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RESEARCH ARTICLE

EVALUATION OF ANTI UROLITHIATIC ACTIVITY OF *WITHANIA SOMNIFERA* IN ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

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ABSTRACT

Objective*: Evalution of Anti-Urolithiatic activity of Withania somnifera in ethylene glycol induced urolithiasis in rats.*

Abstract: *Ethylene glycol (0.75%) and Ammonium chloride (1%) in water was used to induce urinary calculi for 28 days. Five groups were taken and six animals were taken in each group. From14thday test drug Withania somnifera was given at 200mg/kg and 500mg/kg along with standard drug cystone 750mg/kg and all the group was compared with the positive control group. After completion of treatment after 24 hrs urine was collected and blood was collected by retro-orbital puncture and kidney histopathology was done. calcium, oxalate, inorganic phosphate, magnesium were analysed in urine and creatinine, BUN, uric acid in serum and calcium, oxalate and phosphate in kidney homogenate.*

Results and Discussion*: Methanolic extract of Withania somniferashowed increase in urine volume and pH. All the treated groups showed decrease in calcium, oxalate, phosphate, creatinine, urea, and uric acid level in ethylene glycol induced lithiatic acivity.*

Conclusion*: Methanolic extract of Withania somniferashowed significant anti-urolithiatic activity which is associated to its diuretic, anti-inflammatory and antioxidant property.*

Keywords: *urolithiasis, ethyleneglycol, ammonium chloride, Withania somnifera.*

1. INTRODUCTION

Urolithiasis is a common chronic disorder in humans and the most common type of renal stone made of calcium oxalate in which formation of stones take place in kidney & urinary bladder. Most calculi in the urinary system arise from a common component of urine, calcium oxalate (CaO), representing up to 80% of analyzed stones[1] Urolithiasis, also called calculi or uroliths, is a condition which involves the process of stone formation in the kidney, bladder, and/or urethra. Kidney stones are a general cause of blood in the urine and pain in the abdomen and flank, with a reported incidence about 12% in the general population. The majority of kidney stones are made up of calcium oxalate (CaOx) crystals in the urinary system of patients with urolithiasis[2]

Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys. The recurrence of urolithiasis represents a major problem as patients who have formed one stone are more likely to form another. [5]

Urolithiasisis a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation andretention within the renal tubule[3].Levels of urinary supersaturation co-relate with the type of stone formed .Any cellular dysfunction that can affect various urinary ions and other substances can also influence calcium oxalate supersaturation and crystallization in the kidney.Formation of renal stone starts with the transient super- saturation that occurs within kidneys while excretion of millions of urinary crystals through them.However, supersaturation is only one step in the process of stoneformation.It further needs crystals to be retained and cause ulceration within the kidneys[4]

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Between 1,200 and 1,400 per 100,000 will develop urinary stones each year with a male/female ratio of 3:1. A number of known factors of influence to the development of stones.Urolithiasis (kidney stone formation) is a very common kidney disorder in all over the world which is an estimated lifetime risk of 2%-5% in Asia, 8%-15% in Europe and America and around 20% in the Middle East. It is associated with high rate of recurrence, which is around 10%-23% per year, 50% in 5-10 years and 75% in 20 years, due to an imbalance between promoters and inhibitors in the kidneys. The majority of stones, up to 80%, found very often.[11]

The primary agents in medical management for urolithiasis, includes calcium channel blockers, steroids, non-steroidal anti-inflammatory drugs (NSAIDs), and adrenergic receptor antagonists. However, these treatment regimens are not free from side effects. Therefore, alterative treatment modalities with phyto-therapeutic agents have become the mainstay of medical therapy[7]

Common drugs used in urolithiasis are: Terazosin, lisinopril, Cipro, Lexapro, Doxazosin, Tamsulosin, naproxen, prednisone, tramadol, prozac, microzide, flexenil, rapaflo.

Withania somnifera is a herbal drug easily available in India has long been named as ashwagandha in Indian traditional medicine and is used traditionally for many diseases like rheumatism, dyspepsia, antihelmintic.[9,10]

Withania somnifera is having pharmacological & traditional uses like diuretic, antioxidant, anti inflammatory and that is why it can be used in the treatment of urolithiasis. *Withania somnifera* contains alkaloids and flavanoids like withanolide, which can be used to give anti urolithiatic effect.[11]

2. MATERIALS AND METHOD

2.1 Collection of plant material:
The whole plant *Withania somnifera* was collected in January from botanical garden of parul institute, Gujarat, India.

2.2 Preparation of plant extract:
The whole plant was collected and washed with distilled water .The plant was dried under shade and the powder will be obtained by grinding and is passed from sieve40 mesh.

Then was extracted with 80% methanol in soxhlet apparatus.It is evaporated under reduced pressure.[12]

2.3Animals:
Healthy male Wistar Albino rats (250-300 gms) were used in the experiments. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Parul Institute Of Pharmacy And Research (PIPR) as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.4 Chemicals
Ethylene glycol and all other chemicals and reagents of analytical grade were procured from Sigma Aldrich, Bombay.

2.5 Antiurolithiatic activity of *Withania somnifera*:
Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce urolithiasis.[12]

Healthy male albino wistar rats weighing 150-200 g were selected to evaluate anti urolithiatic activity.[13] Animals were maintained at standard laboratory conditions 12h light 12h dark cycle. They were provided with regular chow diet and drinking water ad libitum. Animals were divided into five groups containing six animals in each.[15]

Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) and ammonium chloride(1%)[12] in drinking water wasfed to Groups II-V for induction of renal calculi for 28 days. Group III received standard antiurolithiatic drug, cystone (750 mg/kg body weight) from 15 th till 28 th day[8]. Group IV and V received methanolic extract of *Withania somnifera* at high dose (500 mg/kg p.o) and low dose (200 mg/kg p.o) from 15 th till 28 th day.The Extract was dissolved in distilled water was given once daily by oral route .The two dose levels, 200 and 500 mg/kg were used to evaluate the antiurolitiatic potential of the methanolic extract against ethylene glycol induced urolithiasis in rats.

At the end of experimental period , all the animals were kept in metabolic cages and urine was collected and then were sacrificed by by dietyl ether anaesthesia. Blood samples were collected, allowed to clot. Serum was separated by centrifugation and analyzed for biochemical parameters.[14]

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2.6 Serum analysis:
After the experimental period, blood is to be collected from retro-orbital puncture under anesthetic condition and animals will be sacrificed by dietyl ether anaesthesia.

Serum will be separated by centrifugation at 10000*g for 10min and to be analysed for creatinine, uric acid and urea nitrogen.[16]

2.7 Collection and analysis of Urine:
Collection and analysis of urine will be done by keeping all animals in individual metabolic cages and 24H urine samples will be collected on 28th day of calculi inducing treatment. After measurement of urine volume, all urine samples will be analyzed for calcium, oxalate and phosphate content[16]

2.8 Urine volume:
Animals are to be placed in separate metabolic cages for 24 hrs and Total Urine volume is to be measured using the measuring cylinder and should be reported in ml.[14]

2.9 Urine pH:
Uric acid crystals will be found to be deposited most frequently in the concentrated acid urine. Acidity of urine will tested using pH meter. [14]

2.8 Kidney histopathology:
To confirm the incidence of lithiasis, the animals were sacrificed and their kidneys were isolated and subjected to histopathological studies. The kidneys were washed, weighed and fixed rapidly with 10% neutralized formalin (pH7.4), and soaked in paraffin, cut at 5µm intervals and the slices were stained with hematoxylin and eosin.

Tissue slices were photographed using optical microscopy and observed the pathological changes.[17]

2.9 Statistical analysis:
All the results to be expressed as mean ± SEM. The results will be analyzed statistically using one way analysis of Variance (ANOVA).P-values are to be calculated against vehicle and lithiatic control Groups And $p<0.05$ should be considered significant.[14]

3. RESULT

Simultaneous treatment of methanolic extract of *Withania somnifera* significantly increases urine volume, urine pH, urine calcium, urine oxalate, phosphate in ethylene glycol induced urolithiasis in rats.

Model group showed significant decrease in urine parameters when compared to control group. Administration of cystonesignificantly prevented this increase in calcium levels when compared to urolithiatic model group

All value are expressed as mean± S.E.M., n=6 in each group.**$P<0.01$ Significantly different when compared group (group-2); Data were analysed by one way of varience (ANOVA) followed by Dunett's test.

4. DISCUSSION

A number of models using rats have been used to induce calcium oxalate urolithiasis. The most commonly employed and simplest method is to provide ethylene glycol and ammonium chloride in drinking water. EG is readily absorbed along the intestine and is metabolized in the liver to oxalate leading to hyperoxaluria[16]

Stone formation in ethylene glycol-fed rats is caused by hyperoxaluria, which cause increased renal retention and excessive excretion of oxalate in urine. Urinary supersaturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that after 14 days period of ethylene glycol (0.75%, v/v) administration, renal calculi was formed in the young male albino rat composed mainly of calcium oxalate.[14]

Renal calcium oxalate deposition by EG (ethylene glycol) and ammonium chloride in rats is frequently used to mimic the urinary stone formation in humans. Ammonium chloride reported to accelerate the lithiaisis[12]

Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies shown that the amount of stone deposition in female rats was significantly less [12]

In the present study, oxalate and calcium excretion are progressively increased in calculi-induced animals (Group II). Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than

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hypercalciuria, the changes in urinary oxalate levels are relatively much more important than those of calcium. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth.[8]

An increase in urinary phosphate is observed in calculi induced rats (Group II) Increased urinary phosphate excretion along with oxalate seems to provide an environment suitable for stone formation by forming calcium phosphate crystals, which epitaxially induce calcium oxalate deposition.

Treatment with *Withania somnifera* extract significantly ($P<0.001$) lowered the elevated levels of phosphate in both the regimens, consequently reducing the menace of calculi formation.[15]

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid get accumulated in blood.[12]In calculi-induced rats (Group II), marked renal damage was seen by the elevated serum levels of creatinine, uric acid and Blood Urea Nitrogen (BUN).[15]

However, the curative and prophylactic treatment with test extract restored the elevated serum levels of creatinine, uric acid and BUN.

Cystone was found more effective than *Withania somnifera* extract. However, comparable antiurolithiatic effects of the *Withania somnifera* extracts with proven anti-urolithiatic drug authenticated the beneficial effect *Withania somnifera* in preventing calculi formation by supersaturated lithogenic substances in kidneys.

CONCLUSION

It has been concluded that the methanolic extract of *Withania somnifera* at the Doses 200mg/kg and 500mg/kg shows anti lithiatic activity in experimentally ethylene glycol induced urolithiasis in rats, and thereby it can considered as a potent diuretic as it increases urine volume and also it is considered to promote anti-inflammatory properties.

REFRENCES

1. Nizami AN, Md Atiar Rahman, Nazim UA, Md Shahidul Islam "Whole Leea macrophylla ethanolic extract normalizes kidney deposits and recovers renal impairments in an ethylene glycol-induced urolithiasis in rats." abu Nasim Nizami et al./Asian Pacific Journal of Tropical Medicine (2012) PP:533-538
2. Bayir Y, Halici Z, Keles MS, Colak S, Cakir A, Kaya Y, Akcay F "Helichrysum plicatum DC. subsp. plicatum extract as a preventive agent in experimentally induced urolithiasis model". Journal of Ethnopharmacology 138 (2011) 408– 414.
3. Bouanani S., Henchiri C.,Migianu Griffoni E, Aouf N, Lecouve M.,2010. Pharmacological and toxicological effects of Paronychia argentea in experi- mental calciumoxalate nephrolithiasis in rats. Journal of Ethnopharmacology 129, 38–45.
4. Ahmeda A, AbdulWadud A, Jahan A, Bilal A, Hajera S, "Efficacy of *Adiantum capillus veneris* Linn in chemically induced urolithiasis in rats". Journal of Ethnopharmacology 146 (2013) 411–416.
5. Sathish R, .NatarajanK and Mukesh Madhavrao Nikhad "Effect of *Hygrophila spinosa* anders on ethylene glycol induced urolithiasis in rats." Asian Journal of Pharmaceutical and Clinical Research Vol. 3, Issue 4, 2010 pp: 61-63.
6. C. Türk (chairman), T. Knoll (vice-chairman), A. Petrik,K. Sarica, C. Seitz, M. Straub "Guidelines for urolithiasis : UROLITHIASIS" (Update February 2012): pp:329
7. N.R. Rathoda, Biswasa D., H.R. Chitmeb, Sanjeev Ratnac, I.S. Muchandia, Ramesh Chandra "Anti-urolithiatic effects of Punica granatum in male rats." Journal of Ethnopharmacology 140 (2012) 234– 238
8. Karadi RV, Gadgeb NB, Alagawadi KR and Savadi RV, "Effect of *Moringa oleifera* L. root-wood on ethylene glycol induced urolithiasis in rats." J. Ethnopharmacol., 2006; 105: PP: 306-311.
9. Mathur D and RC Agrawal "*Withania coagulans*: A review on the morphological and pharmacological properties of the shrub". World Journal of Science and Technology 2011 | 1(10): 30-37.
10. Qamar Uddin, L. Samiulla, V. K. Singh and S. S. Jamil " Phytochemical and Pharmacological Profile of *Withania somnifera* Dunal: A Review"; Journal of Applied Pharmaceutical Science 02 (01); 2012: PP: 170-175
11. Girdhari Lal Gupta and A. C. Rana "PHCOG MAG.: Plant Review *Withania somnifera* (Ashwagandha): A Review" Pharmacognosy Reviews Vol 1, Issue 1, Jan- May, 2007: PP : 129-136
12. Velu. S and C. Baskaran "Phytochemical analysis and *in-vitro antimicrobial activity of Withania somnifera (Ashwagandha)" J. Nat. Prod. Plant Resour., 2012, pp 711-716*
13. Kalyani Divakar *, A.T. Pawar, S.B. Chandrasekhar, S.B. Dighe, Goli Divakar "Protective effect of the hydro-alcoholic extract of Rubia cordifolia roots against ethylene glycol induced urolithiasis in rats". Food and Chemical Toxicology 48 (2010) 1013–1018.

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14. Elias Edwin Jarald, Pankaj Kushwah, Sheeja Edwin, Suhail Asghar, Showkat Ahmad Patni "Effect of Unex on ethylene glycol-induced urolithiasis in rats".Indian Journal Of Pharmacology Year : 2011 | Volume : 43 | Issue : 4 | Page : 466-468.
15. Vyas BA, Vyas RB, Joshi SV, Santani DD "Antiurolithiatic Activity of Whole-Plant Hydroalcoholic Extract of *Pergularia daemia* in Rats". Journal of Young Pharmacists *2011;3(1): 36-40*
16. Anil T. Pawar, Gayatri D. Gaikwad , Kavita S. Metkari , Kiran A. Tijore , Jaydip V. Ghodasara , Bhanudas S. Kuchekar "Effect of Terminalia chebula fruit extract on ethylene glycol induced urolithiasis in rats". Biomedicine & Aging Pathology xxx (2012) pp: 1-5.
17. R.Sathish, K.Natarajan And Mukesh Madhavrao Nikhad "Effect of *Hygrophila spinosa* t.anders on ethylene glycol induced urolithiasis in Rats". Asian Journal of Pharmaceutical and Clinical Research Vol. 3, Issue 4, 2010 pp:61-63

LIST OF TABLES

Parameters	Group 1	Group2	Group3	Group4	Group5
Urine vol	14.50±0.450**	6.747±0.185	8.88±0.269**	9.845±0.365**	12.22±0.355**
Urine pH	7.052±0.0502**	8.93±0.054	7.09±0.052**	7.11±0.02**	7.048±0.065**
Calcium	1.33±0.429**	22.99±0.699	13.57±0.46**	14.51±0.565**	12.41±0.35**
Oxalate	6.43±0.313**	12.60±0.551	8.484±0.317**	10.67±0.445*	7.58±0.478*
Phosphate	60.431±2.31**	81.37±2.332	62.31±2.47**	59.09±2.164**	49.40±1.740**
Magnesium	2.933±0.229**	1.883±0.153	2.69±0.144*	2.460±0.261*	2.99±0.278**

Table1: Effect of WS on urine volume, pH ,calcium, oxalate, phosphate and magnesium

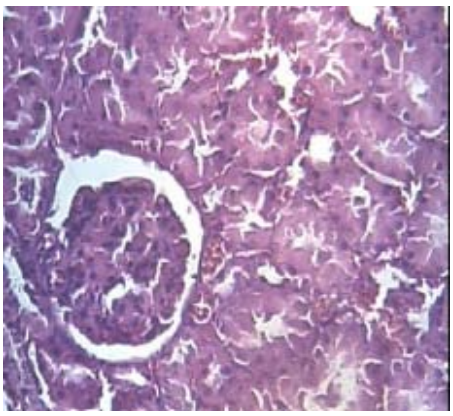
Parameters	Group 1	Group2	Group3	Group4	Group5
Creatinine	0.037±0.003**	0.0070±0.0008	0.0210±0.003**	0.0105±0.01	0.0270±0.003**
BUN	16.03±0.80**	39.84±1.27	20.83±1.078**	18.67±1.054**	15.83±1.078**
Uric acid	4.48±0.375**	7.39±0.296	5.562±0.377**	5.388±0.277**	3.082±0.272**
Calcium	7.268±0.319**	13.64±0.411**	6.313±0.291**	7.683±0.538**	9.140±0.304**
Phosphate	6.158±0.258**	9.357±0.338	7.802±0.460**	8.137±0.330	5.547±0.3484**

Table2: Effect of WS on serum creatinine, blood urea nitrogen, uric acid, calcium and phosphate.

Parameters	Group 1	Group2	Group3	Group4	Group5
Calcium	12.99±0.689**	24.09±1.377	14.08±0.680**	16.15±0.586**	15.79±0.878**
Oxalate	4.370±0.183**	5.568±0.1841	3.787±0.102**	4.242±0.1132**	3.948±0.060**
Phosphate	0.596±0.0447**	0.858±0.070	0.463±0.040**	0.640±0.058*	0.611±0.042**

Table3: Effect of WS on kidney calcium, oxalate and phosphate.

LIST OF FIGURES:



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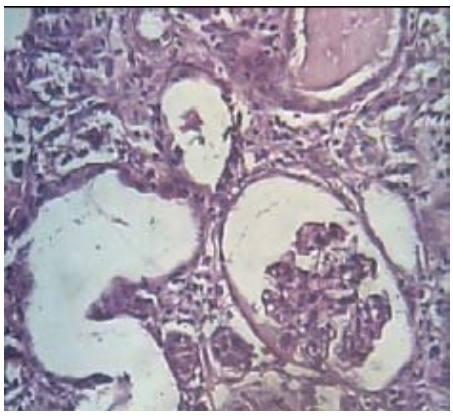

II

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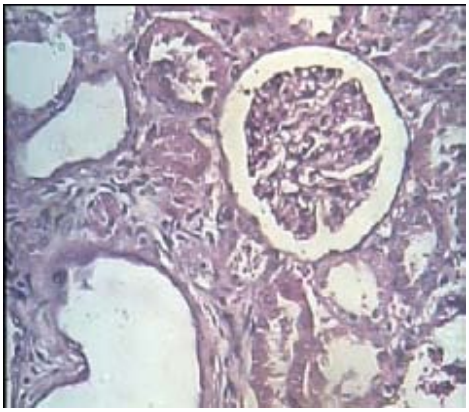

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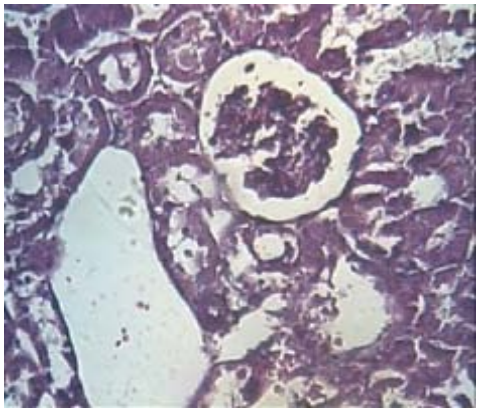

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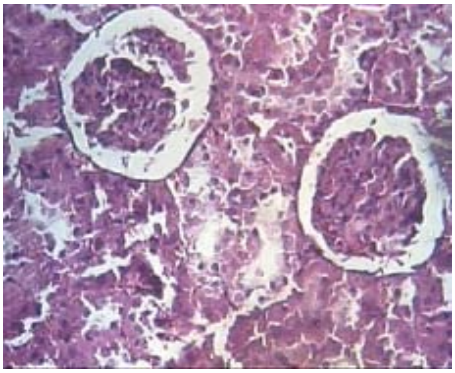

V

Group (I): Normal Control: Normal renal tubules with no dilation and inflammation;

Group (II): Model control: Marked tubules dilation with interstitial inflammatory infiltrate due to crystalDeposits.

Group (III): Cystone treated: Less tubules dilation with interstitial inflammatory infiltrate compared to model group.

Group (IV): *Withania somnifera* methanolic extract 200mg/kg: same changes as seen with std group.

Group (V): *Withania somnifera* methanolic extract 500mg/kg: same as control group.

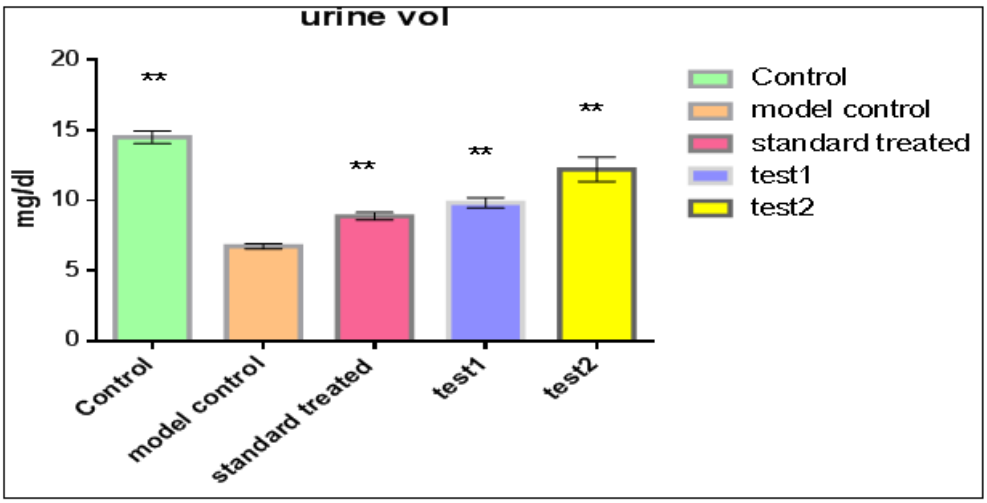

Fig.1: Effect of *Withania sonifera* extract on urine volume

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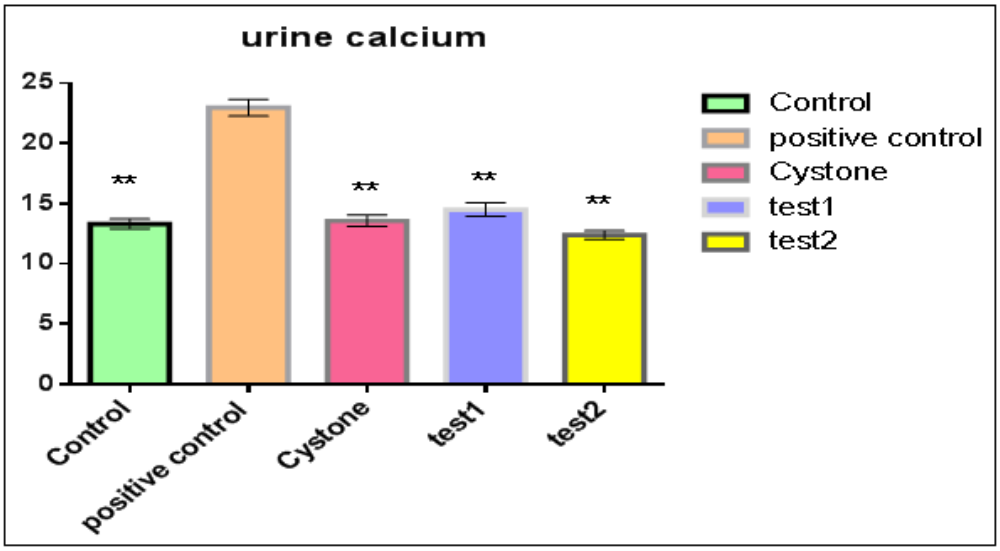

Fig.2: Effect of *Withania sonifera* extract on urine calcium

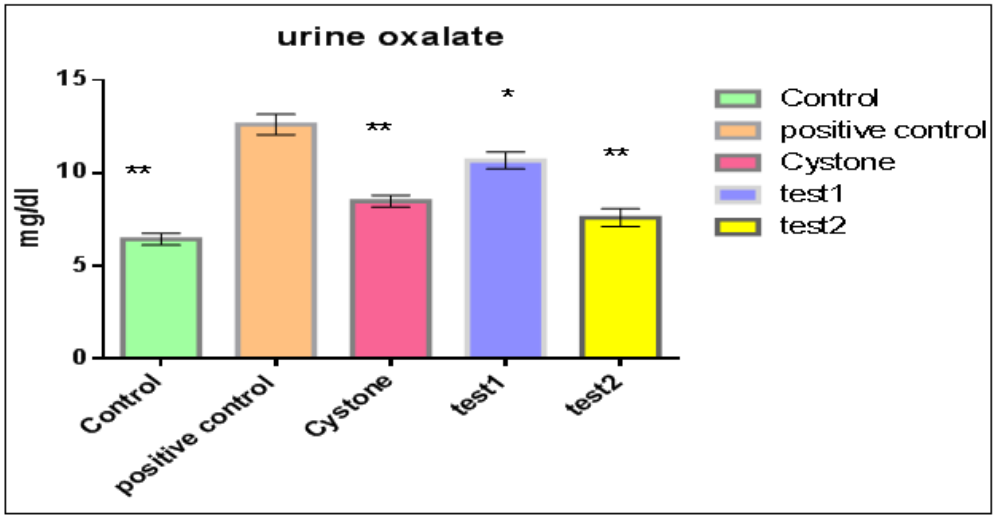

Fig.3: Effect of *Withania sonifera* extract on urine oxalate

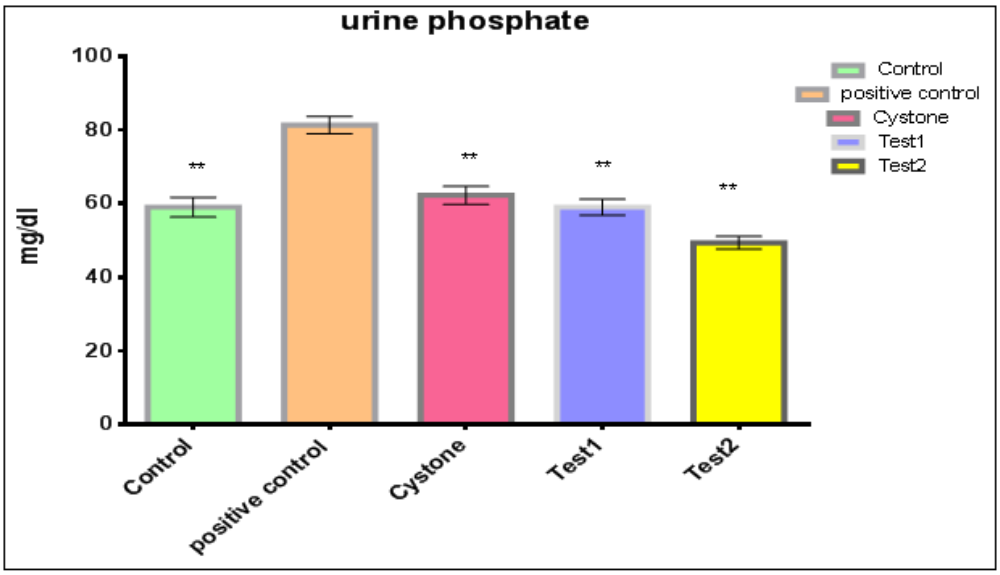

Fig.4: Effect of *Withania sonifera* extract on urine phosphate

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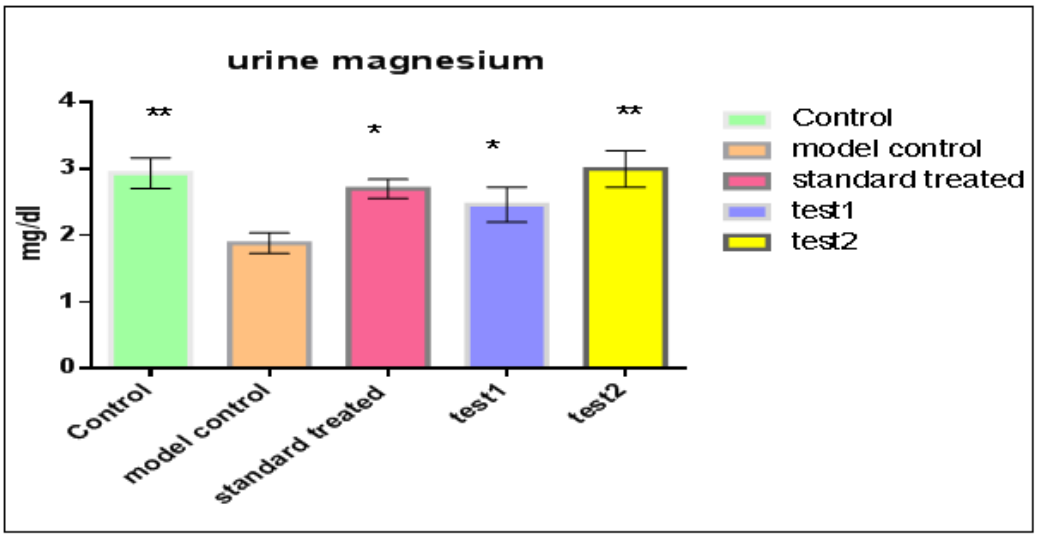

Fig.5: Effect of *Withania sonifera* extract on urine magnesium

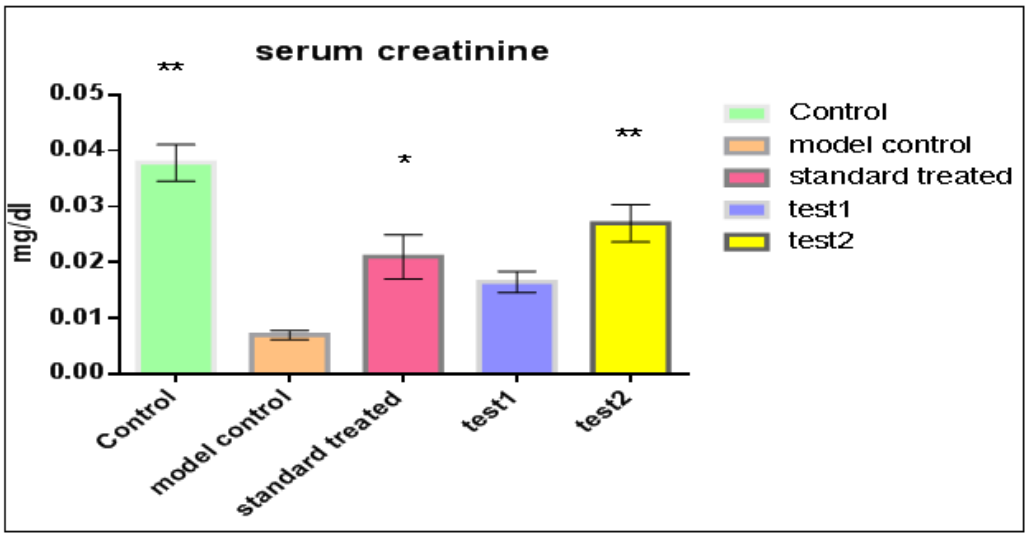

Fig.6: Effect of *Withania sonifera* extract on serum creatinine

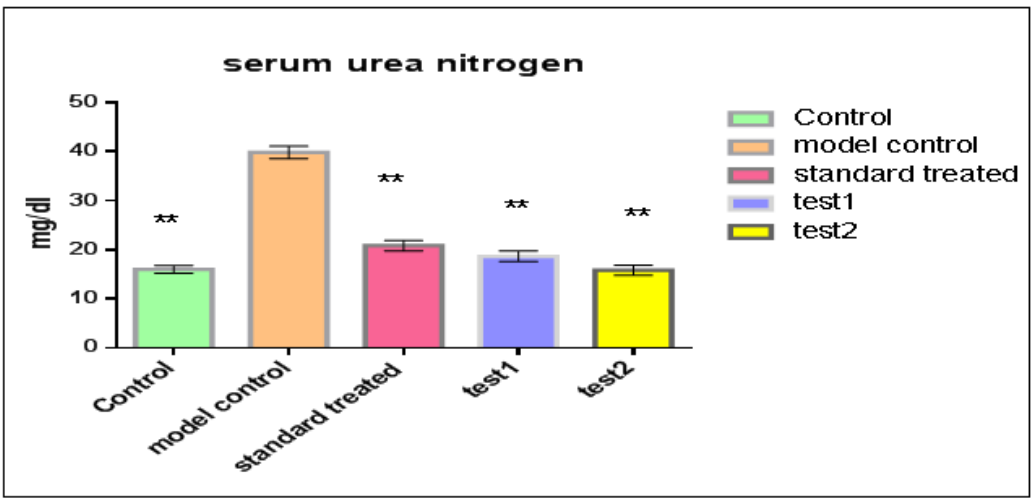

Fig.7: Effect of *Withania sonifera* extract on BUN

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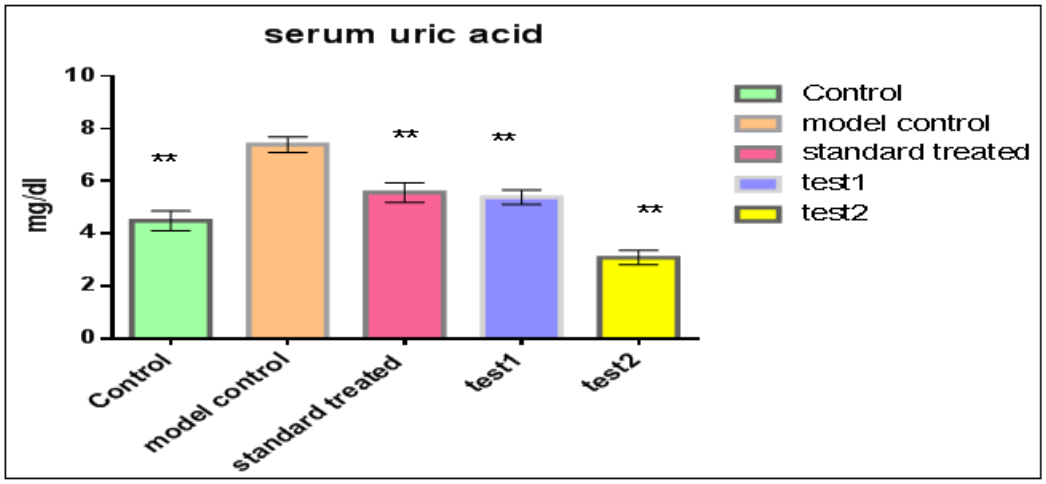

Fig.8: Effect of *Withania sonifera* extract on serum uric acid

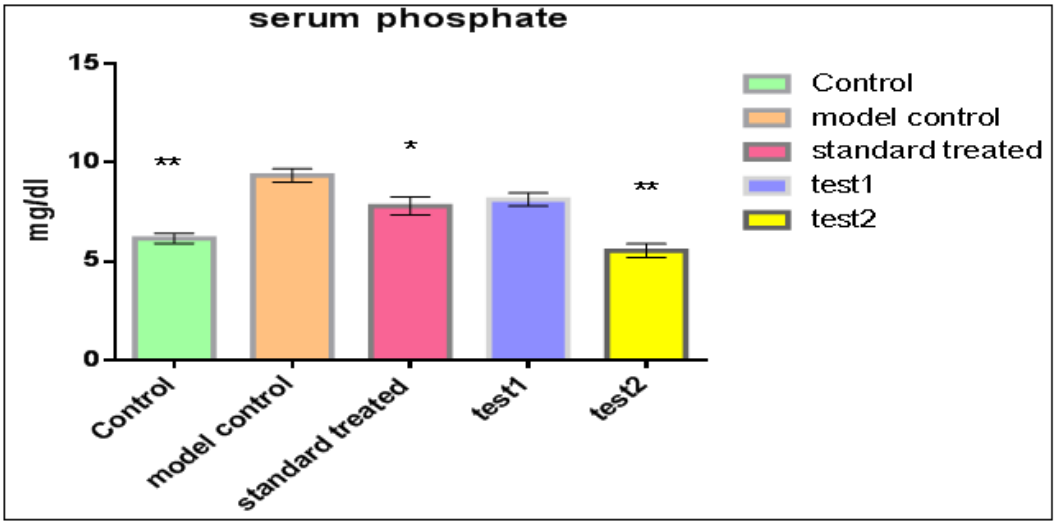

Fig.9: Effect of *Withania sonifera* extract on serum phosphate

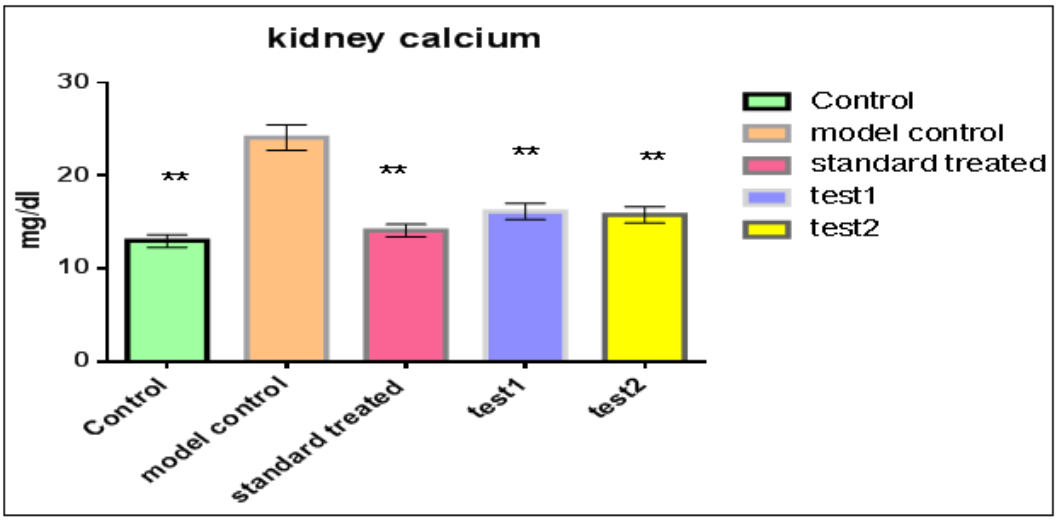

Fig.10: Effect of *Withania sonifera* extract on kidney calcium

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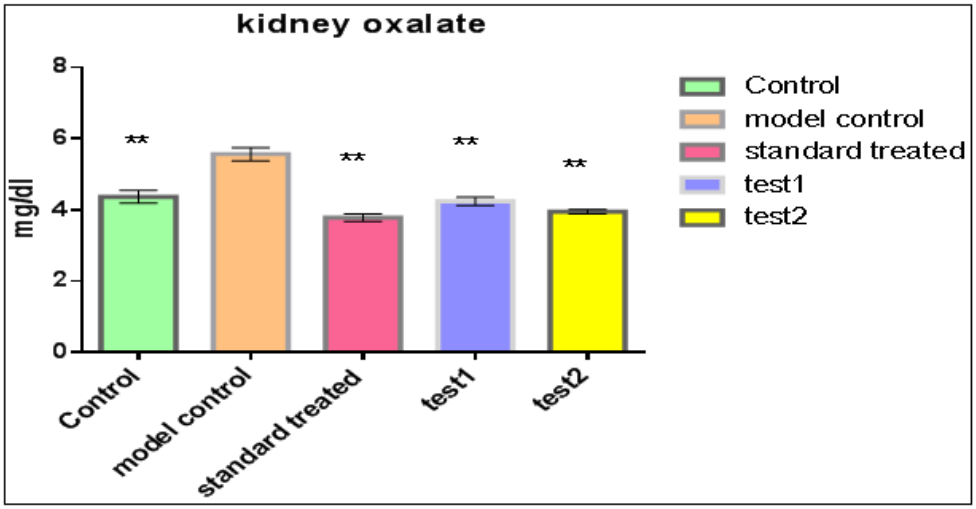

Fig.11: Effect of *Withania sonifera* extract on kidney oxalate

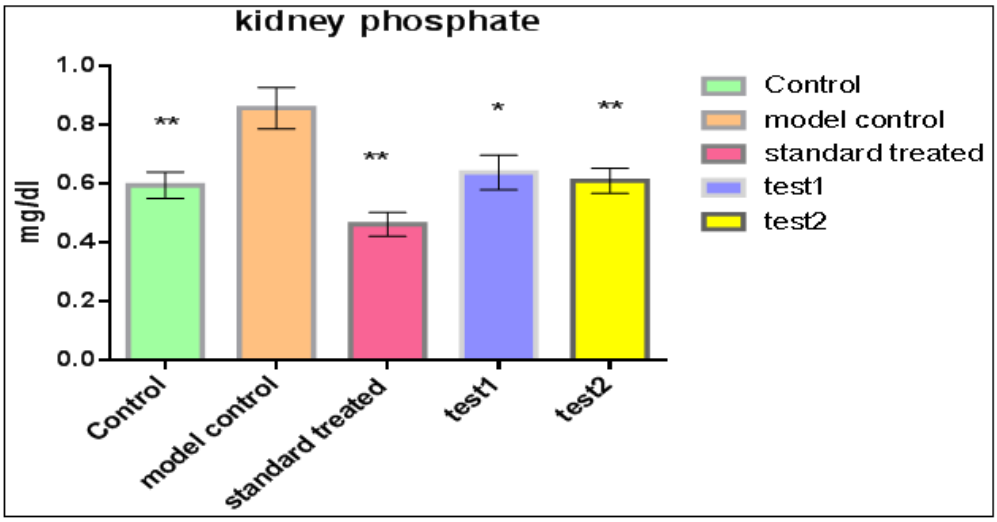

Fig.12: Effect of *Withania sonifera* extract on kidney phosphate

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