WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 870-894 Research Article ISSN 2278 – 4357

ANTIUROLITHIATIC ACTIVITY OF COMBINATION OF *TRIBULUS TERRESTRIS* AND *ASPARAGUS RACEMOSUS* EXTRACTS AGAINST CALCIUM OXALATE INDUCED UROLITHIASIS IN RATES

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Article Received on
05 Dec. 2018,

Revised on 25 Dec. 2018,
Accepted on 16 Jan. 2019

DOI: 10.20959/wjpps20192-13090

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ABSTRACT

Introduction: Urolithiasis is the condition where urinary calculi are formed in the urinary tract by crystal nucleation, aggregation and retention in urinary tract. It is a common disorder on estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females. It causes serious health problems such as severe pain, urinary tract obstruction and infection that adversely affect well-being of individuals. The knowledge of traditional medicine and medicinal plants and their study of scientific chemical principles may lead to the discovery of newer and cheaper drugs. **Objective:** The present study was undertaken to study the effects of a combination of herbal extracts on experimentally-induced Urolithiasis. **Material and Methods:** Calcium Oxalate urolithiasis in male rats were induced experimentally by administration of 0.75% v/v ethylene glycol in drinking water for Fourteen days followed by only 0.75% v/v ethylene glycol for 28 days. The combination of herbal extracts administered to urolithiasis induced test group rats at two doses i.e.Test-I (Combined Hydroalcoholic Extract of *Tribulus terrestris* and *Asparagus racemosus* 200mg/kg) and Test-II (Combined Hydroalcoholic Extract of *Tribulus terrestris* and *Asparagus racemosus* 400mg/kg). These two test groups compared against standard antiurolithic herbal drug Cystone (750mg/kg). **Result:** No mortality or any toxic effects were observed in the animals during acute oral toxicity study as per the OECD no 423. After 28 days, highly significant deposition of calcium oxalate in the kidneys was noticed along with decreased urinary output, increase in the urinary oxalate, calcium and magnesium, phosphorus levels in urolithiasis in control group rats as compared to normal group rats. The serum analysis showed significant increase in the serum creatinine and blood urea in

urolithiasis control group rats. In addition, vehicle treated induction control group rats showed significant increase in the biochemical parameters such as ALP, AST, ALT levels in the kidney homogenate which indicates the induction of urolithiasis. Daily oral treatment with combination of herbal extracts at Test -I & II doses significantly decreased the quantity of calcium oxalate urolithiasis. However 750mg/kg dose of combination of herbal extracts was found to be significant in these regards. Histopathological examination of renal tissues showed drastic reduction in stone formation. **Conclusion:** The presented data indicates that the combination of herbal extracts shows significant antiurolithiatic activity against calcium oxalate induced urolithiasis. The combination of herbal extracts resulted in an increase in urinary volume, urinary pH along with decrease in calcium, oxalate, phosphate and magnesium level which inhibit stone formation. It also resulted in an decrease in serum creatinine and blood urea, SGOT, SGPT, ALP level and kidney weight that leads to normal GFR and tubular damage of kidney tissue. The findings clearly state that, the combination of herbal Hydroalcoholic extract of *Tribulus terrestris L*. and *Asparagus racemosus w*.is a potent nephroprotective agent by preventing the crystal retention in the tissues. Herbal extract treatment is an effective drug in the management of urolithiasis and could be tried in the treatment of urolithiasis.

KEYWORDS: Urolithiasis, Ethylene glycol, Calcium oxalate, Cystone, Kidney homogenate.

1. INTRODUCTION

"Urolithiasis is a condition in which crystals in the urine combine to form stones, or calculi called urolithiasis." It is a painful condition may occurs in urinary tract such as kidney, ureters, urethra, urinary bladder. It may causes variable degree of pain, bleeding, further lead to secondary infection.[1] Urolithiasis is derived from the Greek words "ouron"(urine) and "lithos" (stone).[2] Uroliths (stone) are hard, solid particles, can form when urine contains too much of certain substances and it is composed of calcium oxalate monohydrates & calcium hydrogen phosphate dihydrates(75-90%), Ammonium magnesium phosphate hexahydrate(10-15%),Uric acid & Urate(3-10%) 0.5-1% is composed of cystine,hippuric acid, L-Tyrosine and xanthenes.[3] These Substances can create small crystals that become stones. The stones take weeks or months to Form. Uroliths can be occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys.[4]

Super saturation- It occurs when the concentration of substances forming urinary calculi increases in urine, Urine volume decreases, as well as the absence or reduction in urinary stone inhibitors occurs in urine. Super saturation of the urine constitutes a driving force within the solution which can lead to crystallization and trigger a series of pathophysiologic events that include nucleation, crystal aggregation, growth, and attachment to epithelia. **Nucleation:** It is the formation of a solid crystal phase in a solution. Involves the association of crystalloids in solution to form a sub microscopic particle. There are two types of Nucleation; 1) homogeneous nucleation – occurs *in vivo,* spontaneously in a pure solution. 2) heterogeneous nucleation- occurs at a lower super saturation level than that required for the homogeneous nucleation The nucleation of crystalline components may occur in the lumens of renal tubules, in the basement membranes of tubule cells, or at both sites, perhaps depending on the type of stone.

Crystal Aggregation: The process in which crystal nuclei bind to each other to form larger particles is called aggregation.

Crystal Growth: In this process atoms or molecules from solution are added to the solid phase of growing crystal in a geometrically precise arrangement.

Crystal Retention: If the nucleated crystals were flushed out by urinary flow, crystal retention is, act as a key factor will result if the crystals grow large enough to be trapped in renal tubules.

As far as urinary system is concerned; it is 3rd prevalent disorder which usually starts with obstruction and if left untreated results in severe complications like multiple infections and hemorrhage suggesting need of ideal medical care.[5] Pathophysiologically, urolithiasis occurs as a consequence of the breakdown of a delicate balance to be maintained by the kidneys i.e. excretion of materials that have a low solubility and conservation of water. These two opposing requirements must be balanced during adaptation to diet, climate and various activities. Whenever the urine becomes supersaturated with insoluble material, because excretion rates are excessive and reduced water conservation, crystals are formed, grow and aggregate to form a stones. The impact of these stones increases by many folds in the presence of other complaints like hypertension, obesity hepatic dysfunction etc.[6][7][8] The etiology of this disorder is multifactorial and is strongly related to dietary habits or practices.

The medical management of urolithiasis mainly involves techniques like extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy, however, these treatment options

are too costly and with these procedures recurrence is quite common.[9] The scientific documents revealed that The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years suggesting its need, however continuation of therapy is adversely affected by wide range of undesirable side effects such as hemorrhage, hypertension, tubular necrosis and subsequent renal fibrosis etc.[10][11] The overall outcome gives clear cut indication for exploration of new remedy. In light of this, exploitation of natural sources has been assumed to be of greater potential.[12] In the Indian traditional systems of medicine including Ayurveda, most of the remedies are derived from plants and their traditional applications are proved to be useful chiefly decreasing the recurrence rate of urolithiasis without causing any potential side effects.[13] However, the scientific documentation of their use is not well established through systematic scientific documentation making it worthwhile to explore.[1][7] Ayurvedic drugs such as Chandraprabha bati, calcury, Patherina tablet.,Ber Patthar Bhasma, Trinapanchamool, Rencare Capsule are widely used in treatment of Urolithiasis.[14] Herbal treatment is safer as compared to the synthetic drug and no need of drug monitoring.

So, Herbal medicinal plants investigated and used for the treatment of Antiurolithiatic activity such as, *Armoracia lopathifolia, Apium graveolens, Asparagus racemosus, Achyranthus aspera,* Amni visnaga, *Bergenia ligulata, Barbarea vulgaris,* Cranberry juice, *Capsella bursapastori, Cynodon dactylon, Eleusine indica, Ficus carica, Tribulus terrestris* Rosmarinus officinalis, *Pergularia daemia, Momordica charantia, Lemonade juice, Moringa oliefera* ,Olea europeae, *Quercus salicina, Phyllanthus niruri,* Vetiveria zizanioides, Raphanus sativus.[14][15] It may be possible that single drug cannot give that much effect as compare to combined one.

Therefore, herbal drugs combination or suspension was formulated, evaluated and studied for antiurolithiatic activity. It was prepared from fruits of Tribulus terrestris L. (zygophyllaceae), roots of Asparagus racemosus w.(lilliaceae) Tribulus terrestris have antimicrobial and antifungal activity[16], diuretic activity due to concentration of potassium salts present in it. Antiurolithiatic and is reported to inhibit stone formation.[17][18] It has immunimodulatory[19], analgesic[20] and anti-inflammatory activity.[21] It relaxes spasm of smooth muscle.[22] while Asparagus racemosus have Antioxidant, Phytoestrogenic and Antidiarrhoeal activity[23], Antiinflammatory, Adaptogenic Antidyspepsia activity[23][24], Anticancer, Antibacterial, Antitussive activity[23][24][25] The present study aimed to evaluate the antiurolithiatic activity

of combined hydroalcholic extract of Tribulus terrestris and Asparagus racemosus on wistar male Rats.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Cystone was purchased from local drug store and Ethylene Glycol was purchased from Merck Laboratories, Mumbai. All other reagents and solvents used were of analytical grade and were obtained from various other commercial sources.

2.2 Collection of Plant Material

The plants were collected from Solapur region of Maharashtra in the month of October-November 2017. It was authenticated by Department of Botany from Shri. Shivaji Mahavidylaya, Barshi.

2.3 Plant Material Preparation

The plants were dried under shade and powdered by using grinder mixer. The Combined Hydroalcoholic extract of *Tribulus terrestris and Asparagus racemosus* was prepared by Soxhlet of the powdered material in 80% ethanol and 20% distilled water. Then it was dried. The final semisolid mass of *Tribulus terrestris and Asparagus racemosus* was stored in 4ºc in refrigerator till it was used for further study.

2.4 Formulation of extract for administration

Required quantity of the extract was weighed and dissolved in distilled water with sonication. All the preparations were freshly prepared and administered by oral route.

2.5 Experimental Protocol

Male wistar rats (150-200gm) were used for the study. The animals were maintained in polypropylene cages at a temperature of 22°C ± 3°C and relative humidity of at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Under 12 h light/dark cycle. The animals had free access to standard rat/mice pellets and purified water *ad* libitium.

All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) (Protocol No. YSPM/YTC/PHARM/25/2017) and were conducted according to the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals, India.

2.6 Acute Oral Toxicity Studies

Acute oral toxicity of combined hydroaloholic extract of *Tribulus terrestris* and *Asparagus racemosus* was performed as per the OECD Guideline No.423. In brief, nulliparous and non-pregnant female mice weighing between 25-35 g was used for the study. Only female have been selected for the AOT study because females are more sensitive than the male. Animals were fasted for 3 h and then administered with single dose of extract 2000 mg/kg. Animals were observed for the toxic signs with special emphasis to changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Alteration should be directed to observations of termed convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality initially for 48 h (short term toxicity), animals found alive were further observed for 14 days (long term outcomes). Lethal dose 50% was calculated using AOT 423 stat program.[26]

2.7 Antiurolithiatic activity in ethylene glycol induced urolithiasis

Ethylene glycol induced hyperoxaluria model was used. Healthy male wistar Rats (150-200gm) were selected and divided into five groups of six animals each for the activity. From the first day, Group 1st i.e. control group received saline solution, regular feed and drinking water ad libitum. Groups II to VI were given ethylene glycol EG (0.75%) in drinking water for 28 days, to induce renal calculi.

The groups were assigned as:

Group I – Normal control – administered with Saline solution

Group II – Negative control – given EG only (0.75%v/v p.o.)

Group III – Positive control (standard Group)– EG + Cystone 750 mg/kg (p. o.) from 15th to 28th day

Group IV – Test I (Low Dose Group) – EG + combined Hydroalcoholic Extract of T.T. &A.R. (200mg/kg p.o.) from 15th to 28th day

Group V – Test II (High Dose Group) – EG + combined Hydroalcoholic Extract of T.T. &A.R. (200mg/kg p.o.) (p. o.) from 15th to 28th day(Dixit & koti,nishi saxsena)

Collection and analysis of urine

On the 28th day of calculi induction treatment, all animals were kept in individual metabolic cages and urine samples of 24 h were collected. The collected urine samples were measured for following parameters.

1) Urine volume

Animals were placed in separate metabolic cages for 24 hr and total urinary volume was measured using the measuring cylinder and reported in ml.[27][28][1]

2) Urine pH

Uric acid crystals were found to deposit most frequently in the concentrated acid urine. Thus, the acidity of the urine was tested using the pH meter.[1][27][28]

3) Urinary oxalate

The 1 ml of urine was acidified beforehand by concentrated HNO3 to solubilize crystals and then adjusted to pH 7 by NaOH in the presence of color indicator, the bromothymol blue. About 2ml of saturated CaSO4 and 14 ml of pure ethanol were added to precipitate oxalate overnight. The samples were centrifuged at 450 × *g* for 10 min and then filtered on filter paper. The precipitate obtained was solubilized in 10 ml of water acidified by 2ml of concentrated sulfuric acid. The samples were titrated by a solution of $KMnO^4$.[1][27][28]

4) Urine calcium

It was estimated by using commercially available standard kit of Biolab diagnostic Pvt.Ltd. Tarapur India as per o-cresolphthalein complexone method. Determination of Urine Calcium was done by autoanalyser.[1][28]

5) Urine magnesium

It was estimated by using commercially available standard kit supplied by Biolab diagnostics Pvt. Ltd. Tarapur (India) as per Calmagite method. Determination of urine magnesium was done by using CHARIOT prince autoanalyser.[1][17][28][29]

6) Urine phosphate

Phosphate ions in an acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex has an absorbance in the ultraviolet range and is measured at 340nm. Intensity of the complex is directly proportional to the amount of inorganic phosphorus present in the sample.[30][31][32][33]

Collection of blood and Serum analysis[1][34]

After urine collection period, blood was obtained from the retro-orbital under anaesthetic condition and animals were sacrificed by cervical decapitation. Serum were separated by centrifugation and analyzed for urea, creatinine.

1) Serum creatinine

It was estimated by using commercially available standard kit as per Urease/salisylate method. Determination of serum creatinine was done by using CHARIOT prince biochemistry autoanalyser.

2) Blood urea

It was estimated by using commercially available standard diagnostic kit of Biolab Diagnostics-India using diacetymonoxime colorimetric end-point method. Determination of Blood urea was done by using CHARIOT prince biochemistry autoanalyzer.

Kidney homogenate analysis[35][36][37][38]

The animals were sacrificed on 29th day by cervical decapitation and Abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue Both kidneys were weighed and Right kidneys were disposed with sacrified animal and left kidney is preserved in 10% neutral formalin solution having pH.7.4 embeded in paraffin, sliced 5µm pieces; the slices were stained with hematoxylin and eosin and mounted in diphenyl xylene. Tissue slices were phographed using optical microscopy and the histopathological changes in kidney observed.

Estimation of biochemical markers[1]

The homogenate was used to assay the marker enzymes in serum, urine and tissue constituents like alkaline phosphate (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and were estimated respectively using different types of enzyme marker kits.

STATISTICAL ANALYSIS

Data expressed as Mean S.E.M. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test and $p<0.05$ considered as statistical significant.

3.0 RESULTS

3.1 Acute oral toxicity study

Acute oral toxicity study for the plant extracts was performed as per OECD Guidelines NO. 423. The outcome of the study showed that the combined Hydroalcoholic extract of *Tribulus terrestris and Asparagus racemosus* was safe upto 2000 mg/kg, p.o. Further, no signs of toxicity were observed during short-term (48 hr) and long-term (14 days) observation period.

Evaluation of Hyperoxaluria model by using Urine parameters

1) Evaluation of Urine Volume by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

The average volume of urine and inhibition of urolithiatic response are presented in table 1.1 Antiurolithiatic activity of combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*was screened at two different concentrations i.e. 200mg/kg, p.o. & 400mg/kg, p.o., where as cystone 750mg/kg,p.o. was used as standard drug. In present study, When compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$) to reduce calcium oxalate induced urolithiasis., when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$).i.e. there was increase in urine volume compared to negative control. The maximum inhibition of antiurolithiatic activity (400mg/kg, p.o. and cystone (750mg/kg,p.o.) was observed.

Table 1.1 Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L.and Asparagus racemosus w.on* Urine Volume.

Sr No.	Group	Urine volume values
1	Normal group	8.675 ± 0.3983
2	Induction Group (0.75%v/v)	6.172 ± 0.1802 c
3	Standard Group (750mg/kg)	11.84 ± 0.5959 c r
4	Low Dose (200mg/kg)	9.198 ± 0.1726 r z
5	High Dose (400mg/kg)	9.510 ± 0.2222 r z

Values are mean ± SEM (n=6) p values < 0.05 is considerd as stastically significant , p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

2) Evaluation of Urine pH by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

Evaluation of this test is use to analyze the acidity or alkalinity of urine. It is painless test. low or high pH can indicate there may be chances of kidney stone.Calcium oxalate induced urolithiasis it is analyzed that there is decrease in urine pH it can be overcome by treatment groups and level of urine pH is Regulated. from the Table 1.2 it was observed that Urine pH when compare with Normal group both 200mg/kg and 400mg/kg dose gave significant of ($p<0.01$), when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$) and it

was also observed that cystone (750mg/kg,p.o.) and extractdose of 400mg/kg shows maximum inhibition of Antiurolithiatic activity.

Table 1.2: Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L.and Asparagus racemosus w.on* Urine pH.

Sr No.	Group	Urine pH values
1	Normal Group	6.473 ± 0.1259
2	Induction Group (0.75%v/v)	5.588± 0.1399 c
3	Standard Group (750mg/kg)	6.340± 0.1130 r
4	Low Dose (200mg/kg)	7.093 ± 0.08305 b r z
5	High Dose (400mg/kg)	7.142 ± 0.04512 b r z

Values are mean ± SEM (n=6) p values < 0.05 is considerd as stastically significant , p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group(negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

3) Evaluation of Urine oxalate by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

The changes in antiurolithiatic activity by administration of extract and standard drug are shown in Table 1.3. In the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w.* at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, Urine oxalate when compare with Normal group both 200mg/kg and 400mg/kg dose gave significant of (p <0.01), when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of (p<0.001), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of (p<0.001).

Table 1.3 Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L. and Asparagus racemosus w.on* Urine oxalate.

Sr No.	Group	Urine oxalate values
1	Normal Group	0.4217 ± 0.03894
2	Induction Group (0.75%v/v)	2.685 ± 0.1427 c
3	Standard Group (750mg/kg)	2.183 ± 0.1797 c
4	Low Dose (200mg/kg)	0.7650 ± 0.09629 r z
5	High Dose (400mg/kg)	1.092 ± 0.1182 b r z

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as

compared with Induction group(negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

4) Evaluation of Urine Calcium by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

In the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w*. at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. From Table 1.4. It was observed that when extract group was compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of (p<0.001), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave significant of (p<0.01) also standard drug cystone also shows inhibitory effect on urolithiasis.

Table 1.4 Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L. and Asparagus racemosus w.on* Urine Calcium

Sr No.	Group	Urine calcium values
1	Normal Group	3.563 ± 0.5749
2	Induction Group (0.75%v/v)	7.327 ± 0.7421 c
3	Standard Group (750mg/kg)	4.548 ± 0.2296 q
4	Low Dose (200mg/kg)	1.897 ± 0.2185 r y
5	High Dose (400mg/kg)	1.963 ± 0.2800 r y

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group(negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

5) Evaluation of Urine Magnesium by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

Table 1.5 shows that the observations of effect of Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L*. and *Asparagus racemosus w.* at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, Urine Magnesium when compare with Normal group 400mg/kg dose gave significant of (p <0.01), when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of (p<0.001), when compare with Standard group (positive control group)

200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$) also dose of standard drug i.e.cystone is highly significant ($p<0.001$).

Table 1.5: Effect of combined Hydroalcoholic extracts of extracts of *Tribulus terrestris L. and Asparagus racemosus w.on* Urine Magnesium.

Sr No.	Group	Urine magnesium values
1	Normal Group	0.8100 ± 0.06758
2	Induction Group (0.75%v/v)	2.647 ± 0.2141 c
3	Standard Group (750mg/kg)	2.623 ± 0.1521 c
4	Low Dose (200mg/kg)	1.772 ± 0.08716 b q y
5	High Dose (400mg/kg)	1.637 ± 0.1825 b r z

Values are mean ± SEM (n=6) p values < 0.05 is considered as stastically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group(negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

6) Evaluation of Urine Phosphorus by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

Urine Phosphorus is a parameter used in Hyperuroloxia model for Antiurolithiatic activity. From Table 1.6 it was shown that Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w*. at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, Urine Phosphorus when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave significant of ($p<0.01$) standard drug cystone is also significant.

Table 1.6 Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L. and Asparagus racemosus w.on* Urine Phosphorus.

Sr No.	Group	Urine phosphorus values
1	Normal Group	3.270 ± 0.2413
2	Induction Group (0.75%v/v)	6.195 ± 0.3414 c
3	Standard Group (750mg/kg)	4.902 ± 0.3674 b p
4	Low Dose (200mg/kg)	3.213 ± 0.2022 r y
5	High Dose (400mg/kg)	3.447 ± 0.2053 r y

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group(negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

Evaluation of Hyperoxaluria model by using serum parameters

1) Evaluation of Blood Urea by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L*. and *Asparagus racemosus w.*

The changes in Urea shown in Table 1.7 standard drug cystone acts as inhibitory action for antiurolithiasis, also when Blood urea compare with Normal group 200mg/kg dose gave highly significant of ($p < 0.001$), when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$).

Table 1.7: Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L.and Asparagus racemosus w.on* Blood Urea.

Sr No.	Group	Urine Blood Urea values
1	Normal Group	51.00 ± 2.668
2	Induction Group (0.75%v/v)	50.33 ± 2.578
3	Standard Group (750mg/kg)	41.67 ± 1.994 p
4	Low Dose (200mg/kg)	27.83 ± 1.302 c r z
5	High Dose (400mg/kg)	33.00 ± 1.291 a r x

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

2) Evaluation of Serum creatinine by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L*. and *Asparagus racemosus w.*

Creatinine determinations with small total error are still required at pathological creatinine concentrations to assess the severity of renal failure. So to recover it was carried out with treatment groups. From Table 1.8 the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w*. at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, Creatinine when compare with Normal group 200mg/kg dose gave significant of ($p < 0.01$), when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave significant of ($p<0.01$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave significant of ($p<0.01$), standard drug cystone acts as highly significant($p < 0.001$) acts as best stone inhibitor.

Table 1.8: Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L. and Asparagus racemosus w.*on Serum creatinine.

Sr No.	Group	Serum creatinine values
1	Normal Group	0.5450 ± 0.03973
2	Induction Group (0.75%v/v)	1.010 ± 0.05086 c
3	Standard Group (750mg/kg)	0.4967 ± 0.04660 r
4	Low Dose (200mg/kg)	0.7733 ± 0.005238 a p y
5	High Dose (400mg/kg)	0.7283 ± 0.04102 q x

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

3) Evaluation of Serum Aspartate aminotransferase (SGOT/AST) by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

Serum glutamic oxaloacetic transaminase is used for the blood test to check damage to liver, kidney, and pancreas. From Table 1.9 it was shown that there is change in level of SGOT level in induction group standard group and test groups. In the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w.* at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For SGOT (AST) standard group i.e. cystone drug shows significant effect on urolithiasis, when compare with Normal group 400mg/kg dose gave significant of ($p < 0.01$), when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$).

Table 1.9: Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L. and Asparagus racemosus w.*on Serum Aspartate aminotransferase(SGOT/AST).

Sr No.	Group	SGOT values
1	Normal Group	41.10 ± 0.7450
2	Induction Group (0.75%v/v)	62.15 ± 1.214 c
3	Standard Group (750mg/kg)	49.37 ± 0.4016 c r
4	Low Dose (200mg/kg)	43.67 ± 0.3827 y z
5	High Dose (400mg/kg)	45.57 ± 0.5850 b r y

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test.

4) Evaluation of Serum Alanine aminotransferase (SGPT/ALT) by using Hyperoxaluria model in combined Hydroalcoholic extract of Tribulus terrestris L. and Asparagus racemosus w.

SGPT (ALT) is a parameter used in Hyperuroloxia model for Antiurolithiatic activity. In the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w*. at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, SGPT (ALT) when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), cystone is also shows highly significant effect on urolithiasis all results are shown by Table 1.10.

Table 1.10 Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L.and Asparagus racemosus w*.on Serum Alanine aminotransferase (SGPT/ALT).

Sr No.	Group	SGPT values
1	Normal Group	2.266 ± 0.1432
2	Induction Group (0.75%v/v)	3.956± 0.09607 c
3	Standard Group (750mg/kg)	3.201 ± 0.1064 c q
4	Low Dose (200mg/kg)	2.119 ± 0.2013 r z
5	High Dose (400mg/kg)	2.283 ± 0.09088 r z

Values are mean ± SEM (n=6) p values < 0.05 is considered as stastically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

5) Evaluation of Serum Alkaline phosphate (ALP) by using Hyperoxaluria model in combined Hydroalcoholic extract of *Tribulus terrestris L.* and *Asparagus racemosus w.*

To investigate whether Hydroalcoholic extract of combination of *Tribulus terrestris L.* and has Antiurolithiatic activity, ALP is a parameter used in Hyperuroloxia model for Antiurolithiatic activity. Table 1.11 shows that In the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w*. at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, ALP when compare with Induction group (negative control group), 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$), when compare with Standard group (positive control group) 200mg/kg, 400mg/kg both dose gave highly significant of ($p<0.001$) standard drug cystone shows highly significant effect on urolithiasis.

Table 1.11: Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L.and Asparagus racemosus w*.on Serum Alkaline phosphate (ALP).

Sr No.	Group	ALP values
1	Normal Group	69.89 ± 0.4951
2	Induction Group (0.75%v/v)	79.71 ± 0.6813 c
3	Standard Group (750mg/kg)	74.81 ± 0.7273 c r
4	Low Dose (200mg/kg)	68.10 ± 0.8425 r z
5	High Dose (400mg/kg)	69.23 ± 0.7147 r z

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

Evaluation of Kidney weight by using Hyperoxaluria model in combined Hydroalcoholic extract of Tribulus terrestris L. and Asparagus racemosus w.

The change in kidney weight is a parameter which can shown the effect of calcium oxalate induced urolithiasis in Rats. From Table 1.12 shown that the present investigation Hydroalcoholic extract of combination of *Tribulus terrestris L.* and *Asparagus racemosus w.* at the dose of 200mg/kg and 400mg/kg p.o. was given to the Rats. For, kidney weight when compare with Standard group (positive control group) 200mg/kg, gave highly significant of (p<0.001). 400mg/kg both dose gave significant effect of of (p<0.01) and cystone standard drug act as best significant for Urolithiasis condition.

Table 1.12 Effect of combined Hydroalcoholic extracts of *Tribulus terrestris L.and Asparagus racemosus w*.on kidney weights.

Sr No.	Group	Kidney weight values
1	Normal Group	0.4617± 0.03156
2	Induction Group (0.75%v/v)	0.6050 ± 0.03939 a
3	Standard Group (750mg/kg)	0.4333 ± 0.02201 q
4	Low Dose (200mg/kg)	0.6450 ± 0.0.02742 b z
5	High Dose (400mg/kg)	0.6233 ± 0.0.03180 b y

Values are mean ± SEM (n=6) p values < 0.05 is considered as statistically significant, p values a<0.05, b<0.01, c<0.001 as compared with normal control; p<0.05, q<0.01, r<0.001 as compared with Induction group (negative control); x<0.05, y<0.01, z<0.001 as compared with Standard group (positive control) (by one-way ANOVA followed by Turkey's Multiple comparison test).

HISTOPATHOLOGY

The histopathological study of the kidney sections also supported the results. The normal group did not showed any crystalline deposits.(1.1 A) Whereas, in all the stone forming rats there was damage to the last part of the nephron, collecting system, renal tubular damage, tubular dilatation and presence of calcium oxalate crystals in the induction control rat kidney architecture. (1.2 B). However, in combination of Herbal extracts (combined Hydroalcoholic extracts of Tribulus terrestris and Asparagus racemosus 200mg/kg) treated group Test I shows little renal destruction (1.3 C). But in combination of herbal extracts (combined Hydroalcoholic extracts of Tribulus terrestris+ Asparagus racemosus 400mg/kg) treated group Test II showed significantly less number of calcium oxalate crystal and less damage as compared to induction control group (1.4 D) Cystone treated group also showed significantly less number of calcium oxalate crystal. (1.5 E).

Histopathological view of kidney

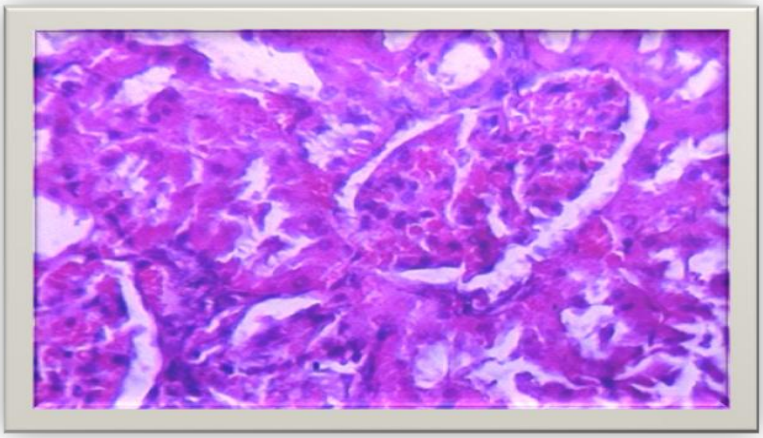

Fig No. 1.1 A) Normal Group.

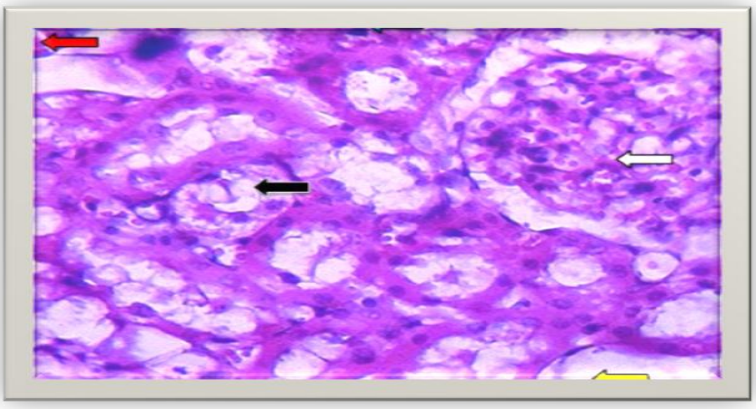

Fig No. 1.2 B) Induced Group (Ethylene Glycol).

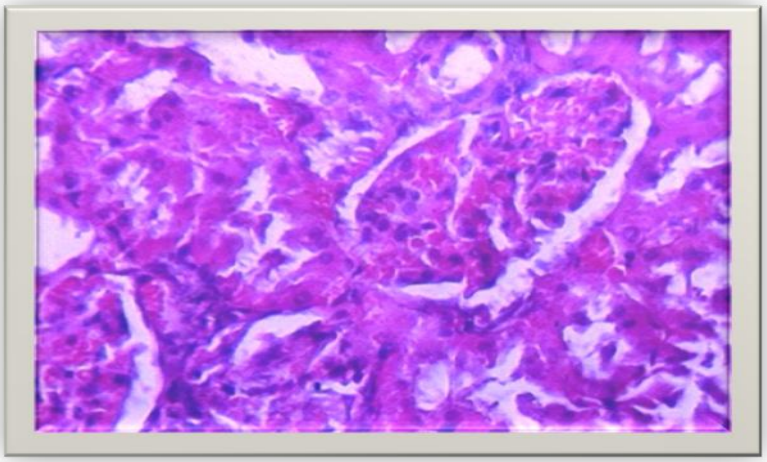

Fig No. 1.3 C) Cystone Treated Group.

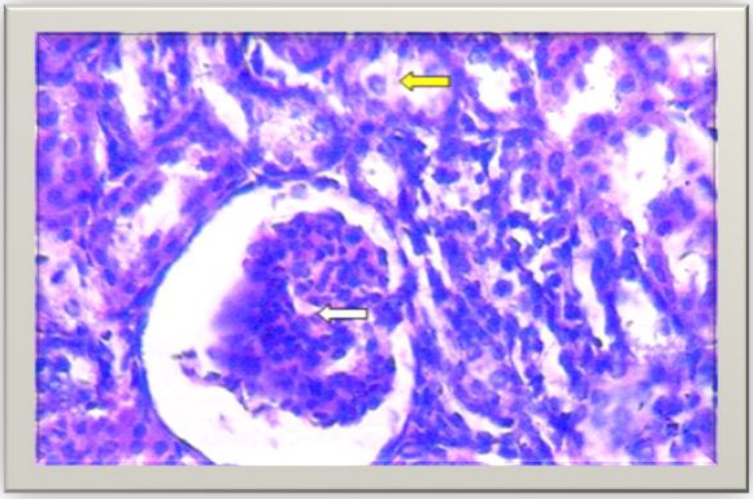

Fig No. 1.4 D) Low Dose Group(200mg/kg).

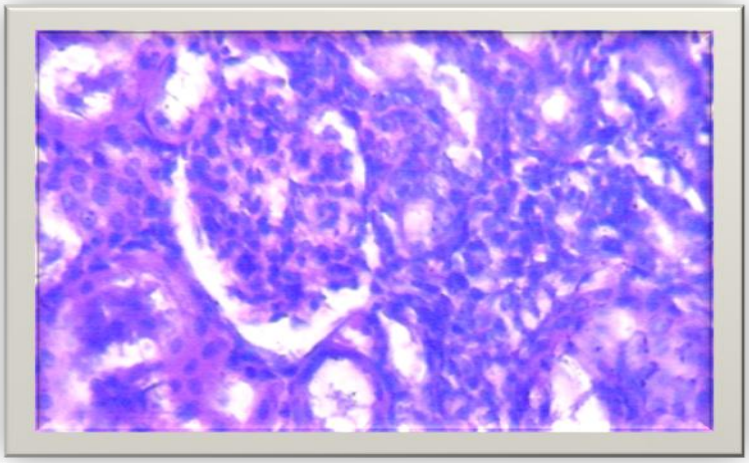

Fig No. 1.5 E) High Dose Group(400mg/kg).

4.0 DISCUSSION

Formation of Stone in the kidney is one of the oldest and most wide spread disease in human being. Urolithiasis is characterized by formation of stone in kidneys or urinary tract.[38] Urolithiasis is derived from Greekword "ouron" (urine) and "lithos" (stone).[39] Specially it is non metabolic minerals in urinary tract. Stone result due to phase change whereby dissolved salts condensed into solids because of supersaturation.[40] Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females.[41] Occurrence of urolithiasis requires formation of nidus, its reaction and growth in the urinary tract which may cause obstruction of the ureter.[42] In Indian patients, upper and lower urinary tract stones occur frequently, but the incidence depends on regional, climatic, and socioeconomic conditions. Urolithiasis is third prevalent disorder of urinary system which may cause serious medical consequences such as extreme obstruction, hydronephrosis, infection and haemorrhage in urinary tract system(43)(44)(45).Approximately 80% are of calcium-containing stones are in the form of pure calcium oxalate (CaOx) (50%) or calcium phosphate (1%) and a mixture of both (45%), and other stones are *Struvite* (10%), uric acid (9%), and cystine (1%)(44)(46). However the treatment present in modern medical system such as Lithotripsy, Kidney Dialysis and Surgical operations local disruption using high power laser are associated with acute renal injury leading to decrease in renal function, are too costly or not without side effects and does not shows the 100% efficacy & the re-occurance is common.[46] All these facts indicate the need to develop a suitable alternative therapy for treatment of urolithiasis.[44]

A large group of plants used in medicine or veterinary practice for therapeutic or prophylactic purposes. The therapeutic properties of medicinal plants are having the presence of different active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins and coumarin. These compounds are biologically active in relation to the causative agents of various diseases. More than 30,000 tons of raw materials from approximately 220 species of medicinal plants are used annually by the supervision of physician.[47] Hence the search for antilithiatic drugs from natural sources such as plants has assumed greater importance nowadays, since from ancient times people are taking benefit from the nature for curing many number of diseases. The Indian plants are constantly being explored for possible antilithiatic effects.[48]

In this study Ethylene glycol used as an inducing agent by oral administration which indicate that it gets metabolized to oxalate.The principal precursor of oxalic acid is glycoxalic acid.[49] The enzymatic oxidative conversion of glycolate to oxalate via glyoxylate is the major metabolic pathway involved in endogenous oxalate synthesis. The enzymatic disturbances causes idiopathic hyperoxaluria.[50] It causes increased renal retention and excretion of oxalate, increasing urinary calcium favouring the nucleation, precipitation of calcium, oxalate. phosphate from urine and consequent crystal growth.[51]

To overcome the side effects of synthetic drugs, hydroalcoholic extract of A. *racemosus* and *T. terrestris* was investigated for Antiurolithiatic activity. Phytochemical screening of hydroalcoholic extracts of an *Tribulus terrestris L.* and *Asparagus racemosus w.*revealed that presence of some active ingredients such as Alkaloids, Saponins, Glycosides, and Cardiac glycosides, Anthraquinones, Proteins, Carbohydrates, Steroids, Terpenoids and Flavonoids. Identification and characterization of new medicinal plants to cure Urolithiasis. There are more than 120 traditional medicines that are being used for the therapy of urine disorders in Asian countries.

Acute oral toxicity study was performed at the dose of 2000 mg/kg was found to be safe hence therapeutic dose of 200mg/kg as low dose and 400mg/kg as high dose were selected.

In present study Hyperoxaluria model was used to evaluate antiurolithic activity of hydroalcoholic extract of *Tribulus terrestris L.*and *Asparagus racemosus w.* Ethylene glycol induced urolithiasis resulted in significant elevation of calcium, oxalate, phosphorus, magnesium in urine ,it was also seen there was reduction in body weight, Urine volume, Urine pH and increase in blood Urea level with increase in creatinine, SGOT, SGPT, ALP in blood serum. Kidney weight was also increased in Rats when compared with Normal group.

5.0 CONCLUSION

In the present study Hydroalcoholic extract of *Tribulus terrestris* and *Asparagus racemosus* Family zygophyllaceae and Liliaceae was selected for Antiurolithiatic activity. These plants were taken for evaluating activity. Combined Hydroalcoholic extract of *Tribulus terrestris* and *Asparagus racemosus* were prepared with Preliminary phytochemical screening and revealed that presence of some active ingredients such as alkaloids, saponins, glycosides, steroids, Reesin terpenoids, Tannins and flavonoids.

The presented data indicates that the combination of herbal extracts shows significant antiurolithiatic activity against calcium oxalate induced urolithiasis. Further analysis and fractionation of these extracts is needed to predict the phytochemical constituents of combinational extract responsible for the antiurolithiatic activity. The combination of herbal extracts resulted in an increase in urinary volume, urinary pH along with decrease in calcium, oxalate, phosphate and magnesium level which inhibit stone formation. It also resulted in an decrease in serum creatinine and blood urea, SGOT, SGPT, ALP level and kidney weight that leads to normal GFR and tubular damage of kidney tissue. The findings clearly state that, the combination of herbal Hydroalcoholic extract of *Tribulus terrestris L*. and *Asparagus racemosus w*.is a potent nephroprotective agent by preventing the crystal retention in the tissues. Further Histopathological examination of renal tissues showed drastic reduction in stone formation. So, it is concluded that the cystone treatment is still superior for almost all parameters studied compare to combination of herbal extracts used in the present study. Also this herbal extract treatment is an effective drug in the management of urolithiasis and could be tried in the treatment of urolithiasis.

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