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EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF *RICINUS COMMUNIS* L. LEAVES

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ABSTRACT: The present investigation was under taken to evaluate the anti-urolithiatic activity of ethanolic extract of *Ricinus communis* leaves in experimental animals. *Ricinus communis* Leaves were evaluated for anti-urolithiatic activity using Urolithiasis is a common disorder with higher recurrence rate in men. Super saturation of crystals with imbalance between levels of promoters and inhibitors of stone formation results in urolithiasis. Current medical management of urolithiasis is either costly or not without side effects. Therefore, traditionally reported more effective and safer anti-urolithiatic medicinal plants need to be studied. Thus, this study was aimed to evaluate the anti-urolithiatic activity of ethanolic extract of *Ricinus communis* leaves in male albino wistar rats. Ethylene glycol (0.75% v/v in drinking water; 28 days) induced urolithiasis preventive model were used to study the effect *Ricinus communis* leaves dose (200 mg/kg) and. Cystone (750 mg/kg) was used as a standard. At the end of the treatment changes in various physical parameters, promoters, inhibitors, renal function markers in urine and serum samples and histopathology of kidneys were observed. All the treatments significantly prevented the rise in promoters like calcium, oxalate, uric acid, and inorganic phosphate and increased the levels of magnesium and citrate like inhibitors in various biological samples. Thus ethanolic extract of *Ricinus communis* leaves have proved to be an effective drug in prevention of urolithiasis.

INTRODUCTION: Uretierolithiasis is the condition where urinary stones are formed or located in the ureter^{1, 2}. Urolithiasis is the most painful urologic disorder is calculi or stone formation in any location within the urinary tract due to imbalance between promoters (calcium, oxalate, uric acid, phosphate) and inhibitors (citrates, magnesium, potassium, pyrophosphate and urinary glycol-protein) of crystallization in urine.

Urolithiasis is one of the painful urologic disorders that occur in approximately 12% of the global population and its re-occurrence rate in males is 70 - 81% and 47 - 60% in female^{3, 4}. Kidney stone formation is a complex that results from a succession of several physicochemical events including super-saturation, nucleation, growth, aggregation and retention within the kidneys⁷.

Urinary stones can be classified according to stone composition as calcium stone, uric acid stone, Struvite stone, cystine stone and Miscellaneous types of stones-Protein matrix stones, Ammonium urate stones, Xanthine stones and Stones composed of drug. The lifetime risk is about 10 - 15% in the developed world, but can be as high as 20 - 25% in the Middle East.

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Urolithiasis is a recurrent renal disease affecting 4 - 8% in UK, 15% in US, 20% in gulf countries and 11% population in India with a relapse rate of 50% in 5 - 10 years and 75% in 20 years^{8,9}. Presently, the available drug therapy for the treatment of urinary stone includes, antibiotics (for struvite stones), allopurinol (for uric acid stone), opiates and NSAID's (for relieving pain), and diuretics (for renal stone removal). For kidney stones that do not pass on their own by pharmacological management, the most widely preferred technique is the lithotripsy. In this procedure, shock waves are used to break up a large stone into smaller pieces that can then pass through the urinary system. In case of failure with all other treatments, surgical invasive techniques have also been used like percutaneous nephrolithotomy or through ureteroscopy. *Ricinus communis* plant is common and quite wild in the jungles in India and it is cultivated throughout India, chiefly in the Madras, Bengal and Bombay presidencies. Leaves green or reddish-green, broad, palmately lobed, with 5 - 11 lobes, 30 - 60 cm^{11,14,15}.

MATERIALS AND METHODS:

Plant Collection and Authentication: The collection of the leaves of *Ricinus communis* L. was done in the month of october from Jodhpur region, Rajasthan. Since the plants will be enriched with phytoconstituents during that time. The identification and authentication of the plant was carried out by Dr. S. L. Meena Scientist C and Botanical survey of India, Jodhpur (Rajasthan) (No.BSI/AZRC/L.12012/Tech./2014-15/Pl.Id./713) was prepared and preserved Botanical survey of India, Jodhpur, Rajasthan, India.

Preparation of Plant Extract: About 75 gm of dried powder was properly kept in thimble and the soxhlet apparatus was set up. The extraction of powder was done with different solvents with solvents of increasing polarities like petroleum ether (60 -80 °C), chloroform, benzene and ethanol. The flask with the given solvent is heated to a particular temperature. The vapour produced passes through the siphon tube into the thimble kept above where it is condensed and tickles down into the flask again through the thimble dissolving the active constituents in it. The method is described as the continuous extraction. The process is continued until all the soluble constituents get separated. The

extract at the bottom was collected and the solvents were removed using reflex condenser and dried on water bath. Each time, before the extraction with other solvents, the powdered substance is air dried.

Experimental Animals: Wistar rats weighing 180-200 gm were brought from National Toxicological Center. They were housed in well ventilated cages (3 to 4 per cage), under standard laboratory conditions of temperature (22 ± 3 °C) and 12/12 hr light/dark cell at least 1 week before experimentation on animals. Animals had free access to standard pellet diet and water *ad libitum*. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA.

Acute Toxicity Study: Dose was selected by using acute toxicity study (OECD, 423). The acute toxicity study for ethanolic extract of leaves of *Ricinus communis* was performed using rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. To find the LD₅₀ of ethanolic extract of leaves of *Ricinus communis*, four groups of rats, containing six in each group, were given *Ricinus communis* in the doses of 1000, 2000 and 4000, mg/kg orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioural changes / mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of leaves of *Ricinus communis* and therefore was found safe up to dose of 2000 mg/kg^{22,23}.

In vivo Anti-urolithiatic Activity:

Ethylene Glycol Induced Urolithiasis Model:¹¹ Ethylene glycol induced urolithiasis was used to access the anti-urolithiatic activity in wistar albino rats. Animals shall have been divided into four groups containing six animals in each. Group 1 served as control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water will be group 2 to 4 for induction of renal calculi till 28 days. Group 3 will be received standard anti-urolithiatic drug (Cystone 750 mg/kg). Group 4 will be received ethanolic extract suspension in water and all extract will be given once daily by oral route.

Antiuro lithiatic Activity: ^{12, 24}

Collection and Analysis of Urine: Animals will kept in separate metabolic cages and urine sample of 24 hrs will collected on day 14 and day 28. A drop of concentrated hydrochloric acid will add to urine before being store at 4 °C. Urine will be analysis for calcium, phosphate, oxalate, protein, creatinine, magnesium and uric acid content.

Serum Analysis: After the experimental period, blood will collected from the retro-orbital under anaesthetic conditions and the animals will be sacrifices by cervical decapitation. Serum will separate by centrifugation at 10,000 rpm for 10 min

and analyzed for calcium, magnesium, phosphate, oxalate, creatinine, uric acid content.

Histopathological Studies: Kidney sample will be weight and fixed rapidly with 10% neutralized formalin (pH 7.4). Section of kidney fixed in paraffin wills prepared and stained with haematoxylin and eosin observed for histopathological changes.

RESULT: The results of the various experiments conducted so for reveal the following:

Phytochemical Investigation: Table 1.**TABLE 1: QUALITATIVE CHEMICAL ANALYSIS OF PETROLEUM ETHER, CHLOROFORM, BENZENE AND ETHANOLIC EXTRACT OF *RICINUS COMMUNIS* L.**

Extract	Glycosides	Protein	Flavonoid	Alkaloids	Tannins	Carbohydrate	Fats and Oil
Petroleum Extract of <i>R. communis</i> Leaves	-	-	-	-	-	-	+
Chloroform Extract of <i>R. communis</i> Leaves	-	+	-	-	-	+	-
Benzene Extract of <i>R. communis</i> Leaves	-	+	-	-	-	+	-
Ethanollic Extract of <i>R. communis</i> Leaves	+	+	+	+	+	+	-

(+): Present; (-): Absent.

Pharmacological Evaluation: Anti-urolithiatic effects of the ethanolic extract of the plant *Ricinus communis* L. under study was experimented by following studies.

Result of Biochemical Parameters: Table 2, Table 3, Table 4. The results of the anti-urolithiatic study showed that urine and serum levels of calcium, oxalate, phosphate, protine, uric acid and creatinine were increased from the 14th day onwards following 0.75% ethylene glycol

treatment in Urolithiatic control group (GB) contrary to this magnesium level was reduced followed the same treatment as revealed by the values in GB. Simultaneous treatment with ethanolic extract of *Ricinus communis* L. reduced the above mentioned elevated parameters and elevated the reduced parameter (*i.e.*, magnesium). There is no significant difference between the normal and treatment with ethanolic extract indicating that they are equally potent (**Fig. 1- 6**).

TABLE 2: EFFECT OF *RICINUS COMMUNIS* LINN. EXTRACT ON URINE PARAMETERS IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 14th DAY

S. no.	Treatments	Calcium mg/dl	Phosphate mg/dl	Oxalate mg/dl	Protein mg/dl	Uric acid mg/dl	Magnesium mg/dl	Creatinine mg/dl
1.	Group A	24.06±0.012	12.05±0.325	8.11±0.27	10.04±0.013	4.99± 0.09	6.42±0.14	0.66±5.75
2.	Group B	32.2*±0.15	24.05*±1.058	21.38*±0.408	15.00*±0.099	9.03*±0.009	2.63*±0.008	0.95*±0.15
3.	Group C	25.05**±0.013	14.11**±0.25	12.31**±0.277	13.06**±0.007	5.8**±0.009	6.31**±0.01	0.68**±0.15
4.	Group D	26.8***±0.39	16.98***±0.257	14.43***±0.352	14.04***±0.009	5.9***±0.09	5.03***±0.02	0.75***±0.016

Group A: Normal control; Group-B: Disease control;

Group C: Treated with standard (Cystone);

Group D: Treated with test (*Ricinus communis* L.).

Values are expressed as Mean ± SEM for six animals in each group.

* = Values are significantly different from control.

** = Values are significantly different from lithiatic control, (p < 0.0001).

*** = Values are significantly different from lithiatic control, (p < 0.0001).

Values were finding out by using one way ANOVA followed by Newman – Keuls multiple range test.

TABLE 3: EFFECT OF *RICINUS COMMUNIS* LINN. EXTRACT ON URINE PARAMETERS IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 28th DAY

S. no.	Treatments	Calcium mg/dl	Phosphate mg/dl	Oxalate mg/dl	Protein mg/dl	Uric acid mg/dl	Magnesium mg/dl	Creatinine mg/dl
1.	Group A	26.04±0.011	12.01±0.116	9.13±0.32	10.23±0.009	6.98±0.009	6.15±0.009	1.03±0.009
2.	Group B	36.05*±0.36	30.35*±0.311	26.6*±0.37	15.03*±0.003	12.15*±0.010	2.81*±0.009	1.7*±0.009
3.	Group C	26.82**±0.01	14.03**±0.22	12.15**±0.319	14.03**±0.008	7.03**±0.009	6.4**±0.009	1.28**±0.009
4.	Group D	27.31***±0.01	16.58***±0.33	14.1***±0.32	15.09***±0.008	7.98***±0.007	5.8***±0.009	1.90***±0.009

Group A: Normal control;

Group B: Disease control;

Group C: Treated with standard (Cystone);

Group D: Treated with test (*Ricinus communis* L.).

Values are expressed as Mean ± SEM for six animals in each group.

* = Values are significantly different from control.

** = Values are significantly different from lithiatic control, (p < 0.0001).

*** = Values are significantly different from lithiatic control, (p < 0.0001).

TABLE 4 EFFECTS OF *RICINUS COMMUNIS* LINN. EXTRACT ON SERUM PARAMETERS IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 28th DAY

S. no.	Treatments	Calcium mg/dl	Phosphate mg/dl	Oxalate mg/dl	Uric acid mg/dl	Magnesium mg/dl	Creatinine mg/dl
1.	Group A	11.26±0.008	5.28±0.29	2.03±0.28	3.25±0.007	6.07±0.01	0.32±0.009
2.	Group B	23*±0.53	15.16*±0.26	9.16*±0.36	3.90*±0.008	3.02*±0.007	0.62*±0.008
3.	Group C	11.53**±0.14	7.16**±0.30	4.**±0.28	3.47**±0.007	5.34**±0.007	0.41**±0.007
4.	Group D	13.07***±0.010	8.33***±0.30	4.08***±0.23	3.77***±0.007	4.20***±0.009	0.46***±0.009

Group A: Normal control;

Group B: Disease control;

Group C: Treated with standard (Cystone);

Group D: Treated with test (*Ricinus communis* L.).

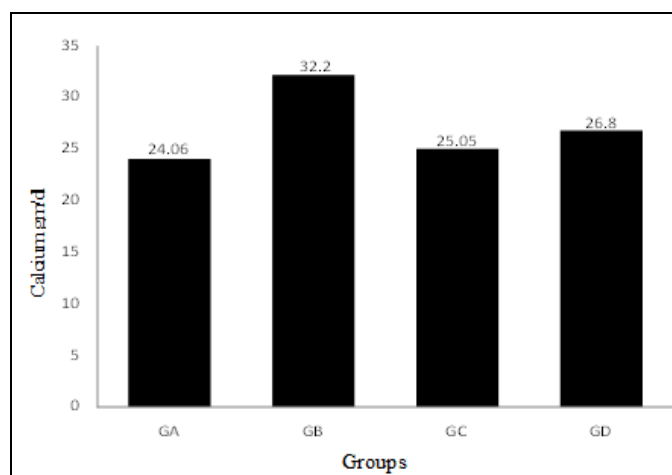
Values are expressed as Mean ± SEM for six animals in each group.

* = Values are significantly different from control.

** = Values are significantly different from lithiatic control (p < 0.0001).

*** = Values are significantly different from lithiatic control (p < 0.0001).

Values were finding out by using one way ANOVA followed by Newman - Keuls multiple range test.

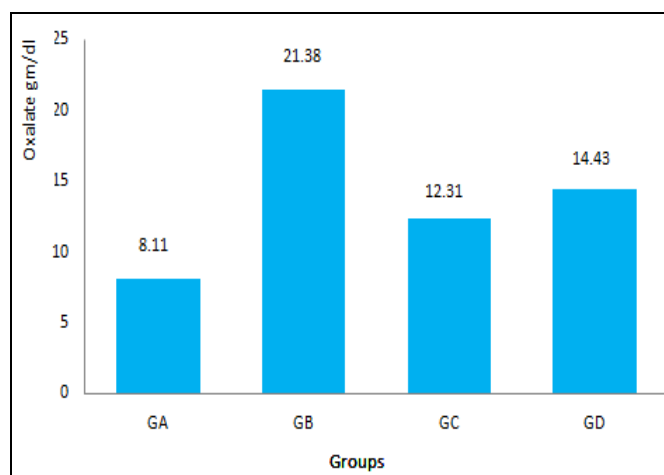
**FIG. 1: EFFECT OF *RICINUS COMMUNIS* L. ON URINE LEVEL OF CALCIUM (mg/dl) IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 14th DAYS**

Group A: Normal control;

Group B: Disease control (0.75% Ethylene glycol);

Group C: Standard (0.75% Ethylene glycol + cystone);

Group D: Treatment (0.75% Ethylene glycol + *Ricinus communis* L.)

**FIG. 2: EFFECT OF *RICINUS COMMUNIS* L. ON URINE LEVEL OF OXALATE (mg/dl) IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 14th DAYS.**

Group A: Normal control;

Group B: Disease control (0.75% Ethylene glycol);

Group C: Standard (0.75% Ethylene glycol + cystone);

Group D: Treatment (0.75% Ethylene glycol + *Ricinus communis* L.)

Histopathological Studies: There were no histopathological changes in tubules, glomeruli and blood vessels in normal group rats (GA). The histopathology of lithiatic group which are the treated with EG (0.75% v/v) sample of kidney showing crystals in animals. These groups also showed congestion and inflammation. On 28th day of treatment the histopathology of kidney samples of rats treated with EG 0.75% v/v and standard drug cystone (750 mg/kg) were showing a mild congestion of the glomeruli. After treatment with *Ricinus communis* L. (200 mg/kg) upto 28 days. The histopathology of kidney sample showed with mild cloudy changes and inflammation (Fig. 7-10).

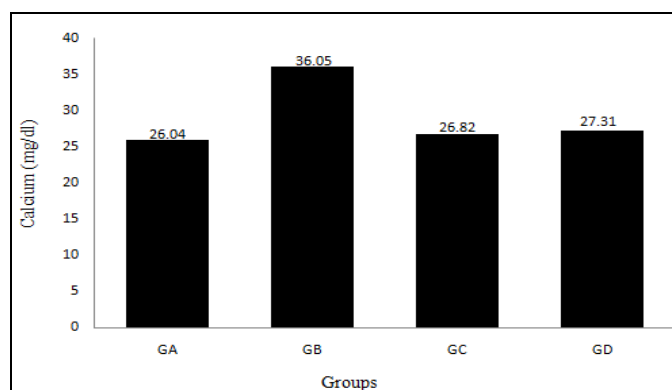


FIG. 3: EFFECT OF *RICINUS COMMUNIS* L. ON URINE LEVEL OF CALCIUM (mg/dl) IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 28th DAY

Group A: Normal control;
Group B: Disease control (0.75% Ethylene glycol);
Group C: Standard (0.75% Ethylene glycol + cystone);
Group D: Treatment (0.75% ethylene glycol + *Ricinus communis* L.)

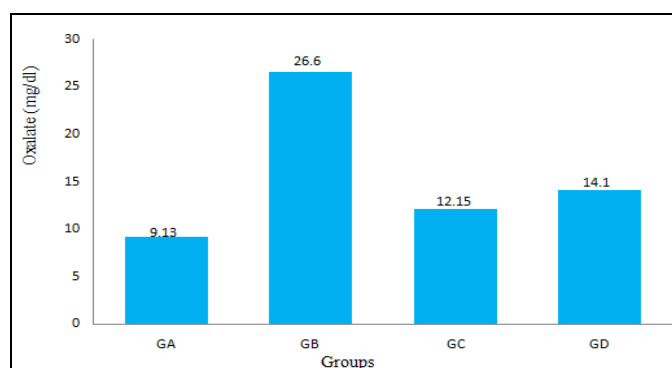


FIG. 4: EFFECT OF *RICINUS COMMUNIS* L. ON URINE LEVEL OF OXALATE (mg/dl) IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 28th DAY

Group A: Normal control;
Group B: Disease control (0.75% Ethylene glycol);
Group C: Standard (0.75% Ethylene glycol + cystone);
Group D: Treatment (0.75% Ethylene glycol + *Ricinus communis* L.)

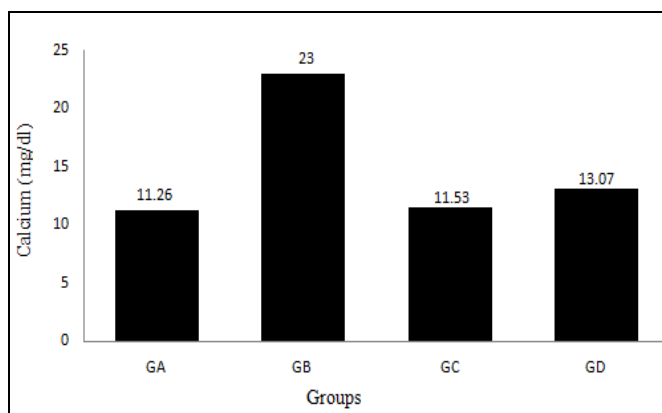


FIG. 5: EFFECT OF *RICINUS COMMUNIS* L. ON SERUM LEVEL OF CALCIUM (mg/dl) IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 28th DAYS

Group A: Normal control;
Group B: Disease control (0.75% Ethylene glycol);
Group C: Standard (0.75% Ethylene glycol + cystone);
Group D: Treatment (0.75% ethylene glycol + *Ricinus communis* L.)

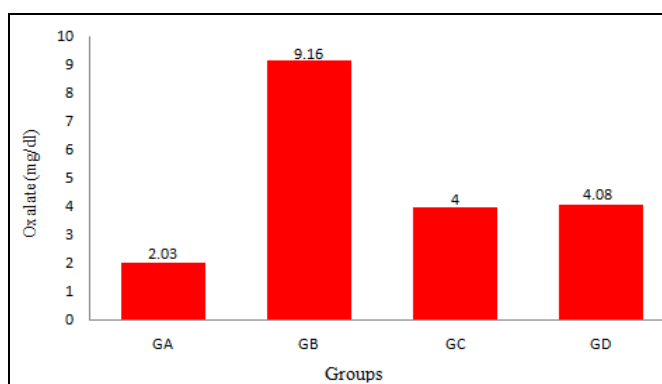


FIG. 6: EFFECT OF *RICINUS COMMUNIS* L. ON SERUM LEVEL OF OXALATE (mg/dl) IN 0.75% ETHYLENE GLYCOL TREATED RATS AFTER 28th DAYS

Group A: Normal control;
Group B: Disease control (0.75% Ethylene glycol);
Group C: Standard (0.75% Ethylene glycol + cystone);
Group D: Treatment (0.75% Ethylene glycol + *Ricinus communis* L.)



FIG. 7: KIDNEY SECTION OF THE GA (NORMAL CONTROL) RAT. NO HISTOPATHOLOGICAL CHANGES IN TUBULES, GLOMERULI AND BLOOD VESSELS

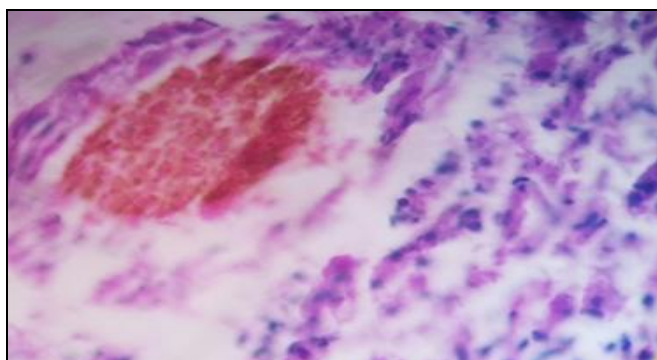


FIG. 8: KIDNEY SECTION OF THE GB (URO-LITHIATIC CONTROL) RAT FROM EG (0.75% v/v) SHOWING CRYSTALS, CONGESTION AND INFLAMMATION

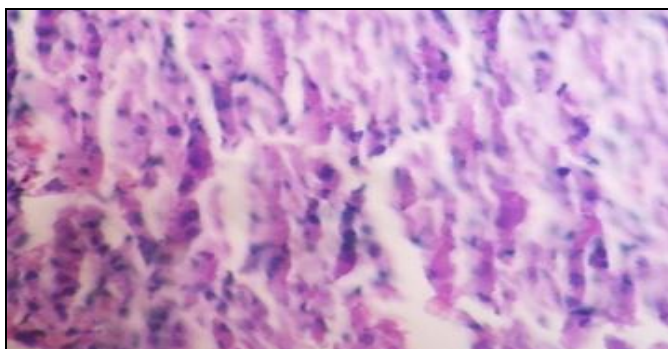


FIG. 9: KIDNEY SECTION OF THE GC (TREATED WITH CYSTONE) RAT FROM EG 0.75% v/v AND STANDARD DRUG CYSTONE (750 mg/kg) SHOWING MILD CONGESTION OF THE GLOMERULI

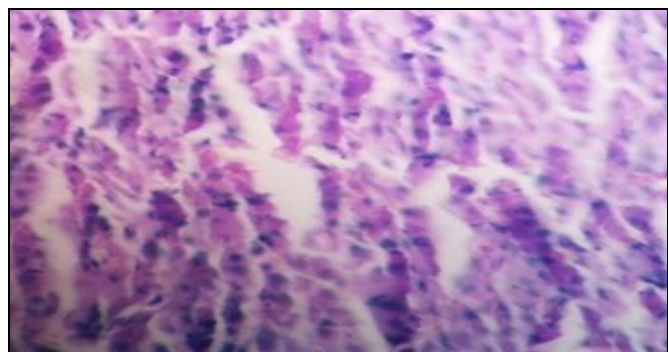


FIG. 10: KIDNEY SECTION OF THE GD (TREATED WITH *R. COMMUNIS* L.) RAT FROM *R. COMMUNIS* L. (200 mg/kg) SHOWING MILD CLOUDY CHANGES, CONGESTION OF INTERSTITIUM AND INFLAMMATION

DISCUSSION: As traditional medicinal are usually taken by the oral route, same route of administration was used for evaluation of anti-urolithiatic effect of the *Ricinus communis* against ethylene glycol induced urolithiasis in rats. The discoveries of the clinical roles of these herbal remedies have made important contributions to the treatment of urinary stone disease as an alternative or adjunct therapy.

Sex hormones have substantial effect on crystal formation in the rat kidney through oxalate metabolism and oxidative cell damage. Testosterone is a promoter and estradiol an inhibitor of such crystal formation. Kidney stone formation or urolithiasis is a complex process that results from a succession of several physico-chemical events including super-saturation, nucleation, growth, aggregation and retention within renal tubules.

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male wistar rats resulted in Hyperoxaluria. Oxalate and calcium excretion were grossly increased in calculi induced animals. Since it is accepted that Hyperoxaluria is a for more significant risk factor in the pathogenesis of renal stones that hypercalciuria, the changes in urinary oxalate levels are relatively much more important than those of calcium. Increase urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth.

From the results of biochemical parameters and histopathological studies, it is obvious that the plant *Ricinus communis* L. has got inhibiting on the calcium oxalate crystal formation so it can be effectively used in animal modal to treat urolithiasis.

Chemical constituents of ethanolic extract of leaves of *Ricinus communis* L. reports as a diuretic in managment. Alkaloids, glycosides, tannins, phenolic compounds, triterpenoids *etc.* are responsible for diuretic activity. Mainly flavanoids and tannins are show the machanism of diuretic activity. These chemical constituents increase the urine output as well as urinary electrolyte concentration. Chemical constituents of leaves of *Ricinus communis* also show antioxidant activity. Mainly flavanoids are responsible for antioxidant activity. The evaluation of calcium and oxalate indicates that these two ions contribute significantly to stone formation. It has been reported, that 90% of all the stones analyzed contain calcium and that 50% to 65% contain mixture of both calcium oxalate and phosphate. Magnesium has an inhibitory action on stone

formation. Magnesium complexes with oxalate, the reducing calcium oxalate super saturation in urine. As a result growth and nucleation rate of calcium oxalate crystals were reduced. The increase in urinary uric acid excretion was 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 crystal 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 of *Ricinus communis* lowered the excretion of uric acid and reduced the risk of stone formation. In urolithiasis, the glomerular filtration rate (GFR) decrease due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous and uric acid get accumulated in blood.

CONCLUSION: The presented data indicate that administration of ethanolic extract of *Ricinus communis* L. Leaves to the rats with ethylene glycol induced urolithiasis reduced the formation of urinary stones and increase the total urinary output. Exact mechanism underlying this effect is not clear, but apparently related to diuretic, antioxidant effect and lowering of the stone forming constituents and suggested to explore the exact pharmacology of the drug and the study was closed with delight.

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CONFLICT OF INTEREST: Nil.

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