DOI: 10.55522/jmpas.V13I4.6547 ISSN NO. 2320 – 7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Prophylactic and curative activity of *Withania somnifera* on experimentally induced calcium oxalate nephrolithiasis

Lalchand Devhare*, Chandani Prasad

Manwatkar College of Pharmacy, Ghodpeth, Chandrapur, Maharashtra, India

Corresponding author: Lalchand Devhar, ✉ lalchand.devhare@gmail.com, **Orcid Id**: https://orcid.org/0000-0003-0579-4949

Manwatkar College of Pharmacy, Ghodpeth, Chandrapur, Maharashtra, India

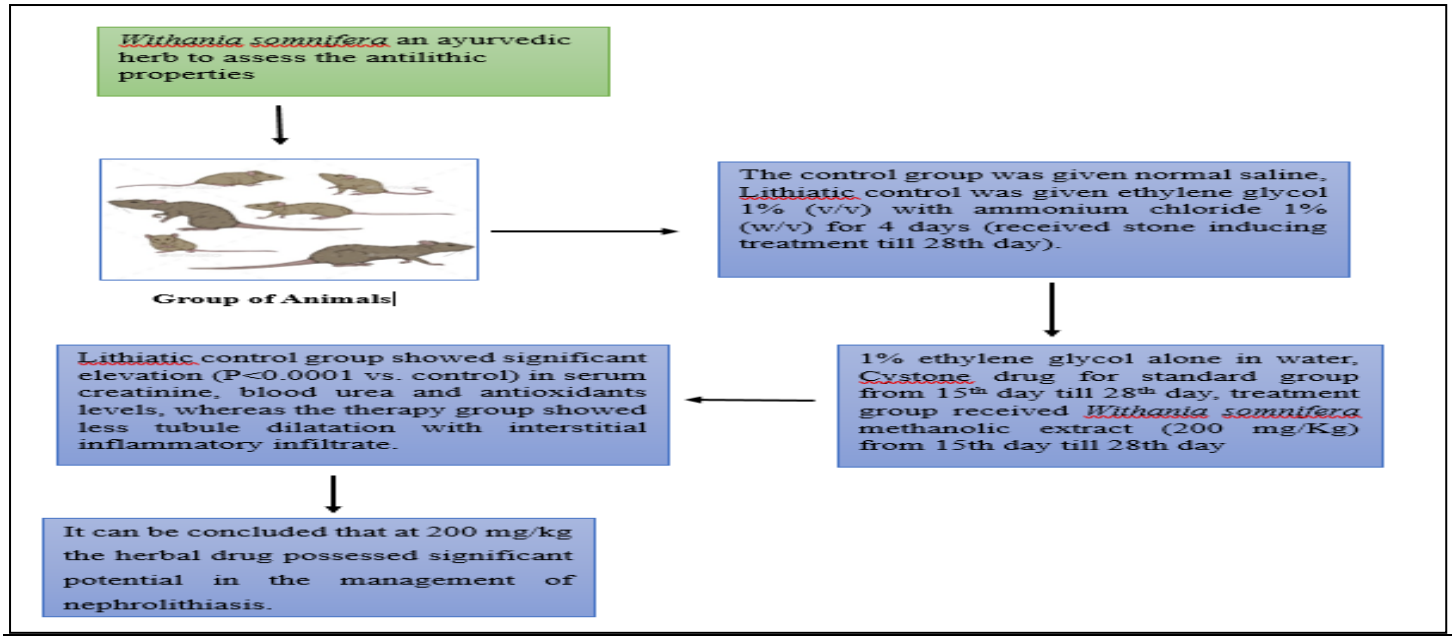
Received - 29-05-2024**, Revised** - 17-06-2024, **Accepted** - 13-08-2024 **(DD-MM-YYYY)**

Refer This Article

Lalchand Devhare, Chandani Prasad, 2024. Prophylactic and curative activity of Withania somnifera on experimentally induced calcium oxalate nephrolithiasis. Journal of medical pharmaceutical and allied sciences, V 13 - I 4, Pages - 6687 – 6695. Doi: https://doi.org/10.55522/jmpas.V13I4.6547.

ABSTRACT

Withania somnifera an ayurvedic herb was utilized to assess the antilithic properties of calcium oxalate (CaOx) Nephrolithiasis in rats fed ethylene glycol (EG) in an experimental setting. There were 5 groups of animals (n = 6). The control group was given normal saline, Lithiatic control was given ethylene glycol 1% (v/v) with ammonium chloride 1% (w/v) for 4 days (received stone inducing treatment till 28th day), follow by 1% ethylene glycol alone in water, Standard group received antiurolithiatic drug (Cystone) (500 mg/Kg) from 15th day till 28th day, treatment group received *Withania somnifera* methanolic extract (200 mg/Kg) from 15th day till 28th day (Curative regimen) and received *Withania somnifera* methanolic extract (WS-200 mg/Kg) from 1st day till 28th day (prophylactic regimen) in wistar rats. Blood and kidney tissue were collected after the 28th day of urine. Ca^{2-}, Mg^{2+}, K^{+} and PO_4^{-} levels were estimated in urine, creatinine and urea level were estimated in serum whereas all oxidative stress was measured in kidney tissue. Additionally, Urinary microscopy and kidney histopathology were estimated. Lithiatic control group showed significant elevation (P<0.0001 vs. control) in serum creatinine, blood urea and antioxidants levels. Due to crystal deposits, the histopathology of Lithiatic group showed tubule dilatation with interstitial inflammatory infiltrate, whereas the therapy group showed less tubule dilatation with interstitial inflammatory infiltrate. It can be concluded that at 200 mg/kg the herbal drug possessed significant potential in the management of nephrolithiasis.



Keywords: Calcium oxalate nephrolithiasis, Prophylactic, Antilithic properties, Withania somnifera, Ethylene glycol (EG).

INTRODUCTION

Throughout human history, nephrolithiasis has been a widespread issue. There is currently no perfect way to stop urinary stones from recurring. The prevalence of nephrolithiasis is estimated to be 12% in the popular population, with a recurrence rate of 70–81% for men and 47–60% for women. 80% of them are male, and they're all between the ages of 20 and 50 [1]. Nephrolithiasis is a clinical disorder distinguish by the formation of crystals aggregating in the urinary tract and resulting in kidney stones. It is caused by a multifaceted process in which solid non-metallic minerals in the kidney and renal tubules supersaturate, nucleate, develop, aggregate, and deposit [2]. Loss of renal function is also linked to some types of nephrolithiasis, particularly those linked to systemic illness.

Nephrolithiasis has been treated with a variety of natural medications. Herbs are nature's gift for diseases like nephrolithiasis since they treat the condition with the greatest therapeutic efficacy and the least amount of hazardous side effects [3]. Here, we assessed the nephrolithiasis potential of *Withania somnifera*, a plant with anti-inflammatory, wound-healing, antioxidant, and diuretic effects. Herb possesses in vivo antioxidant activity, which may support how traditional healers use the herb to cure numerous illnesses [4]. The presence of chemical constituents like triterpenoids, amyrin polyphenols, saponins, and flavonoids found in *Withania somnifera* responsible for its therapeutic efficacy. The precise clinical role and effectiveness of this plant on in-vivo nephrolithiasis have not been documented in the literature [5]. Ayurveda and Unani, two traditional Indian medical systems, have used the roots of *Withania somnifera*, also known as Indian Winter Cherry, for centuries. Withania roots include steroidal alkaloids and steroidal lactones [6]. Withaferin A and withanolides D, 2 main withanolides, account for the majority of the pharmacological activity of this plant. Because *W. somnifera* is readily available across the country and hence not expensive, the current study was designed to investigate its nephroprotective and nephrocurative characteristics [7].

This plant possesses potent aphrodisiac, rejuvenating, and life-extension properties, according to the traditional medical system ayurveda. Numerous conditions, such as nervous weariness, memory problems, insomnia, erectile dysfunction, skin problems, and coughing, are treated with it. Additionally, it possesses restorative and general animating qualities [8]. It enhances learning and memory abilities. Conventional uses of Ashwagandha include enhancing energy, youthful vigour, endurance, fortitude, and wellness as well as nourishing the body's duration elements and promoting the production of cells, vital fluids, muscle fat, blood, lymph, and semen [9]. Emaciation, sluggishness, recuperation, thirst, impotence, chronic exhaustion, weakness, dehydration, bone thinning, loose teeth, and early ageing are all prevented. It helps revive the body's reproductive organs, much as how nourishing a tree's roots revitalizes it [10].

Besides being used as a popular tonic to boost vitality, refine longevity and popular wellness, and prevent disease in athletes. In an effort to validate its reputation as a diverse medicinal agent, Ashwagandha has been the focus of several pharmacological studies [11]. The objective of present investigation is examining the potency of *Withania somnifera* on the level of Serum analysis, Kidney homogenate analysis, kidney histopathology, SOD and GSH, LPO, protein estimation Urinary microscopy in ethylene glycol induce Nephrolithiasis model [12].

MATERIALS AND METHODS

Plant material and preparation of extract

The leaves of the *Withania somnifera* family Solanaceae were collected in plant nursery of Nagpur. Leaves of selected plant by help of *soxhlet* apparatus using appropriate solvent system. Extraction of crude drug by hydro-alcoholic solvent. Before this the plant material (leaves) so cleaned and dried in the shade after being cleaned with distilled water to get rid of any dust. The dried leaves were coarsely powdered and then exhaustively extraction with hydro-alcoholic solvent *Soxhlet* apparatus for 72h approx.

Animals

Wistar rats, weighing of 150-200 gm were taken for the study. The animals were kept at 25˚C and 45-55% relative humidity under 12 hrs. Light-dark cycles, a conventional pellet food, and unlimited access to water were provided for the animals. Animals have unrestricted access to food and water.

Anti-urolithiasis study

Animal were split up into five groups, each group with six animals: -

Table 1: Treatment schedule proposed for anti-urolithiasis activity of *Withania somnifera*.

Group	Treatment
Control	Receive standard diet and water
Lithiasis	1% EG (v/v) with 1% AC (w/v) for 4 days (received stone inducing treatment till 28th day), followed by 1% EG alone in water.
Standard	Received antiurolithiatic drug, Cystone (500 mg/Kg) from 15th day till 28th day.
Treatment 1 (Curative)	Received *Withania somnifera* Methanolic extract (200 mg/Kg) from 15th day till 28th day (Curative regimen).
Treatment 2 (Prophylactic)	Received *Withania somnifera* Methanolic extract (WS-200 mg/Kg) from 1st day till 28th day (prophylactic regimen).

EG – Ethylene glycol; AC – Ammonium chloride

The effect of hydro-alcoholic extract of *'Withania somnifera'* was measured against the ethylene glycol induced urolithiasis. Animals were splited into 5 groups and each group contain six animals and were housed for the duration of the experiment in metabolic cages. Renal calculi were induced in group II and V by ethylene glycol (1% v/v) and ammonium chloride (1% v/v) in drinking water for 28 days. The control group was given normal saline, Lithiatic control was given 1% ethylene glycol (v/v) with 1% ammonium chloride (w/v) for 4 days (received stone inducing treatment till 28th day), follow by 1% ethylene glycol alone in water, Standard group received antiurolithiatic drug (Cystone) (500 mg/Kg) from 15th day till 28th day, treatment group received *Withania somnifera* methanolic extract (200 mg/Kg) from 15th day till 28th day (Curative regimen) and received *Withania somnifera* methanolic extract (WS-200 mg/Kg) from 1st day till 28th day (prophylactic regimen) in wistar rats. After 28th day urine, blood and kidney tissue were collected. Ca^{2+}, Mg^{2+}, K^{+} and $PO4^{-2}$ levels were estimated in urine, creatinine and urea level were estimated in serum whereas all oxidative stress was measured in kidney tissue. Further, urinary microscopy and kidney histopathology were estimated.

Assessment of Antiurolithiatic activity

At the end of day all animals were housed in a separate metabolic cage and 24 h urine sample was acquired by preserving the rats in metabolic cages. During the urine collection period, animals had unrestricted rush to drinking water. Urine samples were tested for the phosphorus, calcium and magnesium. Urine Calcium procedure was based on calcium colorimetric assay kit determination of calcium in urine [13]. Urine Magnesium procedure was based on the calmagnite method in which magnesium combines with calmagnite in an alkaline medium to form a red colored complex [14]. The Gomorri's method, which forms a red-colored complex when magnesium and calmagnite interact in an alkaline medium, is used to detect phosphorus in urine [15].

Assessment of oxidative stress

The kidney tissue (1 g) was homogenized in ice-cold 10% tri- chloroacetic acid (TCA) using a tissue homogenizer. Malondialdehyde (MDA) levels were analysed as an index of lipid peroxidation by monitoring the development of thiobarbituric acid-reactive substances at 532 nm and expressed as MDA content, mmol MDA/mg of tissue protein [16]. A single unit of activity was defined as the quantity of enzyme that prevented 50% of the pyrogallol oxidation, and this method was used to quantify the activity of superoxide dismutase (SOD) at 420 nm for 5 minutes [17]. Similarly, at 412 nm, reduced glutathione (GSH) was measured with the 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) reagent and expressed as µg of GSH/mg of protein [18]. Tissue protein was calculated in each sample using the technique reported by Lowery et al [19].

Serum analysis

After the animals were sacrificed, blood was drawn from the rats in each group, and the serum was separated using centrifugation at 6000 rpm for 15 minutes. Phosphorus, calcium, urea, and creatinine levels were then measured [20].

Kidney homogenate analysis

The animals will sacrifice by decapitating the cervical region, and opening the abdomen to extract the kidneys from each animal and perfuse using phosphate buffer saline (PBS). The isolate kidneys will clean off and left kidney will finely mince and 20% homogenate was prepared in Tris–HCl buffer (0.02 mol/L) of pH 7.4. Total kidney homogenate will use for analyzing tissue phosphorus, calcium and magnesium and oxidative stress markers [21].

Urinary microscopy

5 clean glass slides were collected and labelled as group I, II, III, IV, V. Using a micro-pipette, a drop of collected urine samples was applied to the slides and the urine drop was allowed to spread uniformly throughout the slides. Slides were allowed for drying under ambient temperature in an area free of dust. Then observed through microscope under polarize light (100X) [22].

Histopathology studies

Rats were anaesthetized; decapitated and right kidney was dissected out quickly and promptly washed with saline and preserved in 10% buffered formalin. Sections were cut 5 µm and stained using hematoxylin and eosin. Under a light microscope, the slides were examined to examine the kidney and CaOx deposits. The tubular area with histopathological alterations like cytoplasmic vacuolization, necrosis and stones was examined [23].

Statistical analysis

The results were evaluated as mean ± SEM for nephrological source, oxidative stress parameter and one way analysis of variance (ANOVA) followed with Tukey's test was used to determine significant differences. The statistical analysis was carried out using Graph Pad software.

RESULTS

The photochemical screening of leaves part of WS was analyzed for the confirmation of phytoconstituents present in methanolic and aqueous extract. It was noted that Methanolic extract contain alkaloids, tannins, steroids, amino acid and carbohydrates and It is reported that flavonoids component are associated with active biological principles of most medicinal plants having Nephrolithiasis properties.

Oxidative stress parameter

Withania somnifera effect on GSH, SOD and LPO in the animal to access the effect of *Withania somnifera* on oxidative stress after nephrolithiasis Levels of lipid peroxidation (LPO level) the nephrolithiasis potential of Lithiatic control was confirmed by decreased level of kidney functions marker enzymes. As *Withania*

somnifera methanolic extract possessed a high in vitro antioxidant potential level of enzymes involved in oxidative stress which was also estimated. In Lithia tic control group level of LPO was significantly increased as compared to control and treated group. This was a sign of decrease oxidative stress in the kidney. The activity of GSH and SOD in the kidney tissue was increased when compared with model group; while level of LPO in kidney tissue was reduced. *Withaniasomnifera* methanolic extract (200 mg/kg) induced.

Table 3: Nephrolithiasis occurred in a dose dependent manner with greatest effect of WS

Oxidative stress parameter	Control	Lithia tic control	Standard Cystone	Curative	Prophylactic
LPO (µmol MDA) of protein	2.63±0.22	7.65±0.88***	4.50±0.28*	5.67±0.39***	5.39±0.44**
GSH (µmol/g) of protein	19.24±0.47	6.78±0.81***	14.56±0.99**	9.51±0.76***	12.56±0.76**
SOD (IU/mg) of protein	11.06±0.36	3.49±0.65***	8.07±0.57**	5.23±0.60***	6.41±0.66***

All values are expressed as Mean ± SEM. *** P<0.0001 as compared for control group and Lithia tic control group in all the three parameter, **<0.0001 for SOD, **P<0.01 for GSH and LPO in WS (200mg/kg) treatment group as compared to control.

Serum parameter

Serum function assessment was conducted by measuring serum phosphorus, calcium, urea and creatinine in Lithiatic control urea, calcium, phosphorus level was significantly high as compared to vehicle group. When animals were administrated with WS Methanolic extract at the dose 200 mg/kg respectively. It was observed that the level of all marker enzymes was significantly decreased (p<0.01) as compared to that of vehicle group. WS Methanolic extract significantly decreased the creatinine, urea, calcium, phosphorus level in the treated groups.

Figure 1: Graphs depicting SOD levels

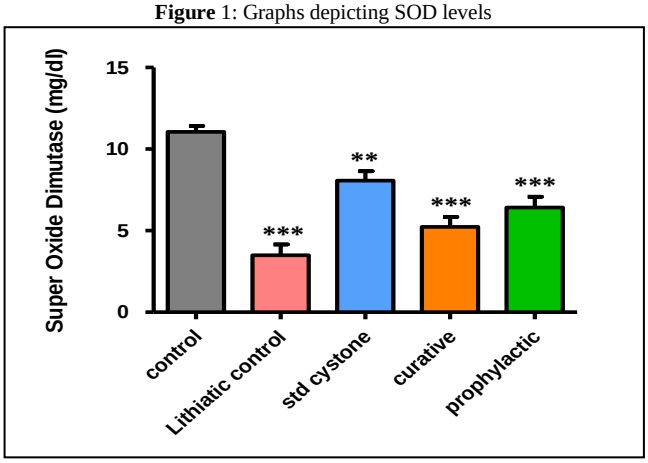


Figure 2: Graphs depicting LPO levels

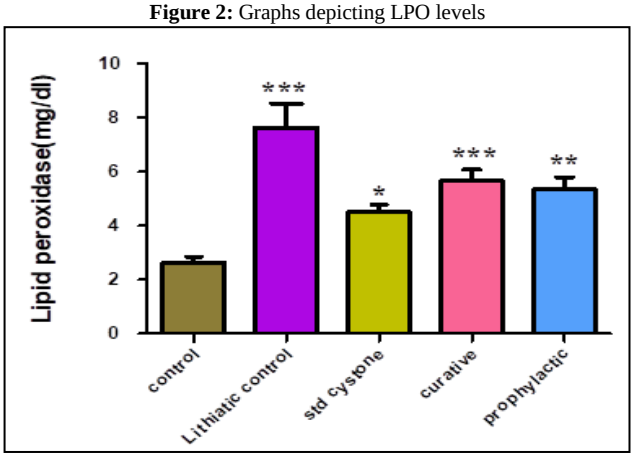


Table 3: Effect of WS (200mg/kg) on Nephrolithiasis of serum analysis

Serum parameter (mg/dl)	Control	Lithiatic control	Standard cystone	Curative	Prophylactic
Calcium	8.75±0.37	15.40±0.88***	11.53±0.33*	12.47±0.82**	11.76±0.67*
Phosphorus	8.13±0.23	15.41±0.65***	11.11±0.54**	13.13±0.48***	12.09±0.62***
Urea	13.58±0.34	26.68±1.25***	17.08±0.71*	18.32±0.92**	17.58±0.75**
Creatinine	0.43±0.04	1.65±0.12***	0.92±0.08**	1.03±0.10***	0.99±0.09***

The value are expressed as Mean ± SEM. ***P<0.0001 compared between control and Lithiatic control group. **P<0.01 for 200 mg/kg treatment group as compare to control.

Urine concentration of urine phosphorus, calcium, magnesium in Lithiatic control calcium, phosphorus level was significantly high as compared to vehicle group. When animals were administrated with Withania somnifera Methanolic extract at the dose 200 mg/kg respectively. It was seen that the level of all marker enzymes was remarkably decreased (p<0.01) as compared to that of vehicle group. WS Methanolic extract significantly decreased the calcium, phosphorus level and increased magnesium level in the treated groups.

Figure 3: Graphs depicting creatinine levels of serum

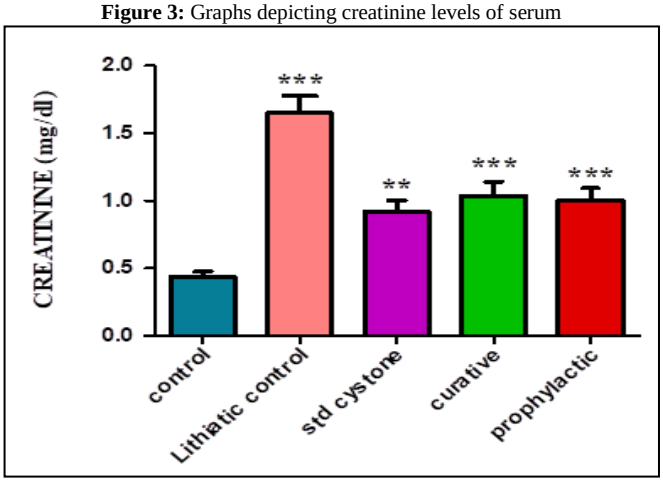


Figure 4: Graphs depicting urea levels of serum

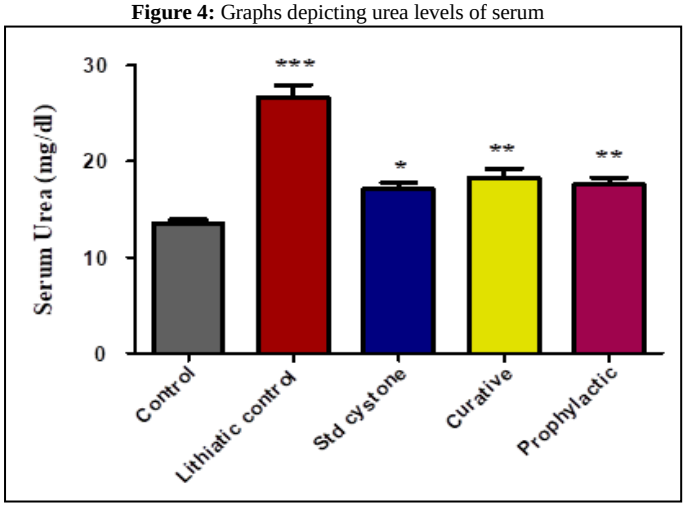


Figure 4: Graphs depicting phosphorus levels of serum

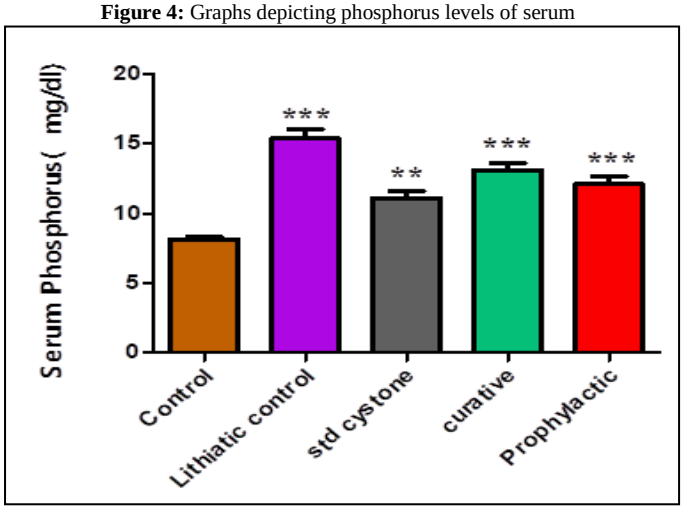


Figure 6: Graphs depicting Calcium levels of serum

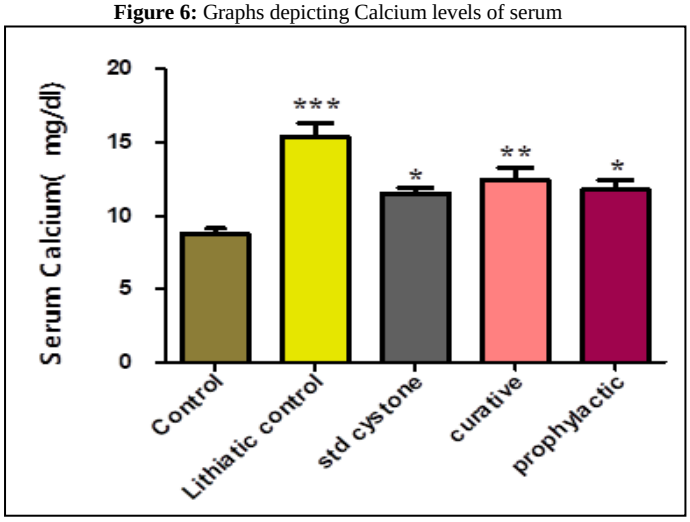


Figure 7: Graphs depicting Calcium levels of Urine

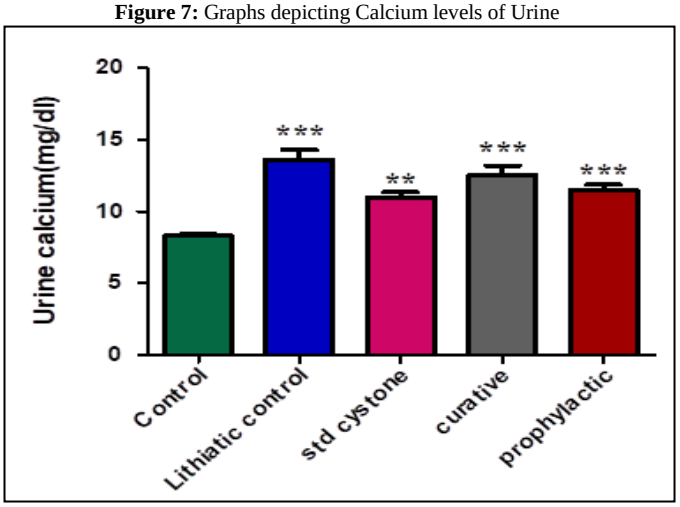


Figure 8: Graphs depicting phosphorus levels of Urine

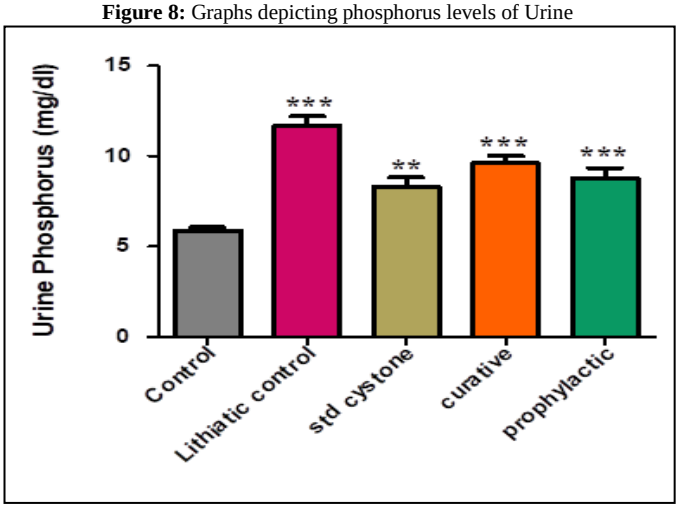


Figure 9: Graphs depicting Magnesium levels of Urine

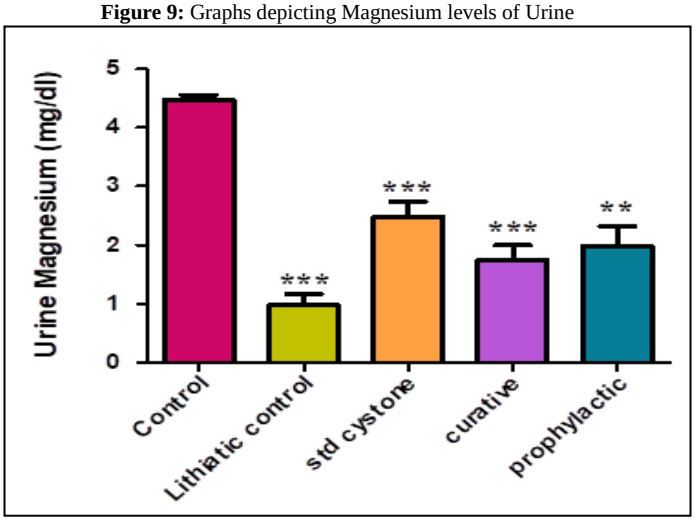


Table 4: Effect of WS (200mg/kg) on Nephrolithiasis of urine analysis

Urine parameter (mg/dl)	Control	Lithiatic control	Standard cystone	Curative	Prophylactic
Calcium	8.28±015	13.63±0.66***	10.98±0.33**	12.53±0.67***	11.48±0.38***
Phosphorus	5.89±0.16	11.67±0.53***	8.28±0.54**	9.61±0.39***	8.78±0.55***
Magnesium	4.46±0.09	0.97±0.18***	2.48±0.25***	1.75±0.23***	1.98±0.33**

The values are expressed as Mean ± SEM. ***P<0.0001 compared between control and Lithiatic control group. **P<0.01 for 200 mg/kg treatment group as compare to control

Histo-pathophysiology

In the urinary microscopy study, the changes of urine microscopy of rat urine section

Control (Group I) animal urine showed the absence of crystals.

Lithiatic control (group II) ethylene glycol + ammonium chloride, urine showed more crystals.

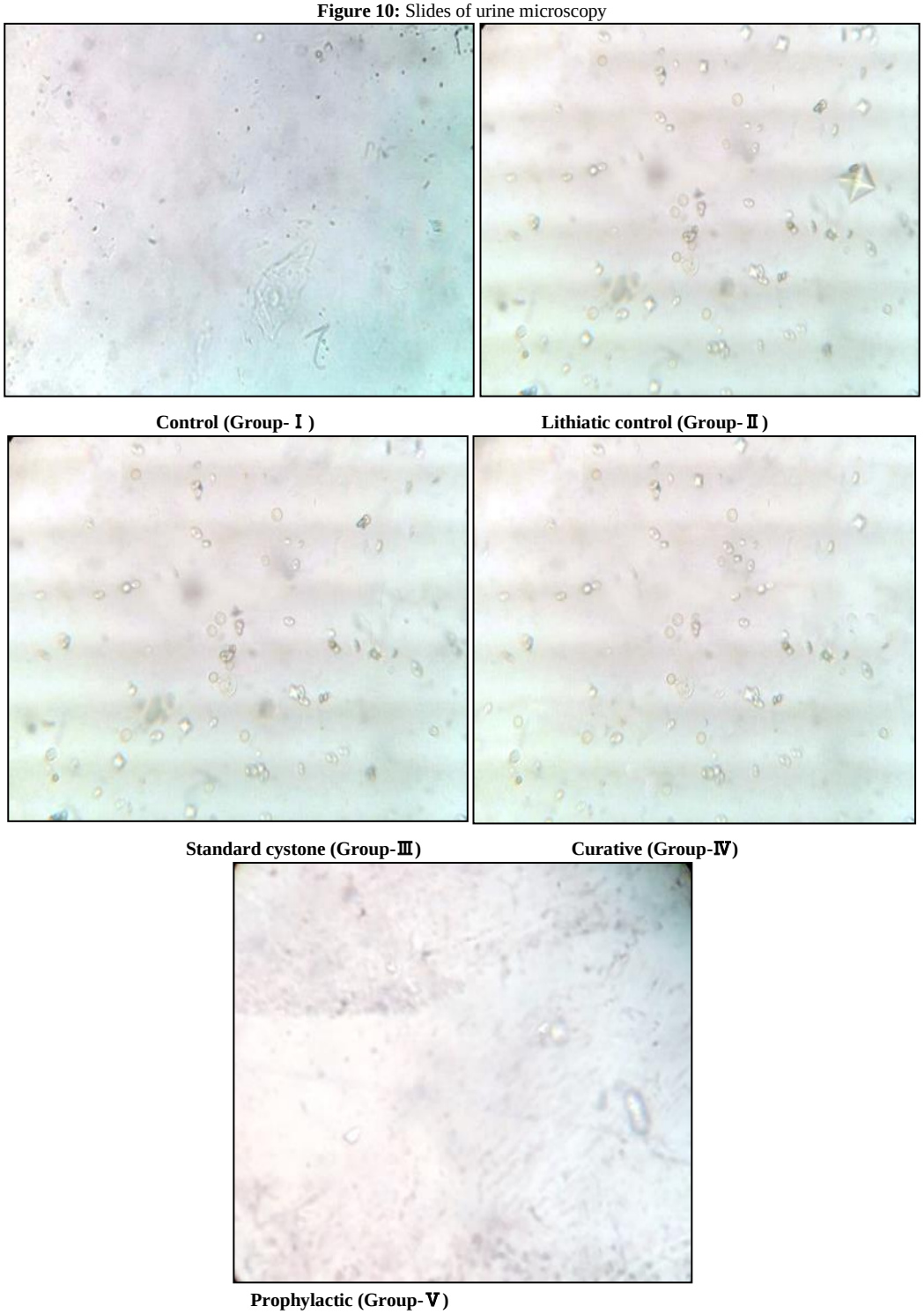
Standard cystone (group III) showed fewer crystals.

Curative (Group IV) better prevention of stone formation.

Prophylactic (Group V) showed better dissolution in performed crystals formation.

The defensive effect of the Withania somnifera methanolic extract was further confirmed by urine microscopy examination of the control extract- treated groups. The Nephrolithiasis of WS (200mg/kg) group 1 there were no calcium oxalate crystals seen in rats on vehicle group, but number of calcium crystal showed on the lithiatic control group 2. Administration with standard group (group 3) and WS Methanolic extract decreased the crystals observed in the urinary microscopic examination.

Figure 10: Slides of urine microscopy



Control (Group-Ⅰ) **Lithiatic control (Group-Ⅱ)**

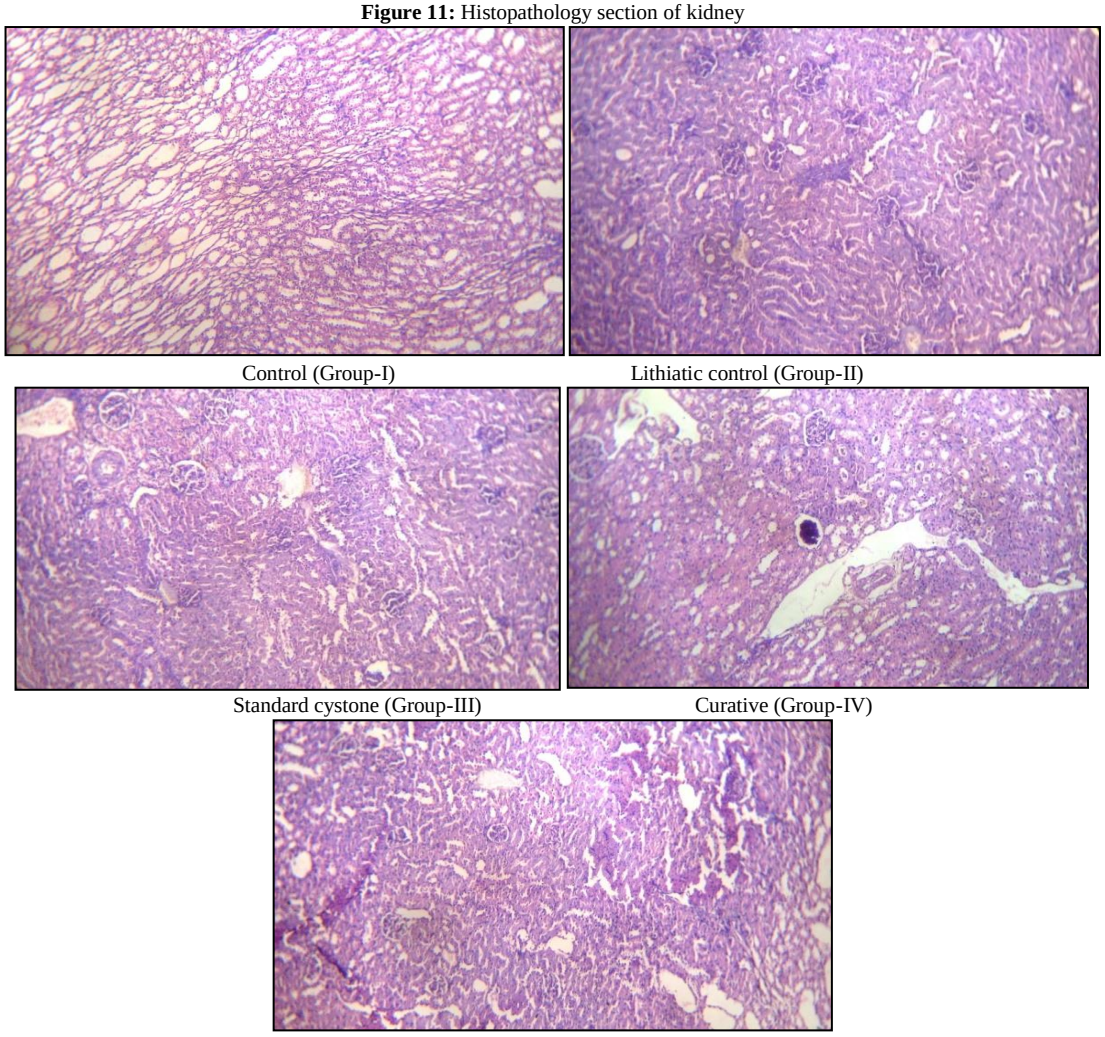
Standard cystone (Group-Ⅲ) **Curative (Group-Ⅳ)**

Prophylactic (Group-Ⅴ)

Effect of *Withania somnifera* on histopathology examination

Control (Group I): animal Normal renal tubules with no dilution and inflammation, Lithiatic control (group II): ethylene glycol + ammonium chloride, tubules dilation with interstitial inflammatory infiltrate due to crystal deposits. When compared to the negative control, standard cystone (group III) less tubules dilated with interstitial inflammatory infiltrate. Curative (Group IV): Some changes as seen with standard group. Prophylactic (Group V): same changes as showed with normal group. The defensive effect of the Methanolic extract of Withania somnifera was further confirmed by histopathology examination of the control (figure) extract- treated groups.

Figure 11: Histopathology section of kidney



Nephrolithiasis of WS (200 mg/kg) histopathology analysis

DISCUSSION

The kidney is primarily responsible for the excretion of metabolic waste products as well as the regulation of the volume of extracellular fluid, the composition of electrolytes, and the acid-base balance.

Nephrolithiasis is calcium oxalate lithiasis. Reactive oxygen species-induced lipid peroxidation damages renal epithelial cells, which in turn stimulates the formation of calcium oxalate lithiasis by supplying cellular debris for crystal nucleation, aggregation, and increased attachment of crystals to other tubular cells. High oxalate levels and calcium oxalate crystals in kidney can produce damage in the epithelial cells. Free radicals are created by renal epithelial cells.

Male rats were selected for this study because the urinary system of male rats similar that of humans, we are not selected female rats because of previous study shown the amount of stone deposition in female rats were significantly less. In rat model, Nephrolithiasis induced by ethylene glycol.

Hydrogen peroxide, superoxide and hydroxyl radicals induce oxidative tissue damage known as lipid peroxidation. This damage results in structural changes to the membrane, the release of cell and organelle content, and the loss of vital fatty acids due to the production of cytosolic aldehyde and peroxide products. SOD is highly expressed in renal tissue damage and cell injuries. *Withania somnifera* had good antioxidant properties, it decreases the all level of oxidative stress and decrease concentration of urinary salt that prevented super saturation of the crystallizing salts.

Urine and waste materials collected in the blood. Serum

neither blood cells, nor a clotting factor. Were study observed calcium, creatinine, phosphorus and urea increased level of lithiatic control group, when treated with *Withania somnifera* calcium, creatinine, urea and potassium decreased level of Nephrolithiasis.

Nephrolithiasis glomerular filtration rate decreased due to the obstruction to the outflow of urine by stones and also include damage to the parenchyma. In urine analysis phosphorus, magnesium and calcium level are increased in Lithiatic control group comparison of control group, other treated group of *Withania somnifera* decreased level of phosphorus, calcium and magnesium.

Urinary microscopic showed no calcium oxalate crystals in rats on vehicle group, but many calcium crystals showed on the negative control group. Administration with standard group and *Withania somnifera* methanolic extract treated group decreased the crystals observed in the urinary microscopic examination.

In the kidneys of the vehicle group, histopathology investigation revealed no abnormalities or calcium oxalate deposits. Regular renal tubules devoid of inflammation and dilution. Comparisons group 2 calcium deposits were observed inside the glomeruli and tubules, ethylene glycol + ammonium chloride, tubules dilation with interstitial inflammatory infiltrate due to crystal deposits. Administration with standard group and *Withania somnifera* methanolic extract treated group decreased the inflammation observed in the histopathology examination; less tubules dilation with interstitial inflammatory infiltrate compared lithiatic control.

From the histopathological study it absolutely was evident that shrinkage of minima interstitial inflammation. The *Withania somnifera* (200 mg/kg) treated group few renal tubules that revealed vacuolar degeneration with no CaOx crystal deposits.

CONCLUSION

The selected Nephrolithiasis agent *Withania somnifera* (200 mg/kg) is used as favored remedies in kidney and protected against Nephrolithiasis. Nevertheless, WS (200 mg/kg) could be a safe and well- tolerated with minimal side effects that suggests that it is administered for a long period of time. Although, *Withania somnifera* (200 mg/kg) has shown promising resulting to Nephrolithiasis model, further studies are required to unravel its specific mechanism of action and therapeutic approach.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENT

Faculty of Pharmacy, Manwatkar College of Pharmacy, Chandani Prasad participated in data collection.

REFERENCES

1. Alelign T, Petros B, 2018. Kidney stone disease: an update on current concepts. Advances in urology. Doi: 10.1155/ 2018/3068365.

2. Kapilraj P K, 2016. Prophylactic and Curative Activity of' Barleria Buxifolia. Linn'on Experimentally Induced Calcium Oxalate Nephrolithiasis in Animal Model. International Journal of Pharmacology and Clinical Sciences, 4(4), Pages 68-75 Doi: 10.5530/ijpcs.4.4.1.

3. Worcester E M, Coe F L, 2008. Nephrolithiasis. Primary Care: Clinics in Office Practice. Nephrolithiasis. 35(2), Pages 369-391. Doi: https://doi.org/10.1016/j.pop.2008.01.005.

4. Soundararajan, Mahesh R, Ramesh T, et al. 2006. Effect of Aervalanata on calcium oxalate urolithiasis in rats. Indian J Exp Biol. 44(12), Pages 981-986.

5. Paul S, Chakraborty S, Anand U, et al., 2021. Withania somnifera (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects. Biomedicine & Pharmacotherapy. 143, Pages 112175. Doi: 10.1016/j.biopha.2021.112175.

6. Lavi D, Glotter E, Shro Y, 1965. Constituents of Withania somnifera Dun, The structure of Withaferin A. Journal Chem Soc. 30, Pages 7517-7531. Doi: doi.org/10.1039/JR9650007517.

7. Charaka Samhita, Chikitsa Sthana, Second Chapter, Chowkambha Publishers, 38 (English Edition), 1997. https://www.carakasamhitaonline.com/index.php/Chikitsa_Stha na

8. Tiwari R, Mishra J, Devhare LD and Tiwari G. 2023 an updated review on recent developments and applications of fish collagen. Pharma Times, 55(6), Pages 28-36. Doi: 10.1080/ 10408398.2020.1751585.

9. Vaidyaratnam PS Varier's. 1994. Indian Medicinal Plants, a compendium of 500 species", (Warrier.P.K. Nambiar V.P.K, Ramankutty Eds.), Part II; 52-55, by Orient Longman Publications, Hyderabad.

10. Chatterjee A, Pakrashi SC. 1995. The Treatise on Indian Medicinal Plants, 4: Pages 208-212.

11. Bone K, 1996. Clinical Applications of Ayurvedic and Chinese Herbs. Monographs for the Western Herbal Practitioner. Australia: Phytotherapy Press. Pages 137-141.

12. Nwobim N L, Adedapo S K, Oyinlade O, et al, 2019. Urinary calcium: a promising predictive biomarker for early recognition of environmental lead exposure in children. International Journal of Research Medical Sciences. 7(6), Pages 2265-2272. Doi: http://dx.doi.org/10.18203/2320-6012.ijrms20192510.

13. Ryan M F, Barbour H, 1998. Magnesium measurement in routine clinical practice. Annals of clinical biochemistry. 35(4), Pages 449-459. Doi: https://doi.org/10.1177/0004563298035004.

14. Tiwari G, Gupta M, Devhare LD, Tiwari R. 2024. Therapeutic and Phytochemical Properties of Thymoquinone Derived from Nigella Sativa. Current Drug Research Reviews. 16(2), Pages Doi: 145-156. 10.2174/2589977515666230811092410.

15. Slater T F, Sawyer B C, 1971. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. Biochemical Journal 123(5), Pages 805-814. Doi: https://doi.org/10.1042/bj1230805.

16. Marklund S, Marklund G, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry, 47(3), Pages 469-474. Doi: 10.1111/j.1432-1033.1974.tb03714.x.

17. Moron M S, Depierre J W, Mannervik B, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et biophysica acta (BBA)-general subjects. 582(1), Pages 67-78. Doi: https://doi.org/10.1016/0304-4165(79)90289-7.

18. Lowery OH, Rosenbrough NJ, Farr AI, et al, 1951.Proteinmeasurement with the folin phenol reagent. J Biol Chem 193, Pages 265–275

19. Das M, Malipeddi H, 2016. Antiurolithiatic activity of ethanol leaf extract of Ipomoea eriocarpa against ethylene glycol-induced urolithiasis in male Wistar rats. Indian Journal of Pharmacology. 48(3), Pages 270. Doi: 10.4103/0253-7613.182886.

20. Zhang A, Sun H, Wang P, et al, 2012. Recent and potential developments of biofluid analyses in metabolomics. Journal of proteomics, 75(4), Pages 1079-1088. Doi: https://doi.org/10.1016/j.jprot.2011.10.027.

21. Becker G J, Garigali G, Fogazzi G B, 2016. Advances in urine microscopy. American journal of kidney diseases, 67(6), Pages 954-964. Doi: https://doi.org/10.1053/j.ajkd.2015.11.011.

22. Troudi A, Amara I B, Soudani N, 2011. Oxidative stress induced by gibberellic acid on kidney tissue of female rats and their progeny: biochemical and histopathological studies. Journal of physiology and biochemistry. 67, Pages 307-316.