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ANTIUROLITHIC ACTIVITY OF AQUEOUS EXTRACT ON ROOTS AND SEEDS OF *ACHYRANTHES ASPERA* ON ETHYLENE GLYCOL INDUCED KIDNEY STONES IN MALE ALBINO RATS

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Abstract:

The kidney stone is one of the most widely spreading renal disorders in the world. The present study was undertaken to evaluate the efficacy of Achyranthes aspera in reducing the growth of calcium oxalate stones in ethylene glycol induced model. Upon administration of Furosemide (20mg/kg), aqueous extract of roots and seeds of Achyranthes aspera (500 and 1000 mg/kg) on hyperoxaluria rats shows the significant activity in decrease kidney stones and serum levels (calcium, phosphorous, creatinine, urea) both not that as standard drug Furosemide.

Key Words: *Achyranthes aspera, calcium oxalate, ethylene glycol, Furosemide*

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INTRODUCTION:

Urinary stone disease occurs worldwide with some geographical and racial variation and is constantly rising in parallel with socio-economic development [1].it is largely a recurrent disease with an approximate relapse rate of 50% in 5-10 years and 75% in 20 years. Urinary tract stones composed of calcium oxalate (coax), either alone or mixed with calcium phosphate, are hitherto the most common uroliths accounting for more than 80% of stones [2]. the crystallization inhibition capacity of urine does not allow urolithiasis to happened in most of individuals, whereas, this natural inhibition is in deficit in stone formers [3].stidies have also that tubular cell injury facilitates calcium oxalate crystal formation and deposition in renal tubules [4].animal and tissue culture studies have demonstrated that both oxalate and calcium oxlate crystals directly induce renal epithelial cell injury mediated through lipid peroxidation and involve oxygen free readical generation [5,6]. Endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionzed the treatment of nephrolithiasis, but do not avoid the possibility of new stone formation [7,8]. Various therapies including thiazide diuretics and alkali-citrate are being used in an attempt to prevent the recurrence of hypercalciuria and hyperoxaluria induced calculi,but scientific evidence for their efficacy is less convincing [9]. Medical plants have played as significant role in various ancient traditional system of medication. Even today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithatic therapy have revealed their therapeutic potential in the in-vitro or in-vivo models [10,11]. Researchers are also trying to isolate potent phytoconstituents and antiurolithiatic potency is being evaluated. The most active protein fractions are isolated from *dolichos biflorus* [12] and *trachyspermum ammi*[13] and their therapeutic use as antilithiatic proteins was established.

Achyranthes aspera commonly called as "puthkanda" in Hindi is being used in ayurived as an herbal drug since ages. There are reports on its antifertility [14,15], antimicrobial [16],anti-inflammatory [17],antinociceptive role [18],and also as an immune stimulator [19,20]. It is an active component of various drugs formulations for kidney stones [21]. However, no scientific basis has been formulated for its antiurolithiatic potency. The present study was undertaken to examine antiurolithic potency of aqueous extract of roots and seeds of *Achyranthes aspera* in male albino rats by using Furosemide as standard.

MATERIALS AND METHODS:

Preparation of *Achyranthes aspera* extract:

Large number of seeds and roots of *Achyranthes aspera* were collected from the village Ibrahimpatanam, R.R Dist,A.P. The plant was identified by Dr.K.P.Shastry assistant public information officer (CIMAP Resource centre, Hyderabad) ref no: 25382.

Aqueous extraction was continuously done everyday throughout the course of the project in order to yield a fresh extract. The powdered material (5gramsof root + 5 grams of seed) was taken in beaker with water of 500ml. it was continuously heated for 6hrs every day. During the heating period, occasional stirring was done. After 6hrs, the solution was filtered through a fine muslin cloth. The filtrate obtained is heated up to evaporation in order to obtain our desired product[22]. The final dried samples were stored in labeled sterile bottle and kept at 20^0c.

Animals:

Healthy male albino rats of Wister strain weighing about 150-200 gms of equivalent age groups were obtained from central animal house of Hyderabad. Rat were acclimatized for one month in polypropylene cages under hygienic conditions and provided with standard animal feed and water *ad libitum*. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals and were approved by the local care of experimental animal committees.Chances for stone formation are more in males when compared to the females because of the absence of oestrogens in males.

Acute toxicity studies:

The aqueous extract of *Achyranthes aspera* was suspended with Na-CMC administered orally in very high doses up to 2000mg/kg body weight of rats, which did not produce any toxic effects. No rats were died within 24hrs, nor have any side effects been observed.

Experimental Procedure:

Ethylene glycol induced hyperoxaluria model was used to assess the antiurolithic activity in albino male rats following procedures as under.

Animals were divided into 5 groups containing 6 animals in each.

Group-1: They were fed with animal feed and drinking water *ad libitum* for 20 days . They served as a normal.

Group-2: they were fed with animal feed and drinking water *ad libitum* mixed with ethylene glycol 0.75% for 20days. They served as controlled group.
Group-3: they were fed with animal feed and drinking water *ad libitum* mixed with ethylene glycol 0.75% .before giving the feed, they were given a dose of 20 mg/kg body weight of standard drug (furosemide) dissolved in Na-CMC. They served as standard group.

Group-4: they were fed with animal feed and drinking water *ad libitum* mixed with ethylene glycol 0.75% .before giving the feed, they were given a dose of 500mg/kg body weight of test drug (aqueous extract of *achyranthes aspera*) dissolved in Na-CMC. They served as low dose test group.

Group-5: they were fed with animal feed and drinking water *ad libitum* mixed with ethylene glycol 0.75% .before giving the feed, they were given a dose of 1000mg/kg body weight of test drug (aqueous extract of *achyranthes aspera*) dissolved in Na-CMC. They served as high dose test group.

The duration of experiment was 20 days. The type of treatment adopted was pre-treatment, where the dose was administered before giving the feed to rats. The rats were evaluated for the following parameters.

SERUM ANALYSIS:
On the 19th day ,the blood from each group were collected and analyzed for calcium, phosphorous, urea and creatinine levels in the blood.th blood was collected from the retro-orbital plexus region and centrifuged at 10000 *g for 10 min. the serum was collected and analyzed.

Serum calcium analysis:
For the determination of calcium in serum or plasma **OCPC method** is employed.CALCIUM in alkaline medium combines with o-cresolphthale to form a purple colored complex. The intensity of the color formed is directly proportional to the amount of calcium present in the sample. The absorbance was measure at 570nm
Calcium in mg/dl= (absorbance of. Test/absorbance of .standard) *10

Serum phosphorous analysis:
For the determination of inorganic phosphorous in serum, plasma and urine **molybdate u.v method** is employed Phosphate ions in acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex has an absorbance in u.v range at 340nm.intensit of complex formed is directly proportional to the amount of inorganic phosphorous present in the sample.

Phosphorous in mg/dl= (absorbance of. Test/absorbance of standard) *5

Serum creatinine analysis:
For the determination of creatinine in serum and urine **alkaline picrate method** is used. Picric acid in an alkaline medium reacts with creatinine and forms an orange-colored complex with alkaline picrate. Intensity of color formed is directly proportional to the amount of creatinine present in the sample and absorbance at 520 nm.

Urine creatinine gm/24hrs=(urine creatinine in gm/l) * volume of urine in 24 hrs
Urine creatinine in gm/lit =(absorbance of test/absorbance of standard) *1

Serum urea analysis:
For the determination of urea in serum,plasma and urine **moderate berthelot** method is employed. Urease hydrolyses urea to ammonia and carbon dioxide. The ammonia formed further react with phenolic chromogen and hypochlorite to form a green colored complex. Intensity of the color formed is directly proportional to the amount of urea present in the sample. The absorbance is measured at 570 nm
Urea in mg/dl=(absorbance of test/absorbance of standard) * 40

Kidney weight:
On the 20th day of the experiment period, all the rats were sacrificed by cervical dislocation. They were dissected by opening the abdomen and both the kidneys of each rat were removed and weighed.
Increase of weight shows the formation of stones

RESULTS AND DISCUSSION:

Serum calcium levels:
From the (graph-1 and table 1) shows that in the normal group (N), the serum calcium levels are maintained at normal levels. In the controlled group (C), the serum calcium levels are abnormally high when compared to the other groups. In the standard group (S), the serum calcium levels are in similar lines with the normal groups. But it comparatively low when compared to test small dose (TSD) and test large dose (TLD).it is very low when compared to controlled group(C).in the test small dose group (TSD),the serum calcium levels are relatively higher when compared to that of other groups. but it is very lower when compared to the controlled group (C).in the test large dose group (TLD),the serum calcium levels are in similar lines with that of normal group

(n)and standard group (S).it is relatively lower when compared to that of test small dose(TSD)and it is much lower than that of controlled group .

Serum phosphate levels:

From the (graph-2 and table -1) shows that normal group (N), the serum phosphate levels are maintained at normal levels. in the controlled group (C),the serum phosphate levels are abnormally high when compared to the other groups.in the standard group (S),the serum phosphate levels are in similar lines with the normal groups.but it is comparatively low when compared to test small dose (TSD)and test large dose (TLD).it is very low when compared to controlled group (C). in the test small dose group (TSD), the serum phosphate levels are relatively higher when compared to that of other groups. But it is very lower when compared to the controlled group (C)in test large dose group (TLD),the serum phosphate levels are in similar lines with that of normal group (N) and standard group (S).it is relatively lower when compared to that of test small dose (TSD)and it is much lower than that of controlled group (C).

Serum creatinine levels:

From the (graph-3 and table -1) shows that normal group (N),the serum creatinine levels are maintained at normal levels. In the controlled group (C) ,the serum creatinine levels are abnormally high when compared to the other groups. In the standard group (S) ,the serum creatinine levels are in similar line with the normal groups. But it is comparatively low when compared to test small dose (TSD) and test large dose (TLD).it is very low when compared to controlled group (C). In the test small dose group (TSD), the serum creatinine levels are relatively higher when compared to that of other group. But it is very lower when compared to the controlled group (C).in the test large dose group (TLD), the serum creatinine levels are in similar lines with that of normal group (N) and standard group (S).it is relatively lower when compared to that of test small dose (TSD) and it is much lower than that of controlled group (C).

Serum urea levels:

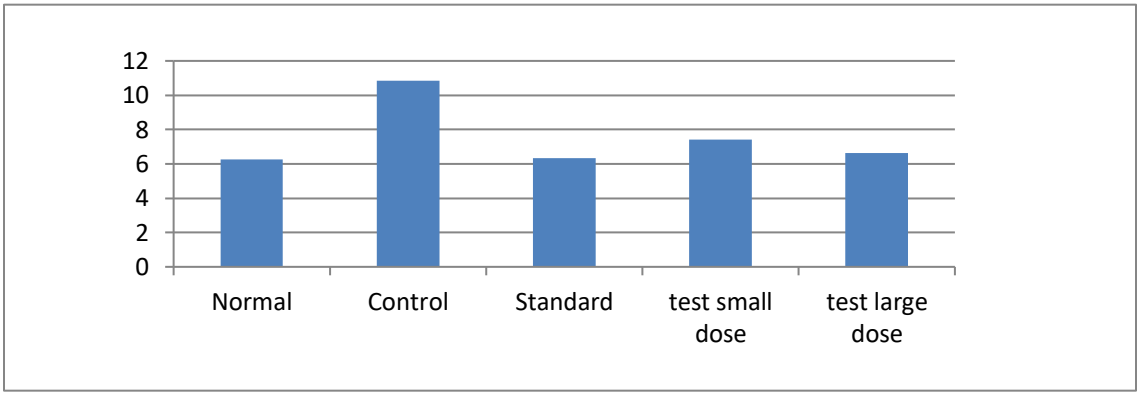
From the (graph-4 and table-1) shows that normal group (N), the serum urea levels are maintained at normal levels. In the controlled group (C), the serum urea levels are abnormally high when compared to other groups. In the standard group (S),the serum urea levels are in similar lines with the normal groups .but it is comparatively low when compared to test small dose (TSD) and test large dose(TLD).it is very low when compared to controlled group (C).in the test large dose group (TLD),the serum urea levels are in similar lines with that of normal group (N) and standard group (S).it is relatively lower when compared to that of test small dose (TSD)and it is much lower than that of controlled group(C).

Kidneys weight:

Weight of kidney is one of the good parameter for evaluation of kidney stone in the body

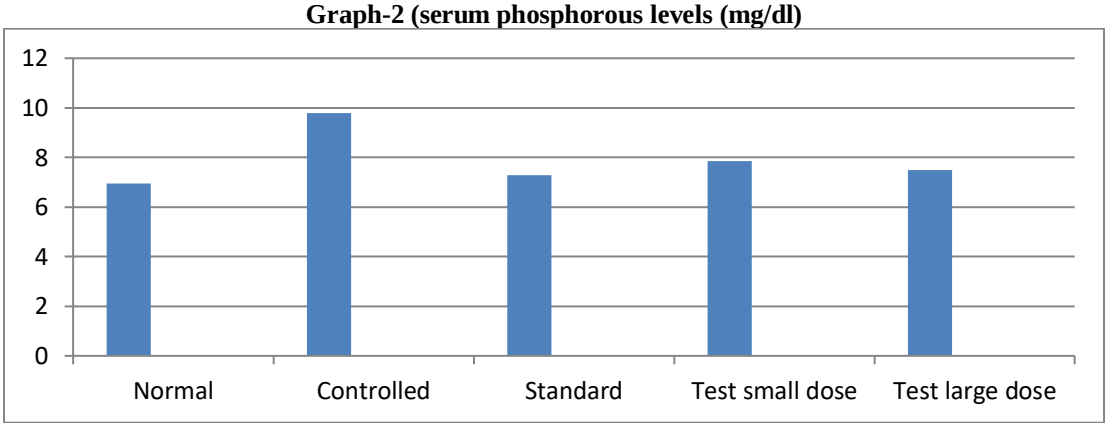
From the (graph-5 and table-2) shows the right kidney weight of the normal group (N)is the lowest when compared to that of the other groups. But the right kidney weight of the controlled group (C) is abnormally high than that of the other groups because of formation of kidney stones in them. The kidney weight of standard group (S) is at an intermediate range between normal and test groups. Both test small dose (TSD)and test large dose (TLD) groups possess similar kidney weights among each other, but their weights are more when compared to n and s groups, less when compared to that of control (C) group. Similar kind of inference as mentioned above can be drawn for left kidney weights in all groups of rats.

Graph-1(serum calcium levels (mg %)



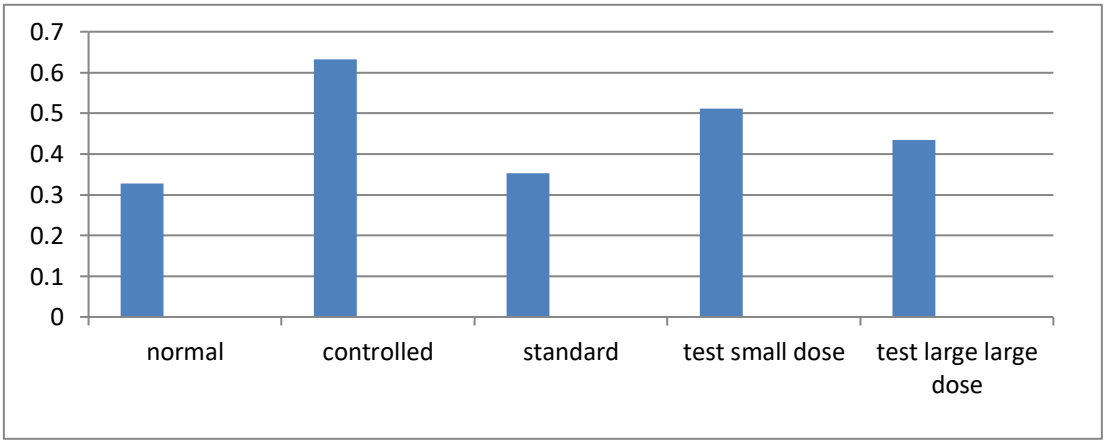
Values are expressed as mean±sem (n=6)

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



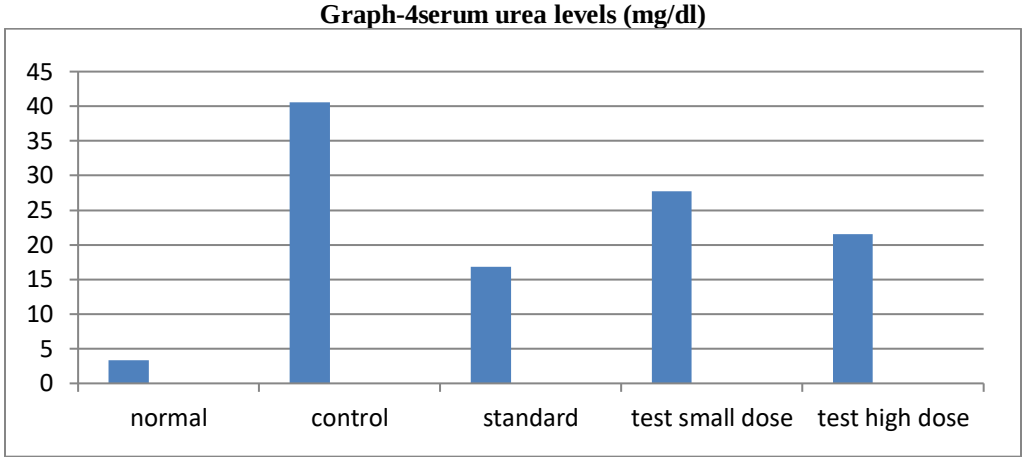
Values are expressed as mean±sem (n=6)

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



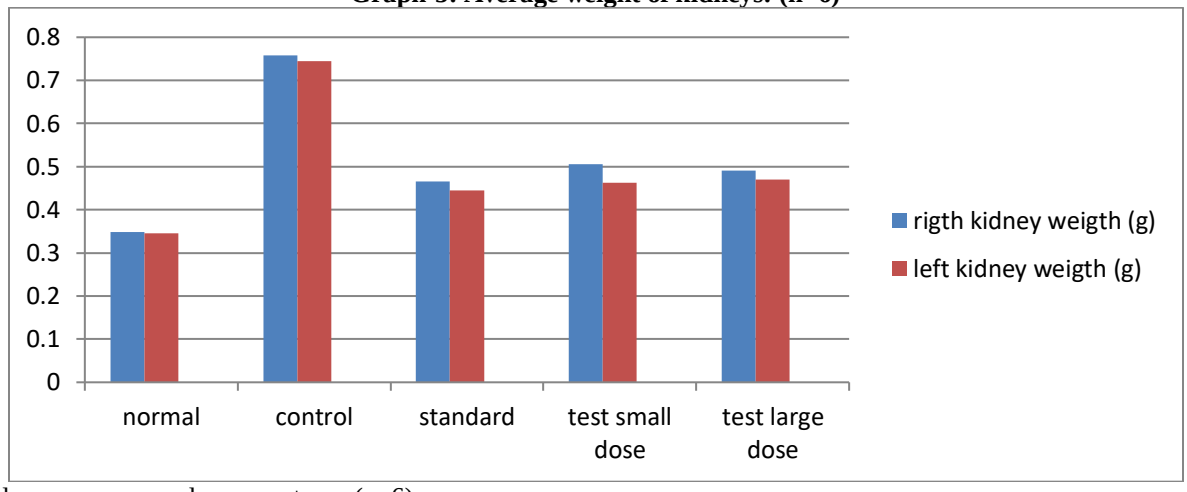
Values are expressed as mean±sem (n=6)

Graph-4serum urea levels (mg/dl)



Values are expressed as mean±sem (n=6)

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



Values are expressed as mean±sem (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%)	Serum Phosphorous Levels(mg/dl)	Serum Creatinine Levels(mg/dl)	Serum Urea Levels(mg/dl)
1	Normal	6.25±0.187	6.95±0.187	0.328±0.073	3.3±0.185
2	Controlled	10.86±0.216	9.783±0.318	0.633±0.025	40.55±0.707
3	Standard	6.35±0.187	7.28±0.146	0.353±0.021	16.80±0.506
4	Test small dose	7.4±0.26	7.85±0.187	0.511±0.023	27.73±0.584
5	Test large Dose	6.65±0.137	7.48±0.231	0.435±0.018	21.51±0.772

Values are expressed as mean±sem (n=6)

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

s.no	Animal treatment	Right kidney (gms)	Left kidney (gms)
1	Normal	0.348±0.024	0.345±0.020
2	Controlled	0.758±0.125	0.745±0.098
3	Standard	0.465±0.018	0.445±0.044
4	Test small dose	0.505±0.068	0.463±0.064
5	Test large dose	0.491±0.027	0.470±0.031

Values are expressed as mean ±sem(n=6)

CONCLUSION:

From the above results and study we can conclude that, *Achyranthes aspera* is having a significant antiurolithic activity, since it has reduced the serum levels of the above chemical constituents in the blood which were increased due to the development of stone in the kidney. The antiurolithic activity of herbal extract is dose dependant..

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