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Antiurolithiatic Activity of *Gum arabic* on Ethylene Glycol Induced Urolithiasis in Rat

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ABSTRACT

Renal stone disease (urolithiasis, nephrolithiasis) represents stone formation in kidney, ureters or bladder. The common component of urinary stone is calcium oxalate (CaOx). *Gum arabic* is an edible, and branches of *Acacia senegal* and *Acacia seyal*, that is rich in non-viscous soluble fiber. Male Wistar rats (150–200 g) were selected and divided into four groups. Ethylene glycol (0.75%) in drinking water was fed to all groups till 28th day to induce calculi. Group–3, 4 served as test group treated with *Gum Arabic (250mg/kg & 500mg/kg)* and group 2 standard (cystone 750 mg/kg) respectively. Blood sample collected and estimate in Serum BUN, creatinine calcium, phosphates and uric acid were also recorded. Body weight, kidney weight and urine volume were also monitored. Histological examinations of the fixed tissue sample. Statistical analysis was performed as the mean± standard deviation (SD).

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INTRODUCTION

Kidney stones are hard, solid particles that form in the urinary tract. In many cases, the stones are very small and can pass out of the body without any problems. However, if a stone (even a small one) blocks the flow of urine, excruciating pain may result, and prompt medical treatment may be needed. Renal stone disease (urolithiasis, nephrolithiasis) represents stone formation in kidney, ureters or bladder. This also includes metabolic disorders, anatomical imperfections of the upper or lower urinary tract, and chronic urinary infection. Individuals consuming diets rich in animal protein (meat, fish and poultry) are at increased risk of stone formation than those on vegetarian diet.[1]

Nature always stands as golden mark to amplify the outstanding phenomenon of symbiosis. Medicinal plants existing even before human being made their appearance on the earth. (Kokate CK, *et al* 1996). An early objective of the World Health Organization's (WHO) traditional medicine program was to promote a realistic approach to the subject. The realism with which countries around the world, both developed and developing, examine their own traditional practices suggests that progress is being made towards this goal.

Gum arabic is an edible, dried gummy exudate from the stems and branches of *Acacia senegal* and *Acacia seyal*, that is rich in non-viscous soluble fiber. It is widely used in pharmaceutical and food industry as an emulsifier and stabilizer[2]. Moreover, herbal remedies are known to contain multiple constituents, acting through multiple pathways needed in urolithiasis, for example, antispasmodic, diuretic, pain relieving.[3,4] Arab folk medicine has recognized the usefulness of GA in regulating the inflammation of intestinal mucosa and as an external soothing agent.[5] In this study, we evaluated *Gum arabic* for its antiurolithic activity and to see if it exhibits calcium oxalate crystallization inhibitory, antioxidant, renal cell protective, which are likely to contribute in its antiurolithic effect.

MATERIALS AND METHODS

Plant material and extraction procedure

The *Gum Arabic* purchased from a Sudan and classified according to the family and species identified by Dr. Naglaa Gamil, Professor, Dubai Pharmacy College, Department of pharmaceutical chemistry and natural products, Dubai, UAE. The sample are cleaned and

poured in powdering machine and dissolved the sample in cold distilled water when test sample administration in rat.

Chemicalsand Reagents

BUN, creatinine calcium, phosphates and uric acid Kit Reagents [Diasys Diagnostic systems, Germany], Alabbar companies Pvt. Ltd, Dubai.Ethylene glycol (EG) was obtained from Merck Laboratories, Dubai.Cystone tablets (The Himalaya Drug Company, Dubai) were used as standard antiurolithiatic drug.

Detection of chemical compounds

Chemical detection was carried out using different reagents to determine the quality of active compounds exists in *Gum Arabic*

Detection of active ingredient using (HPLC)

Quality and quantity analysis performed HPLC technique using C-18 column, 50 × 4.6 mm I.D column, the mobile phase used was 1% phosphate buffer(pH =4.5): acetonitrile: water (60:40), and the flow rate was 1ml/min at 264 nm. The volume of injected extract was 20µl.Peak area was calculated and compared with standard.

Experimental Design

Animals

Male Wistar rats weighing 150–200g were used for the study was obtained from Dubai pharmacy college, Dubai, UAE. All the animals were grouped and kept under constant environmental conditions with a 12/12 light-dark cycle and temperature of 23±2, fed with standard granulated chow, and given drinking water *ad libitum*. The animal experiments were carried out in accordance with the Institutional Protocols of Animal Care. The experimental protocol (Reg no: DPC/AEC/2016-17/ 47) was approved by Dubai Pharmacy college Animals Ethical Committee.

Experimental design: Ethylene glycol-induced hyperoxaluria method was used.[6] Healthy male Wistar rats (150– 200g) were selected and divided into five groups of six animals each. Group-1 served as normal and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to Group-2 to Group-5 for induction of

renal calculi till 28th day. Group-3 received standard antiurolithiatic drug, cystone (750 mg/kg, b.wt.) from 15th day to 28th day.[7] Group-4 and Group-5 served as *Gum Arabic (*100mg/kg and 250mg/kg). All doses were given once daily by oral route.

Collection and analysis of urine: Urine samples (24 h) were collected on 28thday by keeping the animals in metabolic polypropylene cages. Animals had free access to drinking water during urine collection period. The volume of urine from each group of animal was measured. Further microscopy of the urine was performed at 100X.[8] Urine was analyzed for the calcium[9]phosphate[10] and oxalate[11] by using standard methods.

Serum analysis: After the experimental period, blood was collected from the retro orbital puncture under anesthesia and serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

Estimation of Serum Creatinine

The serum creatinine concentration was estimated by alkaline picrate method[12] using the commercially available kit. The 2.0ml of picric acid reagent in a tube was added to 0.2ml of serum for deproteinization of specimen, which was mixed well and centrifuged at 3000 rpm to obtain a clear supernatant. 100 µl of buffer reagent was added to 1.1 ml of supernatant, 0.1 ml of standard creatinine and 0.1 ml of distilled water to prepare test, standard and blank, respectively. 1.0 ml of picric acid reagent was added to blank and standard. The test tubes were mixed well and kept at room temperature for 20 minutes. The alkaline picrate reacts with creatinine to form the orange colored complex, which was read at 520 nm spectrophotometrically.

Estimation of Creatinine:[13] Creatinine reacts with alkaline picrate to produce an orange-yellow colour. Specificity of the assay has been improved by the introduction of an initial rate method. The absorbance of orange-yellow colour formed is directly proportional to creatinine concentration and is measured photometrically at 500–520 nm.

Estimation of uric acid:[14] Uricase converts uric acid to allantoin. The hydrogen peroxide formed further reacts with a phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colouredquinoneimine dye complex. The intensity of Chromogen (Quinoneimine) formed is proportional to the uric acid concentration in the sample when measured at 505 nm (500–540 nm).

Estimation of urea nitrogen:[15] The rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD was proportional to urea concentration in the sample.

Urea (mg/dl) = (Absorbance of Test /Absorbance of Standard) x Concof Standard (mg/dl)

BUN (mg/dl) = 0.467 x Urea concentration (mg/dl)

Estimation of calcium:[15]o-Cresolphthalein complexone (OCPC) reacts with calcium in alkaline solution to form a purple colour complex. The intensity of purple colour formed is proportional to the calcium concentration and is measured photometrically between 540 nm and 600 nm with maximum absorbance at 575 nm.

Estimation of phosphate:[16] Phosphate ions in acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex reacts with metals and is reduced to a molybdenum blue complex. Intensity of molybdenum blue complex formed is directly proportional to amount of inorganic chemical present in the sample

Histopathological Examination The liver and kidneys were sectioned longitudinally into two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. These sections were stained with hematoxylin and eosin and were observed under a computerized light microscope.

Statistical Analysis All values were expressed as mean ± S.D. The data obtained from various groups were statistically analysed using One-Way ANOVA. The P value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of *Gum Arabic* on Serum Creatinine, Blood Urea Nitrogen, demonstrates the effect of *Gum Arabic*the serum creatinine, blood urea and urea nitrogen. There was marked increase in serum creatinine was noted in ethylene glycol administered albino rats as compared to normal albino rats. In addition, the blood urea and nitrogen urea were noted to be increased in ethylene glycol -administered albino rats. However, concomitant administration of *Gum Arabic* in two doses (500mg/kg) significantly reduced urolithiasis effect, levels of serum creatinine and blood urea nitrogen in albino rats shown in (table 1).

Effect of *Gum Arabic*on Serum Uric acid

The effect of *Gum Arabic*about the serum uric acid level itmarked that increase of uric acid level in the ethylene glycol –induced urolithiasis control animal and the treated with *Gum Arabic*datashows significantly decrease uric acid. The kidney is healthy in the test dose (500mg/kg) shown in (table 1).

Effect of *Gum Arabic* on Serum calcium and phosphorous

Gum Arabic 500mg/kg treated animals serum calcium and phosphorous level marked that significantly decreased level compare to control and the treated with *Gum Arabic*significantly decrease as standard drug shown in (table 1)

Table 1: Antiurolithiatic Effect of *Gum Arabic* in urolithiasis induced animals

Group	BUN mg/dl	Creatinine mg/dl	Uric Acid mg/dl	Calcium mg/dl	Phosphorous mg/dl
Normal	19.83±0.03	0.14±0.03	1.06±0.25	7.1±1.17	5.63±1.32
Control	31.28±1.48	1.44±0.10	4.50±0.72	25.33±4.01	24.01±1.96
Cistone standard 750mg/kg	15.49±1.11	0.18±0.03	1.66±0.66	8.80±0.52	10.53±1.90
Gum Arabic 250mg/kg	26.18±0.69**	0.48±0.06	2.76±0.66	20.56±2.07	16.33±1.96*
Gum Arabic 500mg/kg	21.38±1.02***	0.23±0.03**	1.76±0.25***	13.76±1.00**	11.90±1.17***

*Values are represented as mean ±SD, where n=6, ***P<0.001 as compare to normal control, **p<0.01 as compare to control.BUN (blood urea nitrogen), STD (standard)*

Histopathological study of kidney

Histopathological analysis revealed no calcium oxalate deposits or other abnormalities in the nephron segments of vehicle treated group. Many calcium oxalate deposits inside the renal tubules and dilation of the proximal tubules along with interstitial inflammation were observed in the renal tissue of urolithiatic rats. The number of calcium oxalate deposits in the renal tubules of Groups III and V rats was significantly less than the Group II (Figure1).

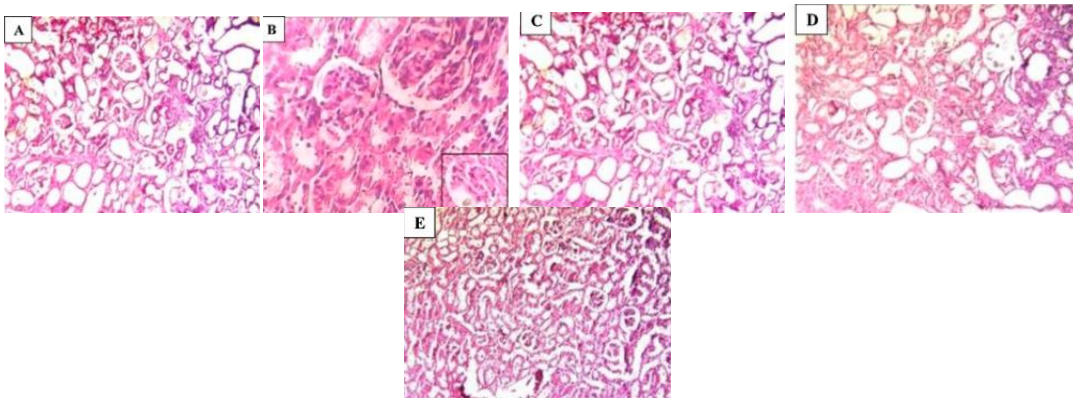

Figure 1: Histopathology studies of Antiurolithiatic Effect of *Gum Arabic* in urolithiasis induced animal'skidney tissues

Figure1. Histopathology of kidney tissue of (A) normal, (B) calculi induced group, crystal of calcium oxalate shown in the box (C) standard drug cystone treated, (D) *Gum Arabic* at the dose 250 mg/kg, (E) *Gum Arabic* at the dose 500 mg/kg. Microscopic magnification: 40×.

CONCLUSION

The stone formation occurs through supersaturation of urine with some specific salts like calcium oxalate. Hyperoxaluria is greater risk factor for pathogenesis of renal stones compared to hypercalciuria. Hence, measuring changes in urinary oxalate levels are relatively much more important than those of calcium. Increased calcium level in urine will trigger nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) and subsequent crystal growth.In urolithiasis, the glomerular filtration rate decreases due to the obstruction to the flow of urine by stones in urinary system. So, the waste productsparticularly nitrogenous substances such as urea, creatinine and uric acid accumulate in blood. In calculi-induced rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid which are markers of glomerular and tubular damage. Cystone prevents the formation of kidney stones and dissolves kidney stones. Deposition and supersaturation of calculogenic chemicals like oxalic acid and calcium hydroxyproline in urine is prevented. Cystone is also a diuretic that flushes out small stones from the kidneys.

Treatment of *Gum Arabic 500mg/kg* showed to prevent the elevation of serum levels of these markers and inhibits the blood urea nitrogen, creatinine, and uric acid.

Increase in calcium levels in the renal tissue of EG treated rats was observed. The *Gum Arabic 500mg/kg* treatment suppresses this increase in intracellular calcium.

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