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Research Article

ESTIMATION OF ANTIUROLITHIC ACTIVITY OF GOKHRU AND COMESTIBLE IN EXPERIMENTAL UROLITHIC RATS

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ABSTRACT

Tribulus terrestris grows widely in the the warmer region of India including west Rajasthan and Gujarat. It contains many active constituents like flavonoids, steroids, terpinoids, phenols, alkaloids and tannins. It is reported to have hepatoprotective, anti-microbial, anti-inflammatory, antihypertensive, diuretic, and urinary anti-infectives. The present study was aimed at evaluating the hydroalcoholic extract of fruits of Tribulus terrestris (Gokhru, HAEG), at dose of 50mg/kg and also along with the comestible for anti-urolithic activity. Anti-urolithic activity of the the hydroalcoholic extract of Gokhru at dose of 50mg/kg along with comestible was evaluated by ethylene glycol (0.75% W/V) induced hyperoxalurea in group II, III, IV and V animals. Group I was taken as normal, group II was taken as control, group III as standard group, group IV and V received HAEG and HAEG along with diet. BUN and creatinine were estimated in serum. Oxalate, calcium, phosphate, magnesium and uric acid were estimated in urine. Histopathological studies were also done for all the groups.The hydroalcoholic extract of Gokhru (HAEG) at dose of 50mg/kg along with comestible was evaluated by ethylene glycol induced hyperoxalurea in rats. Interpretation of the results was done after subjecting the data obtained from various studies to statistical analysis which included one way ANOVA followed by post test (Tukey's). The results suggest that the group that was treated with HAEG along with comestible had shown better protective activity when compared to alone HAEG.

Key words: *Gokhru, Ethylene glycol and anti-Urolithic.*

INTRODUCTION

Urolithiasis is a painful medical condition compounded by a high recurrence rate [1]. It begins with solute supersaturation, crystal formation, and aggregation, followed by retention in the collecting system and further growth. Kidney stones are associated with hypertension and chronic kidney disease in adults and they result in an increase in the financial burden [2].

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The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices [3]. It affects 4-8% of the population in the UK, 15% in the US, 20% in Gulf countries and 11% in India with a recurrence rate from 70 to 80% in males and from 47 to 60% in females [4, 5].

The prevalence of idiopathic urolithiasis is increasing in rich countries [6]. Changes in socioeconomic conditions over time, and the subsequent changes in dietary habits, have affected not only the incidence but also the site and chemical composition of calculi [7]. Treatment procedures for renal stones such as surgical removal, percutaneous techniques and

extracorporeal shock wave lithotripsy (ESWL) are prohibitively costly and with these procedures recurrence is quite common [8]. Complications include residual stone fragments, compromised renal function, acute renal injury and urinary tract infection [9]. Dietary manipulation could contribute to the prevention of both its first appearance and the recurrence of the disease [10]. The target of dietary treatment is to decrease the "urinary lithogenic risk factors" such as low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria, hyperphosphaturia, hypocitraturia, hypomagnesuria and excessively alkaline or acid urinary pH [11].

Tribulus terrestris (L) belongs to Family zygophyllaceae (vernacular names: Gokharu (Hindi), Gokuri (Bengali), Kante gokaru (Marathi), Land caltrops (English)). It is an annual herb found in many tropical and moderate areas of the world, including the U.S.and Mexico, the Mediterranean region, and throughout Asia.

In folklore medicine *T. terrestris* is used as aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithon-triptic and urinary anti-infectives. Previous phytochemical studies have reported the isolation of flavonols, triterpenoids, steroids, tannins and alkaloids being few of its important constituents. Taking into consideration the folklore uses and the active constituents present, the present study aims at pharmacological evaluation of hydro alcoholic extract of *Tribulus terrestris (gokhru)* along with a diet for anti-urolithic activity[12,13].

Materials and Methods

Animals:

Male wistar albino rats weighing 150- 200gm were used for the study, after approved from Industrial animal ethical committee. Animals were acclimatised to laboratory conditions for one week before starting experiment and had free access to water and standard rat feed. The animals were kept 12 hr fasting prior to experiment.

Plant material:

The fruits of gokhru were obtained from Siri herbal suppliers Hyderabad and the plant was authenticated by botany department of St.marys College Hyderabad. A voucher specimen has been deposited at the herbarium for future reference.

Plant extraction:

The authenticated plant fruits were dried in shade and powdered coarsely. Extraction was done according to standard procedure. 500 grams of powder was taken and subjected to continuos solvent extraction in soxhlet apparatus. The powder was packed in filter paper and later a thimble was placed in apparatus and wetted by addition of solvent hydro alcoholic system. It was prepared by mixing of distilled water and ethanol (Ethanol: Water in 50:50 ratio) .The extraction process was continued for four days with occasional shaking. The solution was filtered, lyophilised to get dried powder.

Comestible:

The comestible diet is composition of Magnesium, Potassium, Phosphorus and Calcium in adequate composition. The diet was prepared by mixing 100 gm of brown rice and 70gms of sessame seeds and 89 gms of white beans.

Chemicals:

All the chemicals drugs and reagents were obtined from SD fine chemicals and these are all analytical grade. Cystone standard drug obtained from Himalaya herbals. The analysing kits used for parametres estimation was Spinreact ltd. obtained from united traders.

EXPERIMENTAL DESIGN:

- **Group I rats** - served as vehicle-treated control, received normal saline (2.5mL) through gastric gavages once in 24 h and water *ad libitum* daily for 28 days (normal control).
- **Group II** - rats received stone-inducing treatment for 28 days, which comprised of

0.75%(w/v) EG with 1%(w/v) ammonium chloride for 5 days; following this the water supply was switched to 0.75% EG alone in water, along with saline treatment (positive control urolithic group)

- **Group III** - rats received standard drug-Cystone, (100mg/kg) through gastric gavages and simultaneously received stone-inducing treatment similar to the positive control daily for 28days (treatment group, standard drug).
- **Group IV** - rats received *extract (*50mg/kg) through gastric gavages and simultaneously received stone-inducing treatment similar to the positive control daily for 28 days (treatment group, test dose).
- **Group V** - rats received *extract* (50mg/kg) through gastric gavages and simultaneously received comestible diet rich in cations.

Table I: Effect of Hydroalcoholic extract of gokhru and comestible on urinary and serum parameters.

Parameters	*Group I (saline)*	*Group II (EG Control)*	*Group III (EG + Cystone)*	*Group IV (EG + HAEG)*	*Group V (EG + HAEG +*
Urine Volume (ml/24hrs)	13.15 ± 0.29	5.36 ± 0.30	11.18 ± 0.26***	9.35 ± 0.35**	10.78 ± 0.37***
Urine Ph	6.96 ± 0.07	5.17 ± 0.10	6.34 ± 0.01**	6.29 ± 0.01**	6.37 ± 0.02**
Oxalate (mg/dL)	0.42 ± 0.01	1.87 ± 0.02	0.64 ± 0.01***	0.93 ± 0.01**	0.62 ± 0.01***
Calcium (mg/dL)	3.60 ± 0.03	1.90 ± 0.22	3.32 ± 0.19*	2.90 ± 0.25*	3.76 ± 0.14***
Phosphate (mg/dL)	5.22 ± 0.06	8.77 ± 0.07	5.71 ± 0.07**	6.88 ± 0.21*	5.70 ± 0.05**
Magnesium (mg/dL)	3.32 ± 0.12	2.25 ± 0.17	4.02 ± 0.11**	3.65± 0.10*	4.10 ± 0.12**
Uric acid (mg/24hrs)	0.57 ± 0.02	1.28 ± 0.19	0.64 ± 0.10***	1.00 ± 0.14**	0.75 ± 0.11***
Serum Creatinine (mg/dL)	0.82 ± 0.01	1.43 ± 0.01	0.77 ± 0.01***	0.98 ± 0.02**	0.80 ± 0.01***
BUN[a] (mg/dL)	31.83 ± 0.54	53 ± 1.21	37.17 ± 0.94***	46.67 ± 1.054*	41.33 ± 1.174**

- All of the data obtained from the experimental groups have been compared to control group. Values are expressed as Mean ± S.E.M. (n=6); Values are significant at * p<0.05. *** (p<0.001) compared to control, ** (p<0.01) compared to control. [a]Blood urea nitrogen

ASSESSMENT OF ANTIUROLITHIC ACTIVITY

- ***Serum analysis:*** On day 28 blood samples were collected from the retro orbital puncture under ether anesthesia and Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine and Blood urea nitrogen.

- ***Urine analysis:*** Animals were kept separately in metabolic cages and urine sample was collected for 24 hrs on 28 th day. Volume and pH were measured immediately after collection. Thymol crystals were used as preservative for urine to prevent significant changes in the concentrations of stone risk factors [14] and the collected urine was stored at 4° C. Urine samples were analysed for calcium, oxalate, magnesium, uric acid and phosphate content.

Enzyme assays and lipid peroxidation:

Preparation of kidney homogenate:

The animals were sacrified by cerival dislocation. The abdomen was cut open to remove both kidneys from each animal. The weight of the kidney was measured. Isolated kidneys were cleaned of extraneous tissue and rinsed in ice-cold physiological saline. Homogenate was prepared by using 0.1M phosphate buffer (7.4 Ph), then the supernatant was centrifuged at zero degree C at 10000 rpm

for 10 min. This homogenate was used for estimation of Reduced glutathione, glutathione peroxidise, Superoxide dismutase and lipid peroxidation by using standard kits.

Histopathological study:

Organs were rinsed in ice cold physiological saline and weighed. The right kidney will fixed in 10%neutral buffered formalin, processed in series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 μm, and stained with Haematoxylin and Eosin for examination under polarized light microscope

Statistical analysis: Statistical analysis was performed by one way ANOVA followed by post test Tukey.

RESULT

Serum estimations:

- **Serum Creatinine:**

Creatinine is the biomarker of the renal functioning. Creatinine levels shown significantly more in pathogenic group (group II) when compared to normal group. In group III (cystone treated) levels of serum creatinine were found to be significantly (p<0.001) less when compared to pathogenic group (group II). In both the treated groups IV and V the levels of creatinine were found to be significantly (p<0.001) less but the group V treated with gokhru along with the comestible had showed better effect when compared to group IV.

- **Blood urea nitrogen (BUN):**

BUN levels were significantly more in pathogenic group (group II) when compared to normal group. In group III (cystone treated) levels of serum creatinine were found to be significantly (p<0.001) less when compared to pathogenic group (group II). In both the treated groups IV and V the levels of BUN were found to be significantly (p<0.001) less but the group V treated with gokhru along with the comestible had showed better effect when compared to group IV.

Urine estimations:

Urine volume:

Ethylene glycol (0.75% w/v) administration for 28 days showed significant alteration of the output of urine. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significantly increased ($p < 0.001$) urine output compared to Ethylene glycol alone treated group.

Urine pH:

The administration of Ethylene glycol (0.75% w/v) for 28 days showed significant ($p < 0.001$) decrease of the urine pH. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significantly increased ($p < 0.001$) urine pH compared to Ethylene glycol alone treated group.

Oxalate:

The administration of Ethylene glycol (0.75% w/v) for 28 days showed significant ($p < 0.001$) increase of the urine oxalate. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significant ($p < 0.001$) decrease in urine oxalate compared to Ethylene glycol alone treated group (group II). Group V treated with gokhru along with the comestible had showed better effect when compared to group IV.

Calcium:

The administration of Ethylene glycol (0.75% w/v) for 28 days showed significant ($p < 0.001$) decrease of the urine calcium. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significant ($p < 0.001$) increase in urine calcium compared to Ethylene glycol alone treated group (group II). Group V treated with gokhru along with the comestible had showed better effect when compared to group IV.

Phosphate:

The administration of Ethylene glycol (0.75% w/v) for 28 days showed significant ($p < 0.001$) increase of the urine phosphate. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significant ($p < 0.001$) decrease in urine phosphate compared to Ethylene glycol alone treated group (group II). Group V treated with gokhru along with the comestible had showed better effect when compared to group IV.

Magnesium:

The administration of Ethylene glycol (0.75% w/v) for 28 days showed significant ($p < 0.001$) decrease of the urine magnesium. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significant ($p < 0.001$) increase in urine magnesium compared to Ethylene glycol alone treated group (group II). Group V treated with gokhru along with the comestible had showed better effect when compared to group IV.

Uric acid:

The administration of Ethylene glycol (0.75% w/v) for 28 days showed significant ($p < 0.001$) increase of the urine uric acid. Administration of cystone (100 mg/kg), Gokhru (50 mg/kg) and Gokhru (50 mg/kg) along with the comestible caused significant ($p < 0.001$) decrease in urine uric acid compared to Ethylene glycol alone treated group (group II). Group V treated with gokhru along with the comestible had showed better effect when compared to group IV

Kidney weight:

The weight of the kidneys in groups I found to be normal. In groups II weights were found to be raised. In treated groups (IV and V) weights were significantly reduced.

Enzyme estimation and lipid peroxidation:

Reduced Glutathione (GSH) Estimations:

GSH levels were significantly decreased in case of pathogenic group when compared to normal group. Levels were found to be increased significantly ($p<0.001$) in standard group when compared to pathogenic group. GSH levels in group IV and V significantly ($p<0.001$) increased indicate protective effect compared to normal.

Superoxide dismutase (SOD) Estimations:

The levels of SOD are marker of the antioxidant activity, which prevent the oxidative stress and kidney tissue damage. SOD levels were significantly decreased in case of pathogenic group when compared to normal group. Levels were found to be increased significantly ($p<0.001$) in standard group when compared to pathogenic group. SOD levels in group IV and V significantly ($p<0.001$) increased indicate protective effect compared to normal.

Glutathione Peroxidase (GPX) Estimations:

The levels of GPX are marker of the antioxidant activity, which prevent the oxidative stress and kidney tissue damage. GPX levels were significantly decreased in case of pathogenic group when compared to normal group. Levels were found to be increased significantly ($p<0.001$) in standard group when compared to pathogenic group. GPX levels in group IV and V significantly ($p<0.001$) increased indicate protective effect compared to normal.

Melanaldehyde (MDA) Estimations:

The amount of the malondialdehyde (MDA) formed is a measure of lipid peroxidation.MDA levels were increased significantly ($p<0.001$) in control group when compared to normal group which indicates increased free radicals generation in kidneys, leading to lipid peroxidation and thereby increase in MDA production. MDA Levels are significantly ($p<0.001$) decreased in cystone group when compared to group II. Co-administration of HAEG (50mg/kg b.w.) with diet were significantly decreased ($p<0.001$) MDA levels when compared to pathogenic group.

Histopathological studies:

Histopathological studies (Fig 1) of the kidney parts like cortex medulla and papilla in pathogenic group (group II) showed the deposits of bifringent crystals under microscope which indicates urolithiasis. When group IV animals were treated with HAEG deposits of bifringent crystals were found to be less when compared to pathogenic group. In group V where gokhru was concomitantly administered with comestible showed better protective activity when compared to group IV.

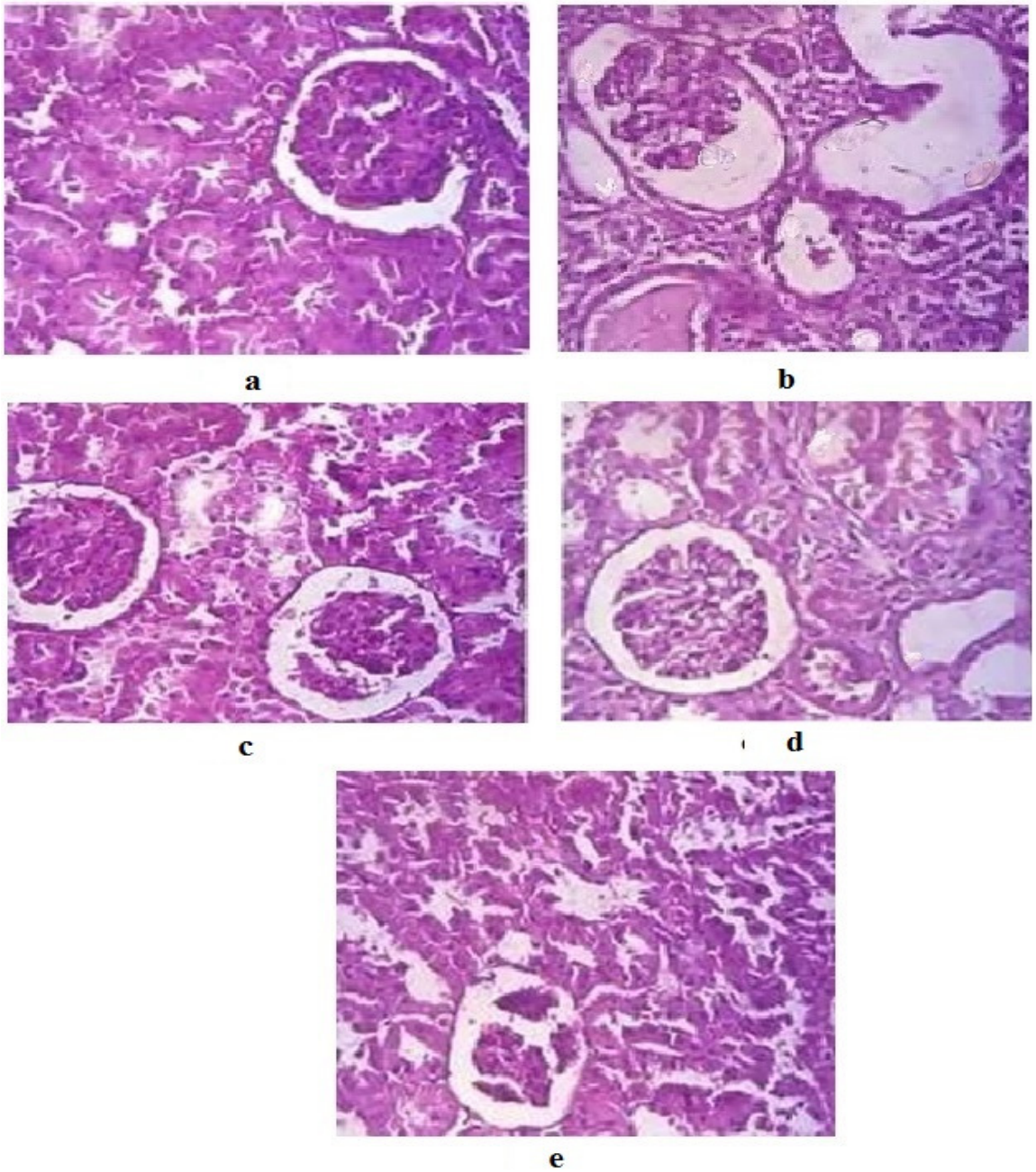

Fig: 1-(a) Normal Microscopic structure of rat's kidney (GpI). (b)Ethylene-glycol-induced urolithic rat's kidney showing crystal formation and inflammation (Gp II). (c) Kidney of urolithic rats treated with Crystone (Gp III). (d) Kidneys of urolithic rats treated with HAEG (Gp IV). (e) Kidneys of urolithic rats treated with HAEG and comestible. (G V).

DISCUSSION

Urolithiasis denotes stones originating anywhere in the urinary tract, including the kidneys and bladder. Kidney stones form as a result of physicochemical or genetic derangements leading to supersaturation of the urine with stone-forming salts or, less commonly, from recurrent urinary tract infection with urease producing bacteria.Hydroalcoholic extract of fruits of gokhru along with comestible was evaluated for anti-urolithitic activity at dose of 50 mg/kg b.w. Ethylene glycol (0.75% W/V) induced urolithiasis successfully in all the groups except normal.

Glycolate oxidase (GOX) is one of the principal enzymes involved in the pathway of oxalate synthesis. It converts glycolate to glyoxylate by oxidation and then glyoxylate is finally converted to oxalate [15]. Ingestion of ammonium chloride was also reported to accelerate the lithiaisis by increasing urinary acidification, which may be responsible for increased deposition of Calcium oxalate crystals in the kidneys [16].Blood Urea Nitrogen and creatinine are indicators of renal health. In serum analysis levels of BUN and creatinine were increased in pathogenic group. On treatment with extract and comestible levels of BUN and creatinine were decreased when compared to group treated with extract alone.

In urinary estimations, the urine output and pH were decreased in pathogenic group. In the extract treated group along with comestible better effect was seen when compared to group

treated with extract alone. In Urinary estimations, the levels of oxalate, phosphate and uric acid were found to be increased and levels of calcium, magnesium decreased in pathogenic group. On treatment with extract and comestible levels of oxalate, phosphate and uric acid decreased and levels of calcium, magnesium were increased when compared to group treated with extract alone.

Ethylene glycol is enzymatically bioactivated to free radical reactive which can damage essential macromolecules including DNA, lipids and protein and forms the metabolite malondialdehyde. It causes reactive oxidation species production and leads to organ damage. To combat with this situation production of enzyme like GSH, GPX and SOD are necessary.In enzyme estimations, levels of GSH, GPX and SOD were decreased in pathogenic group. On treatment with extract and comestible the levels of these enzymes were increased when compared to group treated with extract alone. In lipid peroxidation, there was raise of MDA levels in pathogenic group. Levels were lowered when treated with HAEG and diet.In histopathological studies revealed tissue damage and deposition of calcium oxalate crystals induced by ethylene glycol in pathogenic group. The treatment with gokhru and diet decreased the tissue damage and prevented the crystal formation in the kidney.

Table II: Effect of Hydroalcoholic extract of gokhru and comestible on kidney parameters.

Parameters	*Group I (saline)*	*Group II (EG Control)*	*Group III (EG + Cystone)*	*Group IV (EG + HAEG)*	*Group V (EG + HAEG +*
Kidney Weight (gm)	0.71 ± 0.00	1.25 ± 0.01	0.77 ± 0.00**	1.00 ± 0.02*	0.79 ± 0.01**
GPX[a] (U/ml)	0.56 ± 0.01	0.32 ± 0.01	0.56 ± 0.01**	0.51 ± 0.01**	0.52 ± 0.01**
GSH[b] (U/ml)	16.37 ± 0.28	8.51 ± 0.11	15.55 ± 0.14***	13.77 ± 0.16***	13.77 ± 0.16***
SOD[c] (U/ml)	6.57 ± 0.14	3.58 ± 0.16	6.61 ± 0.29***	6.01 ± 0.21***	6.04 ± 0.27***
MDA (nmol)	0.80 ± 0.08	6.67 ± 0.22	3.01 ± 0.26***	3.11 ± 0.25***	2.95 ± 0.25***

All of the data obtained from the experimental groups have been compared to control group. Values are expressed as Mean ± S.E.M. (n=6); Values are significant at * p<0.05. *** (p<0.001) compared to control, ** (p<0.01) compared to control.
[a]glutathione peroxidise, [b]reduced glutathione, [c]superoxide dismutase.

CONCLUSION

The present study found that the administration of the hydroalcoholic extract of gokru alond with the comestible effectively prevented the development of urolithiasis in rats treated with ethylene glycol. These findings rationalises the importance of proper diet composing in preventing urolithiasis. The results of the experiment have led to the conclusion that the synergetic effect produced when HAEG was given along with the comestible showed better antiurolithic activity.

REFERENCES

1. *Singh SK, Agarwal MM, Sharma S. Medical therapy for calculus disease. BJU international, 2011; 107(3): 356-368.*
2. *Sas DJ. An update on the changing epidemiology and metabolic risk factors in pediatric kidney stone disease. Clinical Journal of the American Society of Nephrology, 2011; 6(8), 2062-2068.*
3. *Colella J, Kochis E, Galli B,Munver R. Urolithiasis/nephrolithiasis: what's it all about?. Urologic nursing, 2005; 25(6), 427-48.*
4. *Pareta SK, Patra KC, Mazumder PM, Sasmal D. Prophylactic role of Boerhaavia diffusa in ethylene glycol induced calcium oxalate urolithiasis. African Journal of Urology, 2011; 17(2).*
5. *Manjula K, Rajendran K, Eevera T, Kumaran S. Effect of Costus igneus stem extract on calcium oxalate urolithiasis in albino rats. Urological research, 2012; 40(5), 499-510.*
6. *Borghi L, Meschi T, Maggiore U, Prati B. Dietary therapy in idiopathic nephrolithiasis. Nutrition reviews, 2006; 64(7), 301-312.*
7. *López M, Hoppe B. History, epidemiology and regional diversities of urolithiasis. Pediatric Nephrology, 2010; 25(1), 49-59.*
8. *Prasad KV, Sujatha D, Bharathi K. Herbal drugs in urolithiasis-a review. Pharmacognosy reviews, 2007; 1(1), 175.*
9. *Begun FP, Knoll CE, Gottlieb M, Lawson RK. Chronic effects of focused electrohydraulic shock waves on renal function and hypertension. J.Urol. 1991; 145(3), 635-9.*
10. *Borghi L, Meschi T, Maggiore U, Prati B. Dietary therapy in idiopathic nephrolithiasis. Nutrition reviews, 2006; 64(7), 301-312.*
11. *Nouvenne A, Meschi T, Guerra A, Allegri F, Prati B, Borghi L. Dietary treatment of nephrolithiasis. Clinical Cases in Mineral and Bone Metabolism, 2008; 5(2), 135.*
12. *Hussain AA, Mohammed AA, Ibrahim HH, Abbas AH. Study the biological activities of Tribulus terrestris extracts. World Academy of Science, Engineering and Technology, 2009; 57, 433-435.*
13. *Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 24th ed. Nirali Prakashan, Pune, 2003; 8.66.*
14. *Nicar MJ, Hsu MC, Johnson T, Pak CY. The preservation of urine samples for determination of renal stone risk factors. Laboratory medicine, 1987; 18(6), 382.*
15. *Shirfule AL, Sangamwar AT, Khobragade CN. Exploring glycolate oxidase (GOX) as an antiurolithic drug target: Molecular modeling and in vitro inhibitor study. International Journal of Biological Macromolecules, 2011; 49(1), 62-70.*
16. *Akila L, Kumar AP, Nirmala P. Effect of a polyherbal Formulations on ethylene glycol induced urolithiasis. International Journal of Pharma and Bio Sciences, 2011; 2(4), 7-24.*