Pharmacognosy Magazine [Phcog Mag]
Vol 5, Issue 19 (Suppl.), Jul-Aug, 2009 Page 83-89
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 04 Apr, 2009
Modified: 05 May, 2009
Accepted: 15 May, 2009

PHCOG MAG.: Research Article

Study the Relative Effect of Spironolactone and Different Solvent Extract of *Tibulus terrestris* on Urolithiatic Rats.

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ABSTRACT

Hyperoxaluria is the foremost initiating cause for urolithiasis in renal cavities, where urolithiasis is a common urinary tract disorder. There is much expectation of finding active anti-urolithiatic compounds from indigenous plant as there are still uses in therapeutic despite the progress in conventional chemistry and pharmacology in producing effecting drugs. Most patients still have to undergo surgery to get rid of painful disease. In our present study on anti-urolithiatic agents from plants which was compared with synthetic chemical substance. As we have selected the sequential extracts of *Tribulus terrestris* with pet ether, chloroform, alcohol and water and compared the activity with the synthetic steroidal drug spironolactone which was used on the lithiatic induced rats and estimated the effect of the extract by doing the invo and invitro analysis alongside by histopathological studies and found the ethanolic extract of the drug was found to be effective than other treatment.

KEYWORDS: *Tribulus terrestris*, Ethylene glycol, Urolithiatic rats.

INTRODUCTION

Kidney stones are painful urinary disorders that start as salt/ chemical crystals which precipitate out from urine. Under normal circumstances, the urine contains substances that prevent crystallization but for patients with this condition, these inhibitory substances are ineffective. Tiny crystals will pass out along with the urinary flow without causing problems. At least few of people will pass a kidney stone during their lifetime, producing some of the most severe pain possible, by increasing the stone concentration in the kindney. If the stone is large enough to block the tube (ureter) and stop the flow of urine from the kidney, it must be removed by surgery or other methods. It is also called Renal Calculus. Symptoms usually begin with intense waves of pain as a stone moves in the urinary tract. Typically, a person feels a sharp, cramping pain in the back and side in the area of the kidney or in the lower abdomen. Sometimes nausea and vomiting occur. Later, pain may spread to the groin. The pain may continue if the stone is too large to pass; blood may appear in the urine and there may be the need to urinate more often or a burning sensation during urination. If fever and chills accompany any of these symptoms, which may lead to infectioins.

The plant drugs survey of *Tribulus terrestris* narrates that, the drug has been utilized for pre-clinical and clinical activities. *Tribulus terrestris* L. is a member of the family *Zygophyllaceae*. It is an annual herb about 30–70 cm high and has pinnate leaves (of unequal length), yellow flowers and characteristic stellate shaped carpel fruits. It is widely distributed in Africa, Western Asia, China, Japan, Korea, Europe, Kuwait and India. The extracts from this plant have been used traditionally in treating a variety

of diseases including hypertension and coronary heart disease, ocular inflammation and infertility in both sexes as an aphrodisiac and diuretics.

Spironolactone is a synthetic 17-lactone drug which is a renal competitive aldosterone antagonist in a class of pharmaceuticals called potassium-sparing diuretics, used primarily to treat heart failure, ascites in patients with liver disease, low-renin hypertension, hypokalemia, and Conn's syndrome as well as high blood pressure. On its own, spironolactone is only a weak diuretic, but it can be combined with other diuretics. As it is been used as a diuretic and most of the diuretic drugs have been used in urolithiasis treatment, so, it is been tried and compared with plant drug extract for the observation.

MATERIAL AND METHODS

Preparation from the dried fruit

The collected drug *Tribulus terrestris* fruits was coarse powdered using mixer grinder, this coarse powder was subjected for sequential extraction process using soxhlet apparatus on using different solvent system like, Pet ether, chloroform, ethanol, and water, and there residual extracts were collected.

Animals

About forty two male, wistar strain weighing between 150-180gms were used in this study.

Methodology and treatment

The rats were divided in four groups of six in each; animals were acclimatized to standard laboratory conditions. The rats were fed with commercially available standard pelleted feed and water *ad libitum* (1,3). The sequential extracts of fruit *Tribulus terrestris* were administered as an aqueous oral suspension using tween 60 as suspending agent by PO route and control animals received water as vehicle. The rats groupings: Group-I Normal control + commercial feed, Group-II Calculi producing agent (CPA) 0.2% ethylene glycol aqueous for 20 days,[2] Group-III 0.2% ethylene glycol aqueous for 20 days +next 10 days 25mg/kgbody wt Spironolactone, Group-IV 0.2% ethylene glycol aqueous for 20 days +next 10 days 25mg/kgbody wt petether extract of *Tribulus terrestris,* Group-V 0.2% ethylene glycol aqueous for 20 days +next 10 days 25mg/kgbody wt chloroform extract of *Tribulus terrestris,* Group-VI 0.2% ethylene glycol aqueous for 20 days + next 10 days 25mg/kgbody wt alcohol extract of *Tribulus terrestris,* Group-VII 0.2% ethylene glycol aqueous for 20 days +next 10 days 25mg/kgbody wt water extract of *Tribulus terrestris*. All the groups were given commercial pellet diet for 30 days and water *ad libitum*.

Parameters investigation
Collection and analysis of urine samples

On day 31, immediately after administration of the respective assigned doses, the rats were housed in metabolic cages for 24hrs urine collection using metabolic cage and measured the urine volume and compared with the normal group. A drop of concentrated hydrochloric acid was added to the collected the urine and urine volume was measured and stored at 4°C. The Levels of Oxalate (4), Calcium (5), Inorganic phosphorous (6), and Magnesium[7] were determined by autoanalyser / UV-spectrometer. Sodium (1) and Potassium (1) were estimated by Ion Selective Electrode (Rosh, name of the instrument).

Assay of renal tissue samples

At the end of the experiment, on day 33, the rats were sacrificed by cervical dislocation after anaesthetization, kidneys and liver were excised, washed with normal saline and kidneys were weighed, and one of the paired kidney was sent for histopathological studies. The other kidney was dried at 80°C in a hot air oven and dried weights were taken.

Kidney ion estimation

A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N HCl for 30 min and homogenized. The homogenate was centrifuged at 5000 rpm for 15 min, and the supernatant separated. The estimation of oxalate (4) and calcium (5) was carried out

Liver enzyme estimation

The livers excised, were washed with cold 0.15 M KCI and homogenized and the supernatant were taken for Lactate dehydrogenase (8) and Glycolate oxidase (4) enzymes estimation.

Statistical analysis

The data of urinary, renal and enzymes parameters were expressed as mean ± SEM. The results were analyzed statistically using one way ANOVA. The minimum level of significance was fixed at $P<0.05$.

RESULTS AND DISCUSSIONS

The 0.2% ethylene glycol a caliculi producing agent (CPA) has successfully induced the oxalate urolithiasis in treated

rats (9). The synthetic drugs spironolactone, which were taken as standard drugs for the contrast with the different extracts *of Tribulus terrestris*. To read out the effects of the extracts and the others drugs, the Group comparison were done, that is Group-I normal compared with the others Groups II-VI and Group II compared with the Groups III- VI

Urine analysis:

Supersaturation of ions is the causative factors to cause urinary caliculi in the kidney and in the renal cavities, on orally inducing the 0.2% ethylene glycol for 20 days resulted in hyperoxaluria due to ready conversions glycolate to oxalate (refer group-II), which received 0.2% CPA. The percentage effect of ethylene glycol was seriously carried out by survival/toxicity studies on animals and found 0.2% as non-toxic and effective dose for the induction of calculi (9).

Oxalate:

On comparision, the alcohol extract of *Tribulus terrestris*, has shown highly significant action to the spironalactone and water extract treated rats in reducing the urinary calculi, the reduction level of calculi in spironalactone was better than water extract and found significant, where the water extract was moderately significant when compared to normal control rats and CPA control rats, others pet ether extract and chloroform extract as not shown the reduction of oxalate ion concentration in urinary calculi. [Table 1]

Calcium and inorganic phosphate

The calcium and inorganic phosphate play vital role in renal calculogenesis, the increase in calcium, inorganic phosphate may be due to defection in tubular reabsorption in the kidney. The calcium, and inorganic phosphate levels were elevated in the rats receiving CPA. The rats receiving alcohol extract of *Tribulus terrestris* reduced more significantly on comparision to Group-I & II rats and Spironoloctone, has decreased the calcium, and inorganic phosphate concentrations significantly, and found effective, the water extract treated animals shown moderate reduction of ions and in the groups receiving pet ether extract, chloroform extract had increased the levels of calcium and inorganic phosphate levels. [Table 1]

Magnesium ions

The magnesium ion content was found to be more in normal group when compared to control group. The groups treated with alcohol extract, water extract and spironoloctone has increased magnesium ion concentration and found reverse in the groups treated with pet ether extract, and chloroform extract.[Table1]

Sodium and Potassium

There was increase in urine volume and shown diuretic effect in the groups treated with alcohol extract, water extract and spironaloctone, were the sodium and potassium ion concentration were found to be increased in the urine, except, in the group treated with spironalactone, the potassium ion concentration was reduced due to retention of potassium in the renal tubules, because, Spironalactone is found to be potassium sparing diuretic and increased out put of sodium, and the rat treated with pet ether and chloroform extract has decreased the urine volume which was in contrast to the output of sodium and potassium ion levels. [Table 1]

Kidney weight analysis

Due to the precipitation of ions the accumulation of ions in the kidney which leads to kidney weight variations on comparison to normal kidney, so, the wet and dry weight of the kidney were taken and compared between the groups,

Table:1 Urine Ananlysis :: Incomplete

Parameters mg/24 hrs urine /rat	Group- I Normal control	Group- II CPA control	Group- III 25mg/ kg body wt spiranolactone	Group- IV 25mg/ kg body wt pet ether extract of Tt	Group- V 25mg/ kg body wt chloroform extract of Tt	Group- VI 25mg/kg body wt alcohol extract of Tt	Group- VII 25mg/ kg body wt water extract of Tt
Oxalate	10.60 ±0.46	21.85^{d} ±0.74	$10.35^{a,d}$ ±0.28	$20.30^{d,a}$ ±0.90	$22.81^{d,a}$ ±0.56	$8.79^{c,d}$ ±0.18	$13.97^{c,d}$ ±0.59
Calcium	4.43 ±0.32	7.83^{d} ±0.21	$4.57^{a,d}$ ±0.22	$9.01^{d,a}$±0.30	$8.13^{d,a}$±0.32	$3.23^{a,d}$ ±0.20	$4.93^{a,d}$ ±0.18
Magnesium	3.15 ±0.26	1.27^{d} ±0.09	$2.94^{a,d}$ ±0.13	$1.3^{d,a}$ ±0.06	$0.93^{d,a}$ ±0.07	$3.019^{a,d}$ ±0.04	$2.02^{d,d}$ ±0.13
In org phos	1.05 ±0.04	2.02^{d} ±0.06	$0.93^{a,d}$ ±0.03	$1.72^{a,d}$ ±0.03	$1.41^{c,d}$ ±0.05	$0.71^{a,d}$ ±0.03	$1.17^{d,a}$ ±0.03
Sodium	10.83 ±0.34	4.86^{d} ±0.15	$12.91^{c,d}$ ±0.47	$5.32^{d,a}$ ±0.49	$6.37^{d,a}$ ±0.39	$12.11^{a,d}$ ±0.41	$9.79^{a,d}$ ±0.37
Potassium	9.62 ±0.44	5.88^{d} ±0.33	$5.68^{d,a}$±0.19	$4.71^{d,a}$ ±0.41	$4.99^{d,a}$ ±0.34	$13.91^{d,d}$ ±0.44	$12.31^{c,d}$ ±0.31

*Comprasion were made between the group I with the groups II,III,IV,V,VI,and VII and the group II with the groups II,IV,V,VI, and VII,; Statistical significance: a = ^{ns} P > 0.05, b = * P<0.05, c = **P<0.01, d = ***P<0.001 { Tt => Tribulus terrestris)*

there was a significant variation were seen in both the wet and dry kidneys weight of animals receiving 0.2% glycolic acid daily of Group-II, which was almost decreased in kidney weight by the drug treated with alcohol and water extract of *Tribulus terrestris,* and the synthetic steroidal drug
Spironolactone on comparision to normal group and found increase in kidney weight in pet ether extract, and chloroform extract treated rats. [Table 2]

Kidney ion analysis

The glycolic acid induction for 20 days resulted in renal tissue damage and deposition of calcium oxalate. The increased deposition of calcium and oxalate in renal tissues is known to lead to papillary calcification and elevated caliculi formation. A similar elevation in renal stone forming constituents in rat fed with CPA (caliculi producing agent). The administration of alcohol and water extract of *Tribulus terrestris,* and the synthetic steroidal drug Spironolactone found more significant reduction in the calcium and oxalate levels
and in the alcohol extract treated rat was more significant and other treated groups pet ether extract and chloroform extract did not reduce the calcium and oxalate ions. [Table 2]

Liver enzymes analysis

The liver is the major site of endogenous oxalate synthesis, where glycolate oxidaze and lactate dehydrogenase enzymes which will convert the glycolate to oxalate through glyoxalate, out of these enzymes the GAO is more actively effective in converting the glycolate to oxalate. The activity of the GAO enzyme was found to be less, in the rats treated with alcoholic and water extract of *Tribulus terrestris,* and the Spironolactone, the other treated groups Pet ether extract and Chloroform extract the activity of GAO enzyme increased in convertion of glycolate to oxalate. where LDH enzyme activity was reduced in the group treated with alcoholic extract and the other groups were ubiquitious distribution. Table 3

Biopsy of Rat kidneys:

Histopathological studies reveal the condition of rat kidneys of normal, 0.2% ethylene glycol which is calculi producing agent (CPA) treated, the synthetic drugs and the different sequential extracts of *Tribulus terrestris* treated rats. The rat kidneys which were stained by Haemitoxylin and Eosin and were examined using microscope under 10X. The accumulation and dissolution of the calcium oxalate precipitates in the rats renal cavities (renal cortex and renal medulla) was recognised and identified in the section and compared with each other, the slides Fig 3,4,5,6,7,. which was compared to the slides of normal Fig: 1and with (CPA) calculi producing agent Fig: 2 and respectively the scoring were done which is mentioned below the figures (4).

Table:2 Kidney ion Analysis/ The Dry weight and Wet weight of kidney

Parameter	Group- I Normal control	Group- II CPA control	Group- III 25mg/ kg body wt Spirano lactone	Group- VI 25mg/kg body wt pet ether extract of Tt	Group- V 25mg/kg body wt chloroform Extract of Tt	Group- VI 25mg/kg body wt alcohol extract of Tt	Group-VII 25mg/kg body wt water extract of Tt
Wet weight g/100g b.wt	0.355 ± 0.01	0.503^{d} ± 0.007	$0.335^{b,a}$ ± 0.02	$0.489^{d,a}$ ± 0.01	$0.491^{d,a}$ ± 0.04	$0.401^{b,a}$ ± 0.02	$0.373^{a,d}$ ± 0.02
Dry weight g/100g b.wt	0.07 ± 0.003	0.115^{d} ±0.002	$0.075^{a,d}$ ±0.004	$0.136^{d,a}$ ±0.008	$0.127^{d,a}$ ±0.003	$0.047^{a,d}$ ±0.008	$0.053^{a,d}$ ±0.007
Oxalate mg/100mg tissue	0.53 ± 0.01	1.11^{d} ±0.004	$0.59^{a,d}$ ±0.002	$1.10^{d,a}$ ±0.01	$1.19^{d,a}$ ±0.06	$0.41^{d,d}$ ±0.09	$0.81^{a,d}$ ±0.06
Calcium mg/100mg tissue	0.15 ±0.003	0.36^{d} ±0.005	$0.17^{a,d}$ ±0.005	$0.33^{a,d}$ ±0.017	$0.42^{d,d}$ ±0.056	$0.16^{c,d}$ ±0.035	$0.21^{a,d}$ ±0.018

*Comprasion were made between the group I with the groups II,III,IV,V,VI,and VII and the group II with the groups II,IV,V,VI, and VII, ; Statistical significance: a = ^{ns} P > 0.05, b = *P<0.05, c =** P<0.01, d = ***P<0.001 { Tt => Tribulus terrestris }*

Table: 3 Liver enzyme analysis

Parameter	Group- I Normal control	Group- II CPA control	Group- III 25mg/ kg body wt spiranolactone	Group- IV 25mg/kg body wt pet ether extract of Tt	Group- V 25mg/kg body wt chloroform Extract of Tt	Group-VI 25mg/kg body wt alcohol extract of Tt	Group- VII 25mg/ kg body wt water extract of Tt
GAO unit/mg protein	**2.56±0.06**	**5.39^{d}±0.19**	**$2.43^{a,d}$±0.12**	**$7.03^{d,a}$±0.17**	**$5.97^{d,a}$±0.06**	**$1.17^{a,d}$±0.09**	**$2.09^{a,d}$±0.05**
LDH unit/mg protein	**0.48±0.18**	**0.51±0.12**	**0.46±0.06**	**0.47±0.09**	**0.49±0.02**	**0.31^{ad}±0.09**	**0.41 ±0.06**

*Comprasion were made between the group I with the groups II,III,IV,V,VI,and VII and the group II with the groups II,IV,V,VI, and VII,; Statistical significance: a = ^{ns} P > 0.05, b = *P<0.05, c =**P<0.01, d = ***P<0.001 { Tt => Tribulus terrestris }*

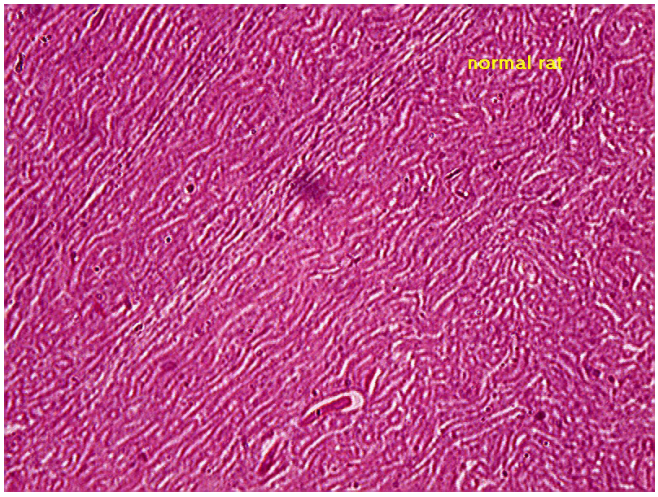

Figure 1: Normal rat referred in renal medulla there is no deposits/ accumulation of calcium oxalate ions and no damages in renal tubules which were intact and Scored: 00

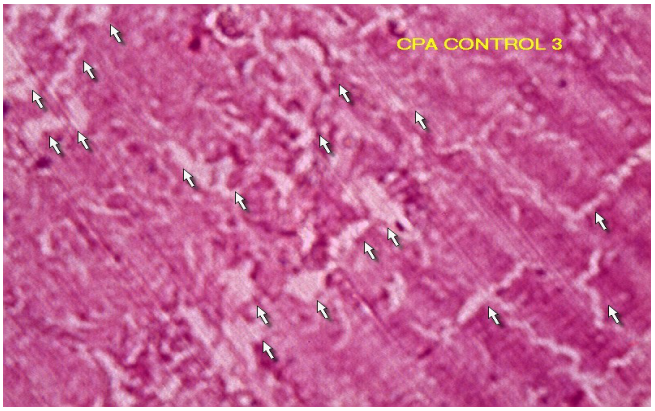

Figure 2: CPA [caliculi producing agent] ethylene glycol treated rat referred in renal medulla there was much deposits/accumulation of calcium oxalate ions and damages to the renal tubules were seen and Scored: +5.

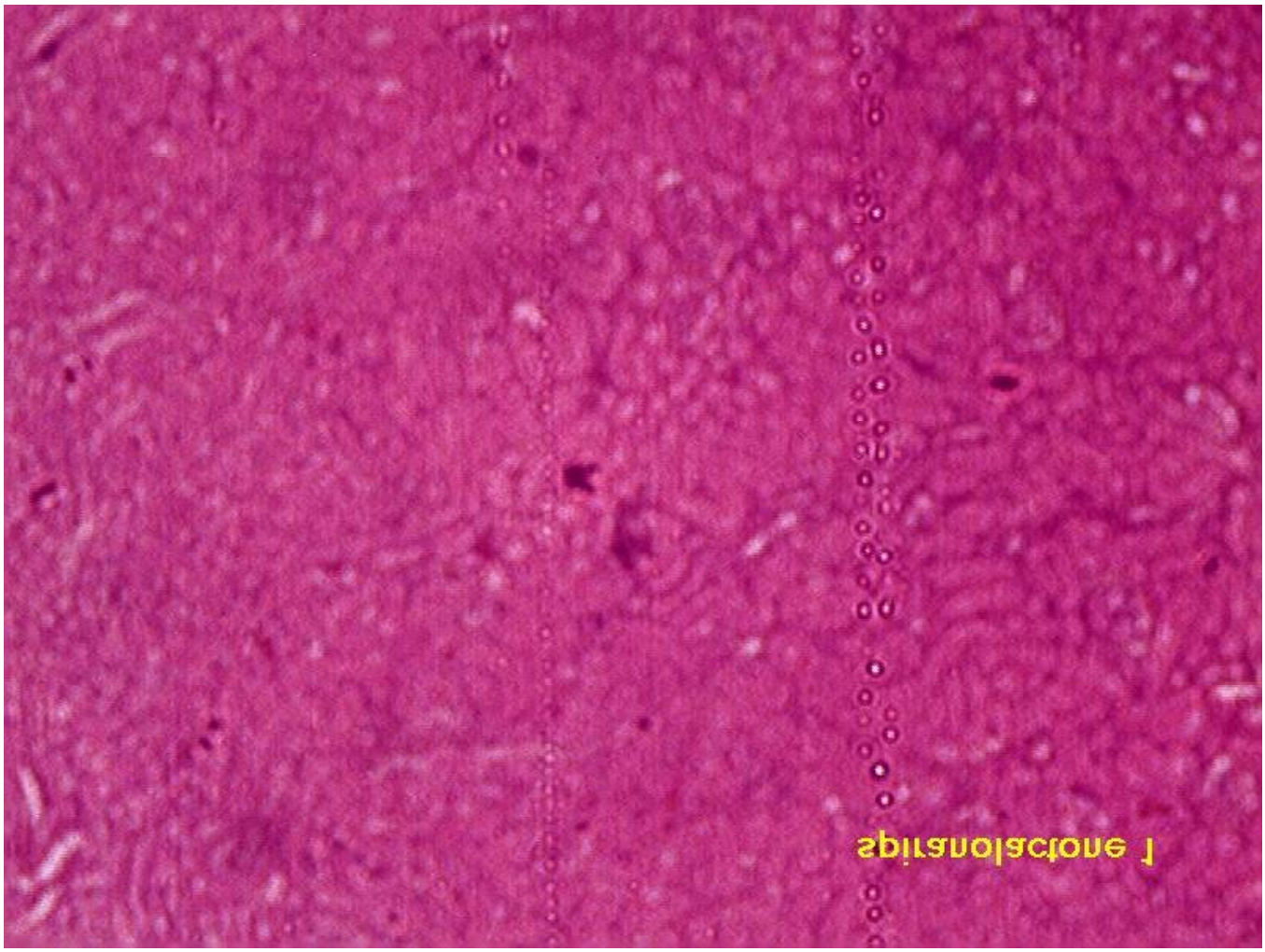

Figure 3: Spiranolactone treated rat, referred in renal medulla there was very very less deposits /accumulation of calcium oxalate ions in renal tubules and Scored: +1

Extracts of Tribulus Terrestris treated Rats

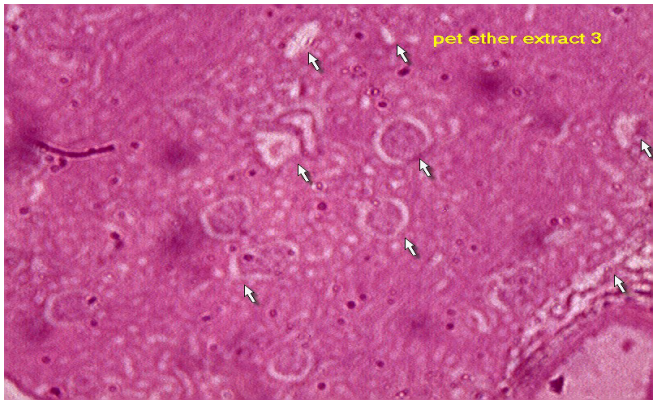

Figure 4: Pet ether extract of Tribulus terrestris treated rat, referred in renal medulla there was deposits/accumulation of calcium oxalate ions and damages were seen in renal tubules, as compared to control it is less and Scored: +3

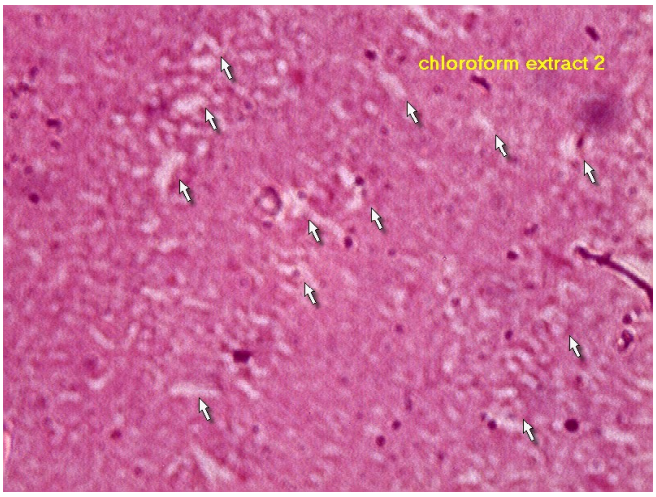

Figure 5: Chloroform extract of Tribulus terrestris treated rat, referred in renal medulla there, was more deposits/accumulation of calcium oxalate ions which was almost equal to the group-II that CPA control rats and scored: +4

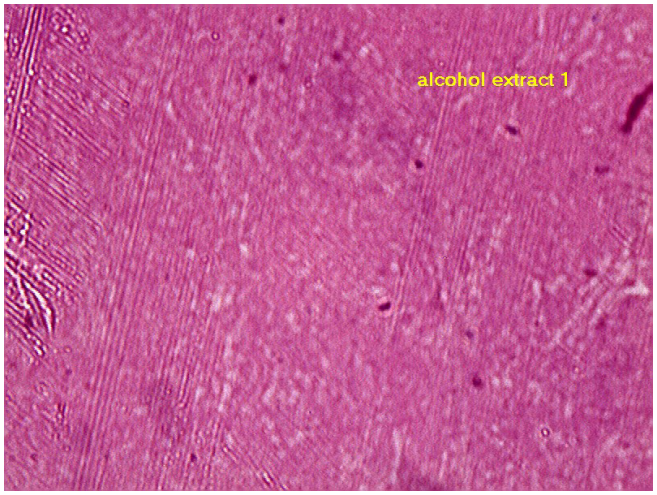

Figure 6: Fig 6: Alcohol extract of Tribulus terrestris treated rat, referred in renal medulla there was very very less deposits/accumulation of calcium oxalate ions no damages to renal tubules which was compared to the normal rat and Scored: 00

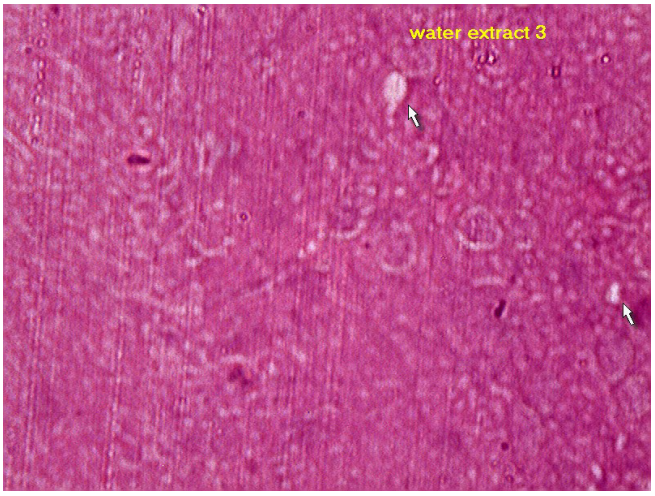

Figure 7: Water extract of Tribulus terrestris treated rat, referred in renal medulla there was less deposits/accumulation of calcium oxalate ions in renal tubules and Scored: +1

CONCLUSION

On detail study, the alcohol and water extracts of *Tribulus terrestris,* was found to be effective then Spironolactone in reducing stone forming constituents both in urine and renal tissues and also reduced, the enzyme activity of GAO and LDH.out of this the alcohol extract of *Tribulus terrestris,* were found to be more effective and highly significant in the reduction of calculi, which can be used as anti urolithiatic agent. The pet ether and chloroform treated groups were found be insignificant.

ACKNOWLEDGEMENTS

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