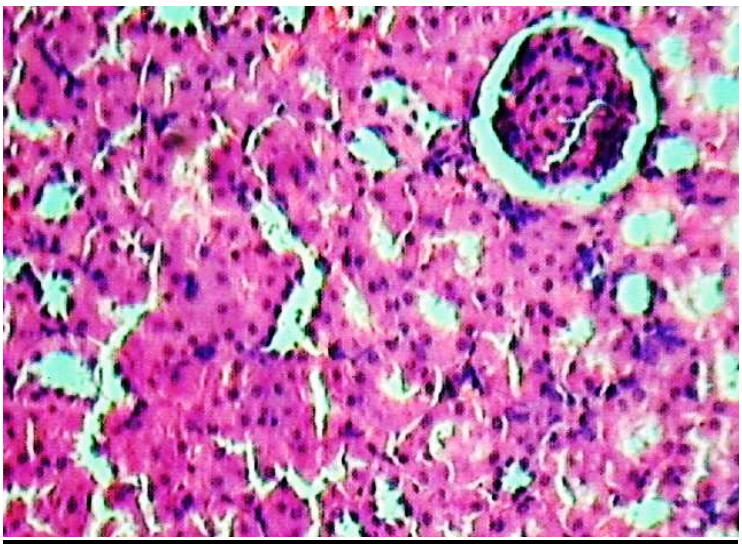
With normal glomeruli and tubules(less crystals in tubular lumen)



Low dose plant extract group animals
With normal glomeruli and tubules(less crystals in tubular lumen)



high dose group animals(no evidence of crystal deposition seen)



Conclusion

In this study we have proved the antiurolithiatic activity of hydroalcoholic leaf extract of coccinia grandis. Further studies are needed to prove antiurolithiatic activity of coccinia grandis leaf extract in other animal models.

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