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UROLITHIASIS ACTIVITY OF METHANOLIC EXTRACT OF *SARACA INDICA BARK* AGAINST ETHYLENE GLYCOL INDUCED IN RATS.

KANAKAM VIJAYABHASKAR¹, GUGLOTH HIMABINDU², KALAKOTA CHAITANYAPRASAD³
PARAKALA ARUN KUMAR⁴

1. Department of Pharmacognosy, Sahasra Institute of Pharmaceutical Sciences, Warangal Telangana, India 506007,
2. Department of pharmacology Dhanvanthari Institute of Pharmaceutical Sciences, Sujatha nagar, Kothagudem, Telangana, India -507120.
3. Department of Pharmaceutical chemistry. Sri Ramananda thirda Institute of Pharmaceutical Sciences, Nalgonda, Telangana. India
4. Department Of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal.

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Abstract: Urolithiasis is one of the third most common afflictions found in humans. The effect of oral administration of methanolic extract of *Saraca indica bark* on calcium oxalate urolithiasis has been studied in male Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium, magnesium and phosphate. Supplementation with methanolic extract of *Saraca indica bark* significantly reduced the elevated urinary oxalate, uric acid and phosphate. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was also significantly lowered by methanolic extract of *Saraca indica bark*. The results indicate that the methanolic extract of *Saraca indica bark* is endowed with antiurolithic activity.

Keywords: *Saraca indica*; Hyperoxaluria; Urolithiasis; Ethylene glycol



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INTRODUCTION

saraca indica belonging family *Caesalpinaceae*. Herbal medicine has such an extraordinary influence that numerous alternative medicine therapies treat their patients with Herbal remedies, Unani and Ayurveda. Approximately 25 percent of all prescription drugs are derived from trees, shrubs or herbs. Nature has bestowed our country with an enormous wealth of medicinal plants therefore India has often been referred to as the medicinal garden of the world. So stand the medicinal plants *Saraca asoca* as one of the foremost plants utilized from antiquity till to date. Asoka or ashoka is a Sanskrit words which means "without sorrow" or which that gives no grief. Ashoka is one of the most legendary and sacred trees of India. Ashoka tree, [1,2]. It is a evergreen tree called in english Asok tree. It is also known as Kankeli (Sanskrit), Ashoka (Assamese), Ashoka (Bengali) , Ashoka (Gujrati), Ashoka (Hindi), Ashokadamara (Kannada) , Ashok (Kashmiri), Asokam (Malayalam), Ashok (Marathi), Ashoka (Oriya), Ashok (Punjabi), Asogam (Tamil), (Balam Kheera) Ashokapatta (Telugu) [3]. Biological and pharmalogical activities are *Antimicrobial Activity* [4], *Anticancer Activity*(5) *Antimenorrhagic Activity* Also employed in menorrhagia, as an emmenagogue, uterine sedative, uterine affections as well as used in several preparations related to female troubles [7,8,9,10]. *Saraca indica* bark, in Pakistan, employed for uterine affection and menorrhagia. *Saraca indica*, in India, dried bark, used as an astringent in menorrhagia, to stop excessive uterine bleeding [11], *Antioxytotic Activity* [6]. *Traditionally* Useful in management of all painful conditions improves complexion of the body, digestion and assimilation, kills all infectious agents. is useful in toxicities and all blood disease and management of excessive bleeding during menstruation(12), *Home remedies* of bark powder should be effective in all types of abnormal discharges per vagina. Ksheerapaka is also beneficial in uterine inertia, uterine pain, urinary calculus, dysurea. [13] bark of plant presence of (-) epicatechin, procyanidin p2,11'-deoxyprocyanidin B, (+) catechin, (24, £)- 24- methyl-cholesta-5-en-3p-ol (22 E, 21£)-24- ethycholesta-5,22 dien-33-ol,(24 £)-24- ethylcholesta-5-en-3-p-ol, leucopelargonidin-3-O-p-Dglucoside, leucopelargonidin and leucocyanidin. The flower part of plant contain Oleic, linoleic, palmitic and stearic acids, P-sitosterol, quercetin, kaempferol- 3-O-P-D- glucoside, quercetin- 3-O- P-D-glucoside, apigenin- 7-O-p-D-glucoside, pelargonidin- 3, 5- diglucoside, cyanidin-3, 5- diglucoside, palmitic, stearic, linolenic, linoleic, p and y sitosterols, leucocyanidin and gallic acid. Seed and Pod contains oleic, linoleic, palmitic and stearic acids, catechol, (-) epicatechol and leucocyanidin [14,15,16]. Five lignan glycosides, lyoniside, nudiposide, 5-methoxy-9-β-xylopyranosyl(-)-isolariciresinol, icariside E3, and schizandriside, and three flavonoids, (-)-epicatechin, epiafzelechin-(4β→8)-epicatechin and procyanidin B2, together with β-sitosterol glucoside, were isolated from dried bark [17]. Kidney stones are one of the most painful urologic disorders, have beset humans for centuries. Scientists have found evidence of kidney stones in a 7,000-year-old Egyptian mummy. Unfortunately, kidney stones are one of the most

common disorders of the urinary tract. Each year, people make almost 3 million visits to health care providers and more than half a million people go to emergency rooms for kidney stone problems. For unknown reasons, the number of people in the United States with kidney stones has been increasing over the past 30 years [18]. Kidney stones may contain various combinations of chemicals. The most common type of stone contains calcium in combination with either oxalate or phosphate. These chemicals are part of a person's normal diet and make up important parts of the body, such as bones and muscles. Kidney stones are composed of crystal and proteins that grow until they break loose and pass into the urine collection system [19]. Stones containing calcium as oxalate, phosphate or both comprise about 80% of total. About 15% contain magnesium ammonium phosphate (struvite; these are often associated with infection), and small numbers of pure cystine or uric acid stones are found. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract [20]. Cystinuria and hyperoxaluria are two other rare, inherited metabolic disorders that often cause kidney stones. In cystinuria, too much of the amino acid cystine, which does not dissolve in urine, is voided, leading to the formation of stones made of cystine. In patients with hyperoxaluria, the body produces too much oxalate, a salt. When the urine contains more oxalate than can be dissolved, the crystals settle out and form stones. Hypercalciuria is inherited, and it may be the cause of stones in more than half of patients. Calcium is absorbed from food in excess and is lost into the urine. This high level of calcium in the urine causes crystals of calcium oxalate or calcium phosphate to form in the kidneys or elsewhere in the urinary tract [21]. In the present study, an effort has been made to establish the scientific validity for the Antiurolithiatic property of Methanolic extract of *Saraca indica* bark using thylene glycol induced hyperoxaluria model in rats.

Materials and methods: The sample was identified Prof. K.Raju, plant taxonomist, Department of Botany, Kakatiya university Warangal, telangana, and the voucher specimen no p-012 was deposited at Department of Botany, K.U. Warangal. *Saraca indica* bark were dried under shade and then were powdered with a mechanical grinder. The powder was then passed through sieve No.40 and was stored in an airtight container for further use. Dried and coarsely bark powdered (350 g) were extracted with petroleum ether, chloroform and methanol respectively using soxhlet apparatus. After complete extraction the solvent was recovered with the help of recovery unit. The extract was stored at room temperature till further use in the experiment. The Percentage Yield (methanolic Extract) was found to be 17.2%.

Preparation of Extract Dose: The animals were fed with pelleted rat chow and had free access to drinking water but were starved for 12 hours prior to testing. The extracts were orally administered as 100, 200, 400, 800, 1600, 3200, 6400, 12800 mg/kg methanolic bark extract of

Saraca indica bark . General symptoms of toxicity and mortality were observed for 24 hours for any sign of delayed toxicity [22]. The extract was well tolerated by the animals as there were no observable signs of acute toxicity effects like restiveness, seizures or dizziness after the administration of 400 mg/kg. However, at 6400 mg/kg, the animals showed signs of toxicity like jerks and writhes with 60% death. At 12,800 mg/kg, there was 80% death of the animals. The LD50 was estimated from a log-dose curve to be 3,981.07 mg/kg[23]. The plant extract was highly soluble in 3% w/v tween 80. The test drug (*Saraca indica bark*) 200mg and 400mg/kg were administered orally as a suspension in 3 % w/v tween 80.2.6 Pharmacological screening for antiurolithic activity:

Animals: Male Wistar rats weighing between 200-250g each, maintained on standard laboratory diet and tap water ad libitum were employed in the present study. They were housed in departmental animal house 12 hr light and 12 hr dark cycle.

Experimental Design:

Ethylene glycol-induced hyperoxaluria method [24] was used to assess the anti urolithiatic activity in Wistar rats. Animals were divided into seven groups containing five animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75% w/v) in drinking water was fed to Groups II, V, VI, and VII for induction of renal calculi for 28 days. Group III received methanolic extract of *Saraca indica* bark (200mg/kg body weight) once daily by oral route for 28 days. Group IV received methanolic extract of *Saraca indica bark* (400mg/kg body weight) once daily by oral route for 28 days. Group V received standard anti urolithiatic drug, cystone (750 mg/kg body weight), from 15th day till 28th day. Group VI received methanolic extract *Saraca indica* bark (200mg/kg body weight) treated once daily by oral route from 15th day till 28th day. Group VII received methanolic extract of *Saraca indica bark* (400mg/kg body weight) treated once daily by oral route from 15th day till 28th day.

Experimental Protocol:

Group I- Control (Treated with normal diet for 28 days),

Group II- Urolithic control [Treated with ethylene glycol (0.75%) in drinking water for 28 days],

Group III-Test drug[Treated with methanolic extract of *Saraca indica* bark (200mg/kg) once daily by oral route for 28 days],

Group IV -Test drug [Treated with methanolic extract *Saraca indica* bark (400mg/kg) once daily by oral route for 28 days],

Group V - Cystone (std.) + urolithic control (Treated oncedaily by oral route from 15th day till 28th day),

Group VI- Urolithic control + methanolic extract of *Saraca indica* bark (200mg/kg, treated once daily by oral route from 15th day till 28th day),

Group VII - Urolithic control + methanolic extract of *Saraca indica* bark (400mg/kg, treated once daily by oral route from 15th day till 28th day).

The test drug (*Saraca indica* bark extract) and standard drug (cystone) were administered orally as a suspension in 3 % w/v tween 80.

Assessment of Antiurolithic Activity:

Collection and Analysis of Urine:

Wistar male rats were kept separately in metabolic cage and 24 h urine samples were collected on 0, 7,14, 21 and 28th days of calculi induction treatment. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine samples were analyzed for calcium [25], uric acid [26], magnesim [27], phosphate [28], and oxalatecontent [29] by autoanalyzer.

Serum Analysis:

At the end of the experiment, blood samples were collected from the retro-orbital plexus under anaesthetic conditions and animals were sacrificed by cervical dacepitation. Serum was separated by centrifugation at 4000 rpm for 10 minutes and analyzed for creatinine and uric acid [30] levels by auto analyzer.

Kidney homogenate analysis:

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 400 C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 mL of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min. and the supernatant was separated. The calcium [25], phosphate [28], and oxalate [21] content in kidney homogenate were determined by auto analyzer.

Liver Histopathology:

The abdomen was cut open to remove liver from each animal. Isolated livers were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The livers were fixed in 10% neutral formalin and transferred to a container designed to allow reagents to freely act on the

tissue inside. This cassette is immersed in multiple baths of progressively more concentrated ethanol to dehydrate the tissue followed by xylene and finally extremely hot paraffin. During this 12 to 16 hour processed paraffin was replaced the water in the tissue turning soft moist tissues into a sample miscible with paraffin. The processed of embedded then allows the sectioning of tissues into very thin (2 – 7 micrometer) sections using a microtome. The microtome slices the tissue ready for microscopic examination and stained with haematoxylin and eosin (H and E) for histopathological examination. The histological slides were examined under a microscope by a pathologist.

Statistical analysis:

The results were expressed as the mean \pm SEM and analyzed using one multiple comparison tests. Data were computed for statistical analysis using Graph Pad Prism Software and 0.05; $P < 0.01$ and $P < 0.001$ were considered to be statistically significant.

Biochemical parameters:

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Auto analyser.

Result:

Urinary output determination Control rats (Group I) did not show any significant variation in the urinary oxalate level throughout the experiment period. The urinary output was increased significantly ($P < 0.01$) in urolithic control. In the methanolic extract of *Saraca indica* bark treated groups, the urine output was lower than that of the urolithic control but significantly ($P < 0.01$) higher than that of vehicle treated rats. Results are shown in Table 1.

Urinary oxalate determination:

In present study the male Wistar rats has shown hyperoxaluria. There was an increased oxalate Group II. Administration of ethylene glycol (0.75% w/v) led to elevation of urinary oxalate levels in groups II, V, VI and VII as compared to control group I, which was maintained over an experimental period of 28 days. Both the doses, 200 mg and 400 mg/kg/day of methanolic extract of *Saraca indica* bark for 15 days significantly reduced the urinary oxalate level as compared to the urolithic control.

Urinary phosphate determination

In present study the urinary phosphate control rats (Group I) did not show any significant variation in the urinary phosphate level throughout the experiment period. Both the doses 200mg and 400 mg/kg/day of methanolic extract of *Saraca indica* bark for 15 days significantly).

Reduced the urinary phosphate level as compared to the urolithic control. The methanolic extract of *Saraca indica* bark at doses of 200 mg and 400 mg/kg/day have reduced the urinary phosphate level as compared to standard (cystone 750 mg/kg/day). Results are shown in Table 2 and 3.

Urinary uric acid determination

The urinary uric acid control rats (Group I) did not show any significant variation in the urinary uric acid level throughout the experiment period. Both the doses 200 mg and 400 mg/kg/day of methanolic extract of *Saraca indica* bark for 15 days significantly reduced the urinary uric acid level as compared to the urolithic control. The methanolic extract of *Saraca indica* bark at doses of 200 mg and 400 mg/kg/day reduced the urinary uric acid level as compared to the standard (cystone 750 mg/kg/day). Results are shown in Table 4.

Urinary calcium determination

The present study of urinary calcium control rats (Group I) did not show any significant variation in the urinary calcium level throughout the experiment period. Both the doses 200 mg and 400 mg/kg/day of methanolic extract of *Saraca indica* bark for 15 days significantly increased the urinary calcium level as compared to the urolithic control. The methanolic extract of *Saraca indica* bark at doses of 200 mg, 400 mg/kg/day have increased the urinary calcium level as compared to standard (cystone 750 mg/kg/day) and restores it to near-normal value. Results are shown in Table 5.

Urinary magnesium determination

The present study of urinary magnesium control rats (Group I) did not show any significant variation in the urinary magnesium level throughout the experiment period. The methanolic extract of *Saraca indica* bark for 15 days significantly increased the urinary magnesium level as compared to the urolithic control. The methanolic extract of *Saraca indica* bark at doses of 200 mg and 400 mg/kg/day have increased the urinary magnesium level as compared to standard (cystone 750 mg/kg/day) and restore it to near normal value. Results are shown in Table 6.

Kidney parameters (calcium, oxalate and phosphate) determination

The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate and calcium, was increased in the stone forming rats (Group II). The methanolic extract of *Saraca indica* bark at doses of 200 mg and 400 mg/kg/day treatment significantly ($P > 0.001$) reduced the renal content. Results are shown in Table 7.

Serum parameters (creatinine and uric acid) determination

The serum creatinine and uric acid was remarkably increased in urolithic control (Group II). The methanolic extract of *Saraca indica* barkat doses of 200mg and 400 mg/kg/day treatment significantly ($P < 0.001$) lowered the elevated serum levels of creatinine and uric acid. Results are shown in Table 8 .

Histopathology of liver section:

Liver section of a control rat, It is composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution.

Liver section of urolithic control rat, in most hepatic lobules, the trabecular structure is lightly blurred and, in the remaining lobules, distinctly blurred. The cytoplasm of some cells shows rare empty vacuole -type spaces. A considerable number of Kupffer cells are observed in the sinusoid walls. Liver section of group III and IV, in most hepatocytes, the structure of nuclei is normal. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution is normal. Liver section of group V, VI, VII, the tubercular structure of the lobules is blurred in places. The cytoplasm of some hepatocytes is enlarged, light, with vacuoles. In most hepatocytes, the structure of nuclei is normal.

DISCUSSION

Currently available drug regimens for management of kidney stone have certain drawbacks. Therefore there is a need for safer and more effective antiurolithic drugs. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. *Saraca indica* is a multipurpose medicinal plant with many attributes and considerable potentials.

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 [30]. Urinary supersaturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Previous studies indicated that, upon 14 days administration of ethylene glycol to the young albino rats resulted into the formation of renal calculi composed mainly of calcium oxalate. The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Renal calcium oxalate deposition by

ethylene glycol in rats is frequently used to mimic the urinary stone formation [31], [32]. Therefore, this model was used to evaluate the protective effect of methanolic extract of *Saraca indica* bark against urolithiasis. Urinary chemistry is one of the important factors in determining the type of crystal formed and the nature of macromolecules included on the surface of the crystals. Hence, the study of the urinary chemistry related to the calculi forming minerals was provided a good indication of the extent of stone formation. By studying some previous reports [33] stone induction by ethylene glycol caused an increase in oxalate and decrease in calcium urinary excretion in the Group II. The methanolic extract of *Saraca indica* bark treatment for 15 days significantly increased the urinary calcium level as compared to the urolithic control. Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone. It has been reported that oxalate play an important role in stone formation and has about 15-fold greater effect than urinary calcium [34]. In our studies urinary oxalate was increased in ethylene glycol induced urolithic rats. The reduction in oxalate excretion was observed on methanolic extract of *Saraca indica* bark treatment. This decreased excretion of oxalate may be due to the inhibition of formation of oxalate by the plant extract. Normal urine contains many inorganic and organic inhibitors of crystallization, magnesium is one such well-known inhibitors. Low levels of magnesium are also encountered in stone formers as well as in stone-forming rats. The magnesium levels return to normal on the drug treatment [35]. Magnesium complexes with oxalate and reduce the supersaturation of calcium oxalate by reducing the saturation of calcium oxalate and as a consequence reduces the growth and nucleation rate of calcium oxalate crystals. Both the doses 200mg and 400 mg/kg/day of methanolic extract of *Saraca indica* bark for 15 days significantly increased the urinary magnesium level and thus reduces the risk of stone formation. In the present studies an increase in the urinary phosphate level was observed in ethylene glycol treated rats (Group II). Increased excretion of phosphate has been reported in stone formers [25]. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition [28], [24]. Treatment of methanolic extract of *Saraca indica* bark lowered the excretion of phosphate and reduces the risk of stone formation. The increase in urinary uric acid excretion was observed in urolithic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric. Uric acid interferes with calcium oxalate solubility [28]. Treatment of methanolic extract of *Saraca indica* bark lowered the excretion of uric acid and reduces the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste product particularly nitrogenous substances such as creatinine and uric acid get accumulated in blood [29]. The serum creatinine and uric acid was remarkably increased in urolithic control (Group II). The

methanolic extract of *Saraca indica bark* at doses of 200mg and 400 mg/kg/day treatment significantly ($P < 0.001$) lowered the elevated serum levels of creatinine and uric acid.

CONCLUSION

The aim of the study was to assess the antiurolithic activity of methanolic extract of *Saraca indica bark* on ethylene glycol induced hyperoxaluria model in Wistar male rats. Antiurolithic activity of *Saraca indica bark* extract was confirmed by measuring the serum marker (creatinine and uric acid), tissue homogenate marker (calcium, oxalate and phosphate), urinary parameter (calcium, oxalate, phosphate, uric acid and magnesium) and urinary output and thus it significantly reduced and prevented the growth of urinary stones. These studies supported the folk information regarding antiurolithic activity of the plant.

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Table 1: Determination of Urinary output in ml/24 hrs methanolic extract of *Saraca indica bark*

| Days | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------|-------------|-----------------|----------------|-----------------|-----------------|-------------------|-------------------|
| 0 | 8.80 ± 0.45 | 8.43 ± 0.32 | 9.17 ± 0.34 | 9.21 ± 0.17 | 12.71 ± 0.56 | 10.71 ± 0.12 | 9.17 ± 0.33 |
| 7 | 8.74 ± 0.27 | 14.80 ± 0.90a* | 15.25 ± 0.16 | 16.80 ± 0.24 | 9.36 ± 0.17 | 15.66 ± 0.14 | 10.02 ± 0.26 |
| 14 | 9.49 ± 0.31 | 21.80 ± 0.98a** | 10.19 ± 0.79 | 10.46 ± 0.56 | 26.9 ± 0.66a*b* | 23.19 ± 0.11 | 21.09 ± 1.34a** |
| 21 | 9.23 ± 0.38 | 21.67 ± 1.80a** | 15.70 ± 0.44a* | 17.85 ± 0.37a* | 19.81 ± 1.28a** | 27.39 ± 1.56a**b* | 28.05 ± 1.41a**b* |
| 28 | 9.28 ± 0.30 | 21.55 ± 0.56a** | 17.50 ± 0.12a* | 18.05 ± 0.17a** | 20.16 ± 1.32a** | 17.02 ± 0.76a**b* | 19.69 ± 0.97a**b* |

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b values are significantly different compared to the respective ethylene glycol treated when group * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2- Determination of urinary oxalate in mg/24 hrs

| Days | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------|-------------|----------------|-------------|---------------|-------------------|----------------|------------------|
| 0 | 5.07 ± 0.33 | 4.62 ± 0.37 | 4.76 ± 0.23 | 4.99 ± 0.27 | 5.13 ± 0.36 | 5.03 ± 0.21 | 4.97 ± 0.33 |
| 7 | 5.02 ± 0.27 | 6.13 ± 0.28a* | 5.13 ± 0.16 | 6.13 ± 0.34 | 5.76 ± 0.17 | 6.32 ± 0.05a* | 6.48 ± 0.26a* |
| 14 | 5.24 ± 0.21 | 7.92 ± 0.37a** | 5.13 ± 0.25 | 6.72 ± 0.32 | 8.24 ± 0.66a*b* | 8.26 ± 0.21a** | 7.82 ± 0.34a** |
| 21 | 5.18 ± 0.38 | 8.83 ± 0.48a** | 5.32 ± 0.44 | 8.44 ± 0.37 | 7.32 ± 0.28a**b** | 8.98 ± 0.56a** | 8.63 ± 0.41a** |
| 28 | 5.12 ± 0.30 | 9.93 ± 0.42a** | 6.29 ± 0.12 | 8.65 ± 0.17a* | 7.05 ± 0.52a**b** | 9.01 ± 0.26a** | 8.69 ± 0.27a**b* |

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated when group * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 3-Determination of urinary phosphate in mg/24 hrs

| Days | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------|-------------|-----------------|-------------|---------------|-------------------|----------------|-------------------|
| 0 | 5.78 ± 0.33 | 6.08 ± 0.23 | 5.23 ± 0.23 | 5.78 ± 0.27 | 5.24 ± 0.36 | 6.04 ± 0.21 | 6.01 ± 0.33 |
| 7 | 5.64 ± 0.27 | 6.29 ± 0.28a* | 5.13 ± 0.16 | 6.03 ± 0.34 | 5.41 ± 0.17a* | 6.54 ± 0.05a* | 6.48 ± 0.26a* |
| 14 | 5.72 ± 0.21 | 7.12 ± 0.37a** | 5.48 ± 0.25 | 6.92 ± 0.32 | 6.73 ± 0.26a** | 6.76 ± 0.21a** | 6.74 ± 0.34a** |
| 21 | 5.63 ± 0.38 | 7.43 ± 0.48a** | 5.92 ± 0.44 | 6.84 ± 0.37a* | 6.77 ± 0.28b** | 6.84 ± 0.56a** | 6.83 ± 0.41a** |
| 28 | 5.70 ± 0.30 | 7.93 ± 0.42a*** | 5.93 ± 0.12 | 6.39 ± 0.17a* | 6.78 ± 0.52a**b** | 6.87 ± 0.26a** | 6.89 ± 0.27a**b** |

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated when group * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 4: Determination of urinary uric acid in mg/24 hrs

| Days | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------|-------------|----------------|---------------|---------------|------------------|----------------|------------------|
| 0 | 1.88 ± 0.21 | 2.01 ± 0.12 | 1.89 ± 0.13 | 1.91 ± 0.16 | 2.27 ± 0.17 | 2.10 ± 0.13 | 2.07 ± 0.32 |
| 7 | 1.86 ± 0.24 | 2.45 ± 0.08a** | 1.92 ± 0.16 | 2.22 ± 0.14 | 2.30 ± 0.11a* | 2.50 ± 0.05a** | 2.53 ± 0.26a** |
| 14 | 1.93 ± 0.31 | 2.60 ± 0.07a** | 2.02 ± 0.05 | 2.37 ± 0.12 | 2.60 ± 0.16b** | 2.60 ± 0.11a** | 2.59 ± 0.34a** |
| 21 | 1.90 ± 0.27 | 2.71 ± 0.18a** | 2.11 ± 0.14 | 2.44 ± 0.07a* | 2.71 ± 0.08b** | 2.63 ± 0.9a** | 2.67 ± 0.41a** |
| 28 | 1.91 ± 0.19 | 2.97 ± 0.12a** | 2.13 ± 0.12a* | 2.47 ± 0.10a* | 2.77 ± 0.05a*b** | 2.71 ± 0.06a** | 2.70 ± 0.27a**b* |

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 5: Determination of urinary calcium in mg/24 hrs

| Days | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------|-------------|----------------|-------------|----------------|-------------------|----------------|------------------|
| 0 | 0.61 ± 0.02 | 0.66 ± 0.03 | 0.71 ± 0.04 | 0.61 ± 0.02 | 0.73 ± 0.08 | 0.71 ± 0.08 | 0.72 ± 0.03 |
| 7 | 0.68 ± 0.03 | 0.41 ± 0.02a** | 0.67 ± 0.06 | 0.59 ± 0.04 | 0.66 ± 0.05b** | 0.43 ± 0.05a* | 0.44 ± 0.06a** |
| 14 | 0.67 ± 0.03 | 0.30 ± 0.03a** | 0.64 ± 0.05 | 0.52 ± 0.02 | 0.61 ± 0.06b** | 0.32 ± 0.06a | 0.31 ± 0.04a** |
| 21 | 0.67 ± 0.05 | 0.28 ± 0.06a** | 0.62 ± 0.04 | 0.46 ± 0.07a* | 0.43 ± 0.01a*b** | 0.29 ± 0.01a** | 0.30 ± 0.01a** |
| 28 | 0.69 ± 0.01 | 0.25 ± 0.09a** | 0.61 ± 0.02 | 0.34 ± 0.08a** | 0.41 ± 0.05a**b** | 0.26 ± 0.05a** | 0.31 ± 0.07a**b* |

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001

Table 6: Determination of urinary magnesium in mg/24 hrs

| Days | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------|-------------|----------------|---------------|---------------|-------------------|----------------|----------------|
| 0 | 2.67 ± 0.20 | 2.12 ± 0.13 | 2.59 ± 0.18 | 2.23 ± 0.17 | 2.48 ± 0.19 | 2.61 ± 0.17 | 2.35 ± 0.12 |
| 7 | 2.61 ± 0.18 | 2.05 ± 0.08 | 2.48 ± 0.16 | 2.13 ± 0.14 | 2.38 ± 0.06b** | 1.99 ± 0.05a** | 1.92 ± 0.14a** |
| 14 | 2.53 ± 0.13 | 1.84 ± 0.17a** | 2.38 ± 0.05 | 2.07 ± 0.12 | 2.33 ± 0.09b** | 1.87 ± 0.11a** | 1.69 ± 0.19a** |
| 21 | 2.49 ± 0.09 | 1.73 ± 0.18a** | 2.31 ± 0.14 | 1.96 ± 0.07 | 2.28 ± 0.13a*b* | 1.64 ± 0.13a** | 1.62 ± 0.16a** |
| 28 | 2.56 ± 0.11 | 1.49 ± 0.07a** | 2.28 ± 0.12a* | 1.91 ± 0.10a* | 2.24 ± 0.05a**b** | 1.78 ± 0.06a** | 1.67 ± 0a**b* |

Values are expressed as Mean \pm SEM for five animals in each group. a values are significantly different compared to control when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b values are significantly different compared to the respective ethylene glycol treated when when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 7 - Determination of Kidney parameters (calcium, oxalate and phosphate) mg/g. Kidney mg/g

| Parameter | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|-----------|------------------|--------------------|-------------------|-------------------|-----------------------|-----------------------|-----------------------|
| Calcium | 0.25 \pm 0.04* | 0.41 \pm 0.07a* | 0.27 \pm 0.02 | 0.24 \pm 0.14 | 0.28 \pm 0.03b** | 0.37 \pm 0.08a** | 0.33 \pm 0.01a**b** |
| Oxalate | 1.58 \pm 0.14 | 6.46 \pm 0.11a** | 1.74 \pm 0.12 | 1.77 \pm 0.15a* | 2.01 \pm 0.17a**b** | 3.91 \pm 0.11a**b** | 2.81 \pm 0.11a**b** |
| Phosphate | 2.85 \pm 0.05 | 4.24 \pm 0.14a** | 2.57 \pm 0.17a* | 2.87 \pm 0.09a* | 2.82 \pm 0.14b** | 3.09 \pm 0.08a**b* | 2.99 \pm 0.14a**b** |

Values are expressed as Mean \pm SEM for five animals in each group. a values are significantly different compared to control when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b values are significantly different compared to the respective ethylene glycol treated group when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 8 - Determination of Serum parameters (creatinine and uric acid) mg/dl. Serum mg/dl

| Parameter | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|------------|-----------------|--------------------|-------------------|-----------------|-----------------------|-----------------------|-----------------------|
| Creatinine | 0.75 \pm 0.04 | 1.15 \pm 0.06a** | 0.77 \pm 0.02 | 0.74 \pm 0.14 | 0.82 \pm 0.03b** | 1.02 \pm 0.08a**b** | 3.75 \pm 0.11a**b** |
| Uric acid | 2.38 \pm 0.11 | 5.83 \pm 0.09a* | 2.74 \pm 0.12a* | 2.37 \pm 0.15 | 2.51 \pm 0.17a**b** | 4.67 \pm 0.11a**b** | 3.75 \pm 0.11a**b** |

Values are expressed as Mean \pm SEM for five animals in each group. a values are significantly different compared to control when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b values are significantly different compared to the respective ethylene glycol treated when when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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