

ANTILITHIATIC EFFECT OF CRINUM GIGANTEUM ANDREWS BULB EXTRACT ON ETHYLENE GLYCOL-INDUCED NEPHROLITHIATIC RAT MODEL

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ABSTRACT

Ethylene-glycol (EG) induced nephrolithiasis is a known model of kidney stone in experimental rodents. Nephrolithiasis is treatable with an antilithiatic and lithotriptic drug. Decoction of *Crinum giganteum* Andrews (CG) bulb, a medicinal herb is used in folklore medicine to manage urinary tract diseases including kidney stone. The antilithiatic effects of *Crinum giganteum* Andrews bulb extract was investigated using biochemical and histological parameters on ethylene-glycol nephrolithiatic rat model and compared with cystone (a known antilithiatic drug). Twenty rats were randomized into a control group (N=4) which received water (vehicle) and experimental groups (N=16) that received 1% ethylene-glycol in water and subdivided into negative control (only 1% EG in drinking water) and treatment groups which were given 200mg/kg/bw, 400mg/kg/bw of ethanolic bulb extracts of CG and 100mg/kg/bw of cystone orally for 21 days. The EG elevated urinary and serum calcium, protein and creatinine, and reduced magnesium concentrations. These were accompanied by microcrystal deposits in kidney sections. But, the ethanolic bulb extract and cystone treatments reversed the above biochemical and histopathological effects. The ethanolic bulb extract of CG exhibited comparable antilithiatic effect with cystone on ethylene-glycol-induced nephrolithiasis. Thus, the extract showed positive indication of its use in folklore medicine.

KEY WORDS: Microcrystal, calcium oxalate, Histology, Antilithiatic, *Crinum giganteum*, Cystone.

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INTRODUCTION

Nephrolithiasis or kidney stone is a common disease worldwide, affecting males and females of different age range. In mimicking or modelling the human symptoms of nephrolithiasis, ethylene glycol (EG) has consistently demonstrated the features of the disease in experimental studies. Hence, it is the commonest substance used for the induction of experimental rat model of nephrolithiasis [1, 2].

Ethylene glycol is also a known metabolic precursor of oxalate and has been used to study the pathogenesis of kidney crystal deposition particularly the deposition of calcium oxalate [1, 3, 4]. The administration of ethylene glycol to rats is known to cause hyperoxaluria, crystal-luria and calcium oxalate deposition in the kidneys, resulting in decreased glomerular filtration and renal insufficiency [1]. This is characterized by decreased urine volume or

increased excretion of stone-forming constituents such as calcium, oxalate, urate, xanthine, phosphate and decreased magnesium concentration [3]. In pharmacological management, various categories of drugs have been used to remove stone formers these include; antibiotics, alkalinizing agents, corticosteroids, calcium channel blockers and alpha blockers [4]. Cystone is a potent antilithiatic and lithotriptic drug for the prevention and management of kidney stones in humans. However, studies have shown that it dissolves the stones, and flushes out the stone gravel via the urine [5, 6].

Concurrently, in the treatment of various ailments including kidney stone, Africans have consistently demonstrated higher preponderance towards the use of herbal medicine [7, 8]. In addition, herbal plants are believed to be an important source of new chemical substances with potential therapeutic effects [8-13]. Therefore, it is worthwhile to look for an alternative by using medicinal plants or phytotherapy. A number of plants have been used globally which claim efficient cure of urinary stones.

In the traditional system of medicine, *Crinum giganteum* bulb claims to be useful in the treatment of urinary stone. However, no scientific study has been reported so far confirming the antilithiatic or lithotriptic property of the ethanolic bulb extract of *Crinum giganteum*. In this present study we made effort to establish the scientific validity for the anti-nephrolithiatic effect of ethanolic bulb extract of *Crinum giganteum* on urine and serum chemistry and histopathology using EG induced nephrolithiatic rat model. The study also established a comparative therapeutic effect between *Crinum giganteum* bulb extract and cystone. The facts obtained from this study can be articulated by the pharmaceutical industries for the synthesis of cheaper alternative therapy for kidney stone.

MATERIALS AND METHODS

Preparation and extraction *Crinum giganteum* bulbs (CG): The CG bulbs were procured from the open market in Adamawa State, North-East of Nigeria. It was identified by a taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The bulbs were washed with distilled water, cut into

smaller sizes and air-dried under shade for seven days. Thereafter, the dried bulb were pulverised into a fine powder, 500grams of the dried bulb powder was placed in a beaker containing 750ml of 95% ethanol. The mixture was continuously stirred at room temperature and then allowed to stand over 48hours and then filtered through mesh cloth. The filtrate was evaporated into paste using vacuum evaporator. The paste was transferred into a suitable container and kept in the refrigerator at low temperature (4 °C) for the experiment.

Antilithiatic study: Twenty (20) male wistar rats of average weight 190g were procured and randomized into control and treatment groups. The control group G1 (n=4) received water and standard rat chows. The rats in the experimental group (n=16) were subdivided into four groups (G2 –G5, n=4). All received 1% ethylene glycol in water (to induce nephrolithiasis). Group G2 received only 1% ethylene glycol in drinking water ad libitum for 21 days and served as the nephrolithiatic group (negative control model), The G3 group received 1% ethylene glycol in drinking water ad libitum; along with lower dose of ethanolic bulb extract of *Crinum giganteum* (200mg/kg/body weight), G4 received 1% ethylene glycol in drinking water ad libitum; along with higher dose of ethanolic bulb extract of *Crinum giganteum* (400mg/kg/ body weight). While, G5 received 1% ethylene glycol in drinking water ad libitum, along with 100mg/kg/body weight of cystone (standard antilithiatic drug/treatment control). Extracts and drug were administered orally for 21days.

Ethical approvals: The experimental protocol was approved by the Departmental Research Ethics Committee. All protocols were carried out in strict accordance with the guidelines for the care and use of animals for research.

Biochemical study

Urine collection and analysis: The method of Gilhotra and Christina [14] was adapted. Animals were kept in separate metabolic cages and urine samples were collected on the day 22 within 24 hours. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40°C. Urine was analysed for calcium, protein, creatinine and magnesium

contents.

Blood collection and analysis: On the 22th day (day one post treatment) blood samples was collected from each rat via the retro-orbital plexus. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for calcium, phosphate, oxalate, creatinine and magnesium concentrations as described by Gilhotra and Christina [14].

Kidney harvest and histopathological study:

The rats were sacrificed under ether anesthetic chamber, both kidneys were harvested and fixed with 10% buffered formal saline. The fixed kidneys were embedded in paraffin, deparaffinized tissues were cut to 5µm thickness and stained with hematoxylin and eosin. The stained slides were then mounted on glass slide and covered slips using DPX, interpreted and micrographed using the Amscope 3.2 digital microscope, England.

Statistical analysis:

The statistical analyses were done using a SPSS software version 23, one way analysis of variance (ANOVA) and Newman test. The results were expressed as Mean ± SEM and presented in tables while $p < 0.05$ values were considered significant.

RESULTS

Biochemical analysis: Urinary and Serum

analysis: The calcium content of both urine and serum increased significantly in the ethylene glycol untreated G2, compared to the normal control (G1). Likewise, protein and creatinine excretions increased significantly in the ethylene untreated (G2). However the calcium, protein and creatinine contents were reduced in the extract treated groups (G3 and G4) and Cystone treated group (G5). The urinary magnesium decreased in the untreated ethylene glycol group. Simultaneous administration of the extract and cystone in groups G3, G4 and G5 increased magnesium level, but significantly increase was seen in cystone treated group- G5 when compared with the ethylene glycol untreated group (G2) in table 1 and 2.

Histopathological findings: Sections of the kidneys of the rats treated with ethylene glycol showed deposition of micro- crystals (G2). There was marked dilatation of tubules, tubular damage and infiltration of inflammatory cells into the interstitial space. However, the kidney sections of rats treated with extracts (G3 and G4) showed improvement of the above symptoms and reduced crystal deposition as shown in (G3 and G4) but significantly improved with cystone treatment (G5).

Fig. 1: Photomicrograph of section of kidney. G1 normal renal architecture with normal glomeruli (NG), renal tubules (RT) lined with tubular cells (TC). G2 shows severe coagulative necrosis of the glomeruli (SCNG)), tubular necrosis (TN) tubular cell necrosis (TC N). G3 shows extract vacuolation red blood (EVRC). G4 shows mild regeneration of renal tubules (RT) and glomeruli (RNG). G5 shows moderate regeneration of glomeruli (RNG), tubular cell (TC). H & E. X 200.

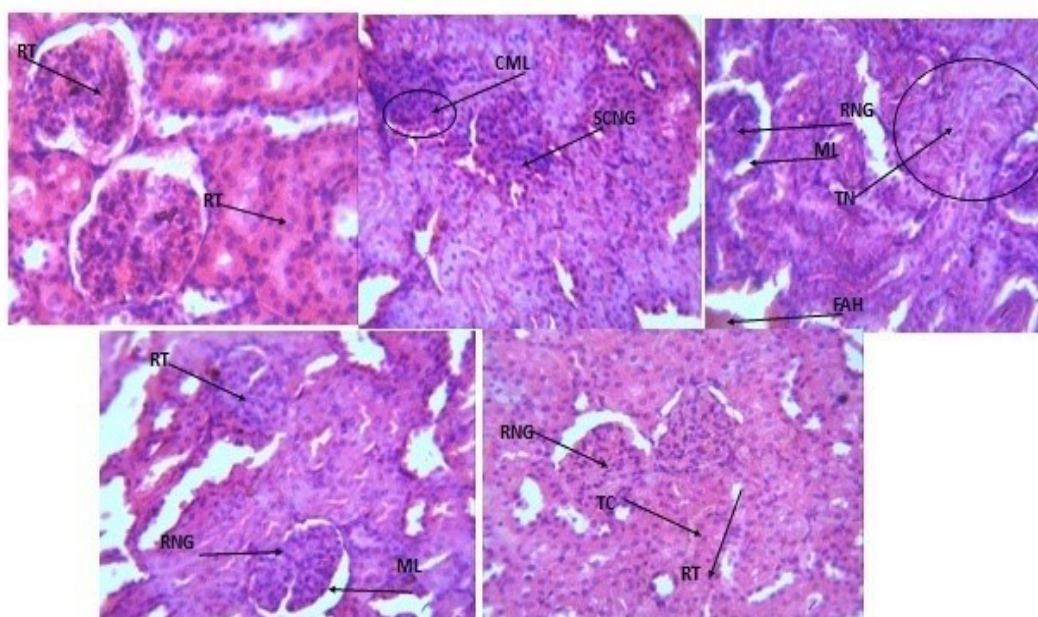


Table 1: Urinary analysis. $p < 0.05$

PARAMETERS	G1	G2	G3	G4	G5	p-value
Total Protein (g/dl)	6.1±2.6	20.1±2.1	10.5±0.7	7.3±4.5	5.4±5.8	0.04
CREATININ (mg/dl)	2.7±0.5	12.1±1.7	10.8±2.2	7.5±0.3	2.7±2.9	0.01
CALCIUM (mg/dl)	2.2±0.4	8.5±1.4	7.0±0.6	6.5±0.1	6.1±0.7	0.03
MAGNESIUM (mg/dl)	8.3±1.6	1.1±0.1	4.6±0.4	5.1±2.3	8.3±0.4	0.01

Table 2: Serum analysis. $p < 0.05$

PARAMETERS	G1	G2	G3	G4	G5	p-value
Total Protein (g/dl)	78.5±8.6	101±13.8	87.1±1.0	91.1±9.5	91.9±2.7	0.01
CREATININ (mg/dl)	1.8±0.1	4.0±4.1	3.3±2.5	0.9±0.2	1.5±1.3	0.03
CALCIUM (mg/dl)	10.8±1.5	13.6±1.1	8.8±0.7	7.8±0.6	7.3±1.3	0.01
MAGNESIUM (mg/dl)	13.2±0.7	9.2±0.7	11.7±1.3	12.6±0.1	12.2±0.8	0.03

DISCUSSION

The antilithiatic effect of the *Crinum giganteum* on ethylene glycol-induced nephrolithiasis in rats was investigated and compared to the cystone an antilithiatic and lithotriptic drug. Evidence from previous studies indicate that in response to 14-28days period of ethylene glycol (1% v/v) administration, young albino rats form renal calculi, composed mainly of calcium oxalate [15-18]. The oxalate ($C_2O_4^{2-}$) is the salt-forming ion of oxalic acid ($C_2H_2O_4$). The oxalic acid forms oxalate salts with cations of sodium, potassium, magnesium, and calcium. These form water soluble sodium oxalate, potassium oxalate, and magnesium oxalate salts and an insoluble calcium oxalate (CaOx). The kidney excretes oxalate via glomerular filtration and tubular secretion. The resultant outcome is increased urinary oxalate excretion (hyperoxaluria), which leads to urinary CaOx supersaturation, with secondary formation and retention of CaOx crystals in renal tissue. These CaOx crystals contribute to the formation of diffuse renal calcifications (nephrocalcinosis) and nephrolithiasis [4, 15-18]

Thus, explains the increased urinary and blood concentrations of calcium, phosphate, protein, oxalate and creatinine and decreased magnesium concentration [15, 18-20]. In addition, studies have been also showed that ethylene glycol could also induced experimental nephrolithiasis alone or in combination with ammonium oxalate [21, 22]. Therefore, this model was used to evaluate the antilithiatic effect of *Crinum giganteum*.

In our present study, urine and serum calcium,

creatinine, and protein concentrations increased in the ethylene glycol treated groups (G2), while magnesium concentration decreased. The outcome agreed with previous reports, it also confirms and validate the reliability of this model. Increased urinary calcium particularly favours the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth [23]. Low urinary magnesium content is a common feature of kidney stone as well [23]. But, the extracts of *Crinum giganteum* and cystone decreased the concentrations of calcium, protein and creatinine and increased that of magnesium when compared to the ethylene glycol untreated group. These reduced the propensity to crystallize, thereby creating an ambience unfavourable for precipitation.

Histopathological examination revealed polymorphic irregular crystal deposits within the tubules which cause dilation of the tubules along with interstitial inflammation that might be attributed to ethylene glycol-induced nephrolithiasis. The *Crinum giganteum* extracts and cystone reversed this renal pathology by causing regeneration of dead cells of the affected tubules. Comparatively, the extract exhibited lithotriptic effect similar to cystone although, the cystone treatment was more effective. In an attempt to explain the possible mechanism of plant extract activities, several studies attributed such to the presence of active principles in the extract. These bioactive agents include flavonoids, polyphenols and triterpenes which have anti-inflammatory and antioxidant effects [24-27].

It can be speculated that the antilithiatic effect of the extract might be via a synergy of anti-inflammatory and free radical scavenging effects of phytochemicals present in the *Crinum giganteum* extract [28, 29, 30], which might be responsible for the lithotriptic effect. But, the antilithiatic activity of cystone is attributable to the presences of *shilapusha* (*didymocarpus pedicellata*) which has antimicrobial, antilithiatic and lithotriptic properties. While the diuretic activities is rendered by the *pasanabhedha* (*saxifraga ligulata*), and small caltrops (*gokshura*) prevents the dysuria, crystalluria and deposition, accumulation and supersaturation of calculogenic substance. These tripartite activities of cystone make it a preferable therapy of choice is the management of nephrolithiasis in recent times. In conclusion, the *Crinum giganteum* extracts has comparable anti-nephrolithiatic effect to cystone, a herbal formulation. This finding supports the folklore claim regarding the antilithiatic effect of the plants in the treatment of urinary ailments. However, the effect was dose dependent and will require dosage adjustment when use in the management of kidney stones.

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Conflicts of Interests: None

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