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ANTIUROLITHIC ACTIVITY OF AQUEOUS EXTRACT ON ROOTS AND SEEDS OF *CRATAEVA NURVALA*. ON ETHYLENE GLYCOL INDUCED KIDNEY STONES IN MALE ALBINO RATS

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Abstract:
Renal calculi have become one of the common kidney related problem presently. These are the hard deposits of salts or minerals that form inside the kidney. The current study focusses on the efficacy of Crataeva nurvala. in controlling the growth of ethylene glycol induced calcium oxalate stones in wistar albino rats. The aqueous extract of seeds and roots of Crataeva nurvala. at doses of 500 and 1000mg/kg showed greater reduction in renal stones in hyperoxaluria rats when compared to the activity of standard Furosemide which is given 20mg/kg.

Key Words: *Crataeva nurvala., ethylene glycol, renal calculi, Furosemide*

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INTRODUCTION:

Renal stones are now a tending problem everywhere around the world showing some geographical and ethnic group variations[1]. These are the hard deposits that form in the kidneys which are often painful while passing through the urinary tract. There are many types of renal stones of which calcium oxalate stones made up of calcium salts are the most common ones[2]. Presence of crystal forming substances in urine lead to the formation of renal stones. These include calcium, uric acid and oxalate and also lack of substances that prevent these crystals from sticking together[3]. Calcium oxalate renal stones may also form due to injury of cells of renal tubules[4]. Free radical generation is seen because of the cell injury caused by calcium oxalate crystals which occurs due to lipid peroxidation[5,6]. There are many methods to crush or prevent the stone formation which include shockwave lithotripsy and endoscopic removal. These processes gained importance in treating renal stones but they failed to eradicate new stone formation[7,8]. Apart from these methods, some drugs like Thiazide diuretics can be prescribed to treat nephrolithiasis. These drugs are known to control the amount of calcium to be released into the urine but researches are going still going on to evaluate their efficacy[9].

Herbal plants play a vital role in curing lots of diseases with less side effects and more efficacy. They are known to be cheaper when compared to synthetic drugs. As a result, research on some of the herbal plants showed anti-urolithic activity[10,11]. Isolation of plant constituents to establish anti-urolithic activity is carried out and proteins are isolated from various sources[12,13].

Crataeva nurvala. commonly called as Varun, has been in use since centuries. It is being used in treating various conditions such as female disorders[14,15], worm infestations[16], gastric irritation[17], dysentry[18], inflammation[19,20] and is a component in drug preparations which are used to treat renal calculi[21]. This study evaluates the potency of this plant to cure nephrolithiasis by using the aqueous extracts of seed and roots of *Crataeva nurvala.* considering Furosemide as standard.

MATERIALS AND METHODS:

The plant material Crataeva nurvala leaves were collected in the month of August 2020 from Visakhapatnam.

Preparation of extract of *Crataeva nurvala.*:

Fresh aqueous extract was prepared everyday throughout the study. 5 grams of seed and 5 grams of root were taken and made into a powder. This powder was mixed with 500ml distilled water in a beaker and was heated for about 6 hours with stirring at regular intervals. After completion of this heating process, the solution was filtered using a muslin cloth and was set to evaporate and the obtained product was collected[22]. This final product was stored in a bottle at a temperature of 20^0C.

Animals:

The animals included in this study were healthy male wistar albino rats weighing 150-200 grams. They have same age and were brought from central animal house of Hyderabad. These albino rats were made to adjust to the new environment for about a period of one month and were stored in polypropylene cages by maintaining proper hygiene. They were provided with standard food and water ad libitum. Male albino rats were preferred because they have more chances of formation of renal stones when compared to females due to the absence of estrogen. Ethical clearance was obtained for safe handling of animals prior to the study from local animal ethics committee.

Toxicity studies:

Crataeva nurvala. Extract was given along with sodium carboxymethylcellulose at a high dose of 2000mg/kg. There were neither any toxic effects nor any side effects. The rats were alive even after 24 hours of administration.

Experimental Procedure:

In this study, the anti-urolithic activity of *Crataeva nurvala.* was studied using ethylene glycol induced renal calculi model.

Group I	Normal group
Group II	Control group
Group III	Standard group – Furosemide at 20mg/kg dose
Group IV	Low dose test group – 500mg/kg of *Crataeva nurvala*
Group V	High dose test group – 1000mg/kg of *Crataeva nurvala*

These 5 groups contain 6 animals each.

Group II to group V were given with 0.75% ethylene glycol for 20days.

The following parameters were evaluated in this study:

SERUM ANALYSIS:

Blood samples of the rats were collected from the retro-orbital plexus region[23,24] of each group and the levels of calcium, urea, creatinine and phosphorus were analyzed. The collected blood samples were centrifuged at 1000rpm for about 10 minutes.

Analysis of calcium in serum:
OCPC method is employed to determine serum calcium levels[25,26]. Calcium combines with o-Cresolphthalein in the alkaline medium to form a purple-colored complex. The strength of the color obtained determines the quantity of calcium present. The absorbance was measured at a wavelength of 570nm.

Analysis of phosphate in serum:
Phosphorus amount in the sample was determined using Molybdate UV method[27,28]. Phosphate ions form a phosphomolybdate complex on reaction with ammonium molybdate in acidic medium. The more the intensity of the complex formed, the more is the quantity of inorganic phosphorus in the sample. The absorbance was measured at a wavelength of 340nm.

Analysis of creatinine in serum:
Alkaline picrate method[29,30] was used to determine serum creatinine levels. Picric acid forms red-orange color when creatinine reacts with picrate ion in alkaline medium. The strength of the color obtained determines the quantity of creatinine present. The absorbance was measured at a wavelength of 520nm.
Urine creatinine gm/24hrs= (urine creatinine in gm/l) * volume of urine in 24 hours
Urine creatinine in gm/lit = (absorbance of test/absorbance of standard) *1

Analysis of urea in serum:
moderate berthelot method[31,32] was used to determine serum urea levels. Ammonia and carbon dioxide are produced on hydrolysis of urea by urease enzyme. A green colored complex is formed when ammonia further reacts with phenolic chromogen and hypochlorite. The strength of the color obtained determines the quantity of urea present. The absorbance was measured at a wavelength of 570nm.
Urea in mg/dl= (absorbance of test/absorbance of standard) * 40

Kidney weight:
Upon completion of the study, all the animals were sacrificed by cervical dislocation. The abdomen was cut open and the kidneys of all the rats were separated and weighed.
Increase of weight of the kidneys shows the formation of stones.

RESULTS AND DISCUSSION:

1. Serum calcium levels
Graph-1 and first column of Table-1 depict serum calcium levels in different groups.
In the normal group, serum calcium levels were found to be normal.
The serum calcium levels were much higher in control group than other groups.
The serum calcium levels were found to be almost similar in normal and standard groups. But it was comparatively lower than that of low dose test group and high dose test group. It was found to be very much lower when compared to control group.

The serum calcium levels were high in low dose test group when compared to others, but were found to be very low when compared to the control group.

The serum calcium levels of high dose test group were similar to that of normal and standard groups, but were found to be much lower when compared to control group.

2. Serum phosphate levels:
Graph-2 and second column of Table-1 depict the serum phosphate levels in different groups.
In the normal group, serum phosphate levels were found to be normal.
The serum phosphate levels were much higher in control group than other groups.
The serum phosphate levels were almost similar in normal and standard groups, but it was lower when compared to low dose test group, high dose test group and control group.
In the low dose test group, the serum phosphate levels were found to be higher than other groups except control group.
In high dose test group, the serum phosphate levels were similar to the normal group and standard group but were lower than low dose test group and control group.

3.Serum creatinine levels:
Graph-3 and third column of Table-1 depict the serum creatinine levels in different groups.
The serum creatinine levels were normal in normal group.
The serum creatinine levels were much higher in control group than other groups.
The serum creatinine levels were almost similar in normal and standard groups, but were lower when compared to the low dose test group, high dose test group and the control group.
In the low dose test group, the serum creatinine levels were lower when compared to control group, but were higher than other groups.

In the high dose test group, the serum creatinine levels were similar to the normal group and standard group, were relatively lower when compared to low dose test group and control group.

4. Serum urea levels:

Graph-4 and last column of Table-1 depict the serum urea levels in different groups.

The serum urea levels were normal in normal group.

The serum urea levels were much higher in control group than other groups.

The serum urea levels were almost similar in normal and standard groups, but were lower when compared to the low dose test group, high dose test group and the control group.

In the low dose test group, the serum urea levels were lower when compared to control group, but were higher than all the other groups.

In the high dose test group, the serum urea levels were similar to the normal group and standard group, were relatively lower when compared to low dose test group and control group.

5. Weight of the Kidneys:

Kidney weight was used as a key parameter to evaluate the formation of stones in rat's kidneys.

Graph-5 and Table-2 depict the wight of kidneys which tell about the formation of stones in different groups.

In the normal group, the weight of right kidney was observed to be lowest than other groups.

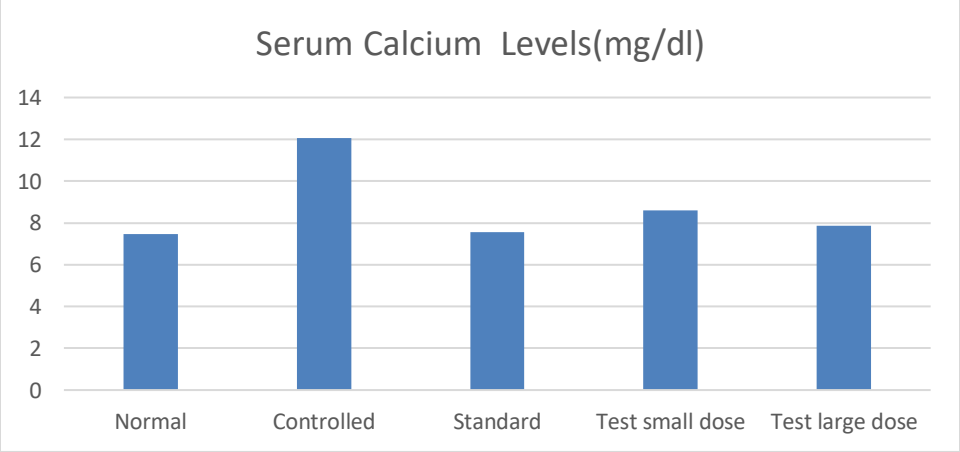
In the control group, the weight of right kidney was found to dominating than other groups. This indicated the formation of stones in this group.

In the standard group, the weight of right kidney was neither found to be high nor low when compared to other groups.

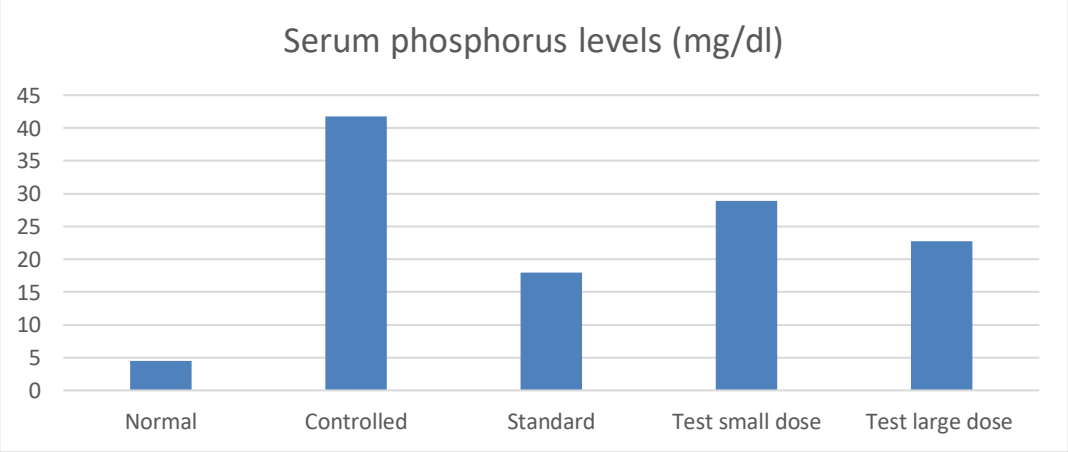
In the low dose and high dose test groups, the weight of right kidney was similar with each other, but was higher than that of normal and standard groups and lower than that of control group.

Similar measurement of weights can be done for the left kidney to draw the required inference.

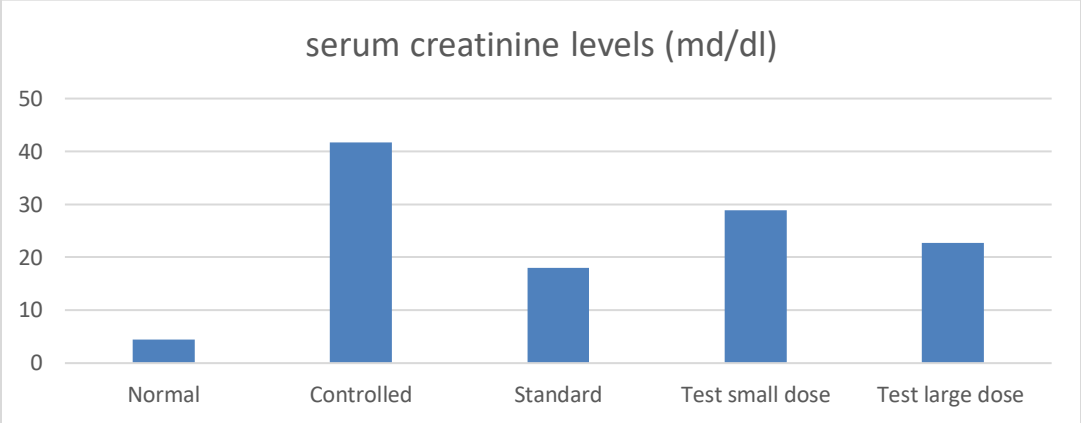
Graph-1(serum calcium levels (mg %)



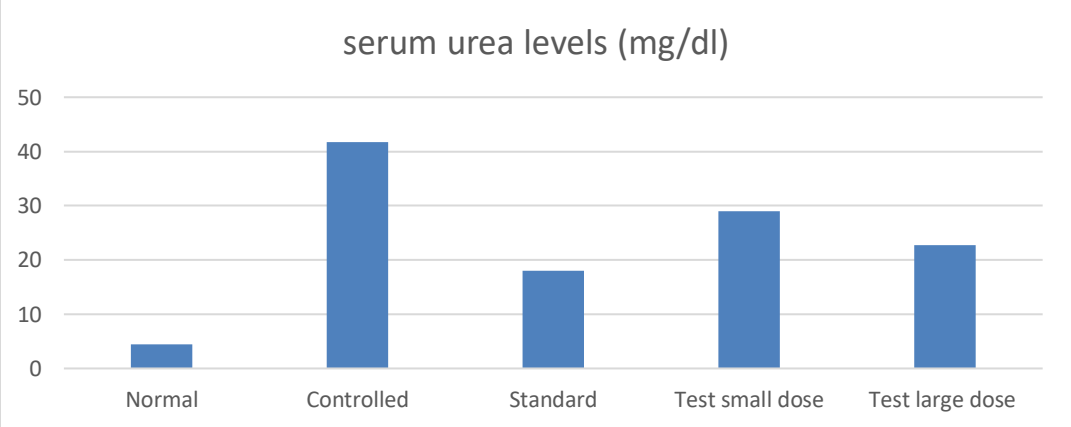
Graph-2 (serum phosphorous levels (mg/dl)



Graph-3 (serum creatinine levels (mg/dl)



Graph-4serum urea levels (mg/dl)



Graph-5: Average weight of kidneys: (n=6)

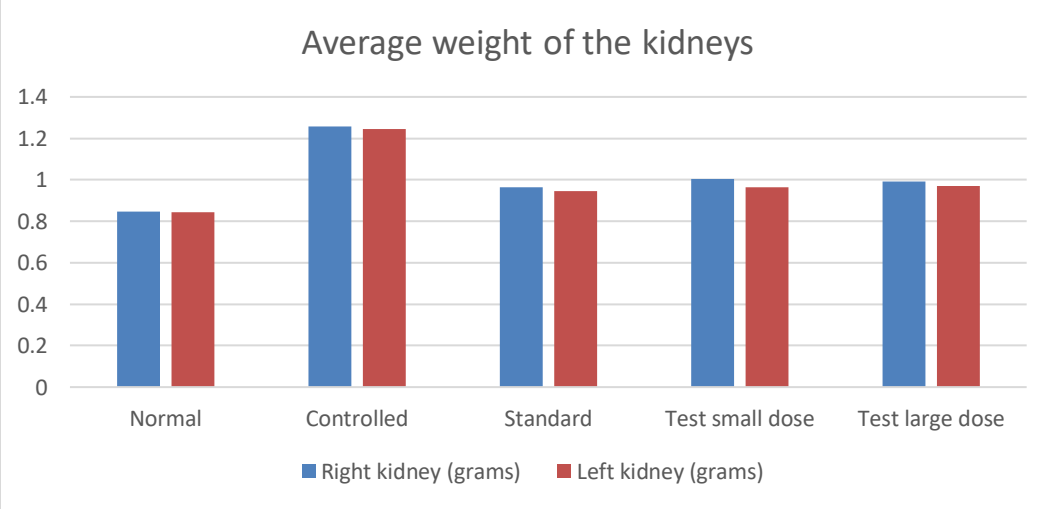


Table: 1 The average of blood serum parameters along with their standard deviations for each group:

s.no	Animal Treatment	Serum Calcium Levels(mg/dl)	Serum Phosphorous Levels(mg/dl)	Serum Creatinine Levels(mg/dl)	Serum Urea Levels(mg/dl)
1	Normal	7.45±0.187	8.15±0.187	1.528±0.073	4.5±0.185
2	Controlled	12.06±0.216	10.783±0.318	1.833±0.025	41.75±0.707
3	Standard	7.55±0.187	8.48±0.146	1.553±0.021	18.0±0.506
4	Test small dose	8.60±0.26	9.05±0.187	1.711±0.023	28.93±0.584
5	Test large Dose	7.85±0.137	8.68±0.231	1.635±0.018	22.71±0.772

Table 2: The average of the kidney weights along with their standard deviation values for each group:

s.no	Animal treatment	Right kidney (grams)	Left kidney (grams)
1	Normal	0.848±0.024	0.845±0.020
2	Controlled	1.258±0.125	1.245±0.098
3	Standard	0.965±0.018	0.945±0.044
4	Test small dose	1.005±0.068	0.963±0.064
5	Test large dose	0.991±0.027	0.970±0.031

CONCLUSION:

From the results of this study, we can conclude that the aqueous extract of roots and seed of *Crataeva nurvala.* possess anti-urolithic activity as it is clear that it lowered the levels of chemical constituents present in serum that are responsible for the formation of stones without causing any toxic or side effects. This indicated the dose dependent nature of *Crataeva nurvala.* plant in treating nephrolithiasis.

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