



International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR | Vol.8 | Issue 3 | Jul - Sept -2024

www.ijpcr.com

ISSN: 2349-5448

DOI : <https://doi.org/10.61096/ijpcr.v8.iss3.2024.250-257>

Research

Exploration of anti-urolithiatic potential of ethanolic root extract of *Pisonia alba* in wistar rats

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	Abstract
Published on: 24 Jul 2024	<p>The root of <i>Pisonia alba</i> (Family: Nyctaginaceae), extract was investigated for its antiurolithiatic activity. Ethylene glycol (0.75% in water) feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Ethanolic extract (250 & 500 mg/kg) of <i>P. alba</i> was given orally in curative and preventive regimens over a period of 28 days. Supplementation with extract significantly ($P < 0.001$) lowered the urinary excretion and kidney retention levels of oxalate, calcium and phosphate. Furthermore, high serum levels of urea nitrogen, creatinine and uric acid were significantly ($P < 0.001$) reduced by the extract. The results were comparable with the standard drug, cystone (750 mg/kg). The reduction of stone-forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of the extract. These findings affirm assertions made regarding the effectiveness of the extract of this plant against urinary pathologies in Indian folk medicine.</p>
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	<p>Keywords: <i>Pisonia alba</i>, ethylene glycol, hyperoxaluria, Lipid peroxidation, urolithiasis.</p>

INTRODUCTION

Nephrolithiasis or renal stone disease remains a significant health problem in the adult population, with serious medical consequences, throughout a patient's lifetime. The worldwide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate.[1] The present-day medical management of nephrolithiasis is either costly or not without side-effects. Invasive procedures for the treatment of nephrolithiasis may cause serious complications and also impose a great load of costs on the healthcare system. [2-5]

In contrast, traditional medicines have offered a substitute for many diseases and also have provided some supplementary information about the pathogenesis of diseases. *Pisonia alba* (Family: Nyctaginaceae), is a perennial herb growing widely along the roadsides of India. Traditionally, the plant is useful as an anthelmintic, laxative, anti-pyretic, expectorant, and used in infantile diarrhea. Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes, and saponins. The plant has been documented for anti-inflammatory, anti-pyretic, and analgesic activities, antifertility, antidiabetic, wound healing, antibacterial, and hepatoprotective

activity. [6-8] The objective of the present study was to investigate and to validate the antiurolithiatic property of *Pisonia alba* root extract in experimentally induced urolithiasis in rats.

MATERIALS AND METHODS

Plant material and extraction procedure

In May 2024, roots of *Pisonia alba* were gathered from Jambai village in the Erode District of Tamilnadu, India, and authenticated. The plant material (500 g) was extracted with 80% v/v ethanol using a Soxhlet apparatus. The extract was concentrated in a rotary evaporator at reduced pressure.

Phytochemical analysis

The extract was screened for various constituents (alkaloids, saponins, tannins, anthraquinones, sterol, flavonoids, terpenoids, glycosides, simple sugars) using standard protocol.

Experimental Animals

Colony inbred strains of Male Wistar rats of 200-250g body weight were used for pharmacological studies, three different groups with 6 animals in each, were housed at controlled temperature ($25\pm 2^\circ\text{C}$), humidity (60–80% relative humidity) and light dark cycle (12/12 hour); on a standard rodent chow (Hindustan lever pvt ltd., Bangalore) and water *ad libitum*. Animals were handled carefully and acclimatized to laboratory conditions for 14 days before experimentation. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee.

Ethylene Glycol-Induced Urolithiasis in Rats

Experimental Design

The animals were split up into five groups, each with six members. Group I was the control group and was given unlimited access to water and ordinary rat food. Groups II–V were given ethylene glycol (0.75%) in their drinking water for 28 days in order to induce renal calculi. From the 15th to the 28th day, Group III was administered the usual antiurolithiatic medication, cystone (750 mg/kg b.w.). [10] For a duration of 28 days, Group V got Extract - II (400 mg/kg b.w.) and Group IV received Extract - I (200 mg/kg b.w.). Every extract was taken once a day by oral route. Following the conclusion of the therapy, anesthesia was administered to each group of animals, and the animals were then sacrificed. [9-17]

Collection of Urine

On days 21 and 28, early morning urine samples were taken. A single pee drop was put on a glass slide so that CaOx crystals could be seen under a light microscope. On the 28th day, urine samples were taken over the course of 24 hours while each animal was housed in its own metabolic cage. Throughout the urine collection time, the animal had unrestricted access to drinking water. Urine was collected and then kept at 4°C with a drop of strong hydrochloric acid added. Urine was used to measure many parameters, including pH, calcium, uric acid, phosphate, magnesium, and urinary output.

Collection of Serum

Glass capillaries were used to draw blood from the wistar rat's retro-orbital plexus while it was under mild ether anesthesia. The blood was drawn into 2-milliliter Eppendorf tubes. It was centrifuged at 5000 rpm for 20 minutes in order to separate the serum after being let to coagulate in the open for 15 minutes. The collected serum was kept at -20°C until other biochemical parameters, including blood urea nitrogen (BUN), urea, creatinine, and uric acid, were estimated.

Kidney Homogenate Analysis

Rats were killed via cervical decapitation at the conclusion of the experiment, and their kidneys were removed and kept separate. They were also washed to remove any unnecessary tissue and dipped in icy physiological saline. A half piece of the kidney that was isolated was stored in crushed ice. They were swiftly blotted with filter paper after being cross-sliced into thin slices using a medical blade and cooled in 0.25 M sucrose. Using a homogenizer running at 2500 rpm and 0.1 M Tris hydrochloride buffer (pH 7.4), a 10% (w/v) homogenate of the tissues was created. A cooling centrifuge was used to whirl the homogenate for 20 minutes at 5000 rpm (-4°C). The different marker enzymes were estimated using the supernatant that was collected after centrifugation. Catalase, glutathione (GSH), and malondialdehyde (MDA) were measured from the clear supernatant after it had been separated.

Estimation of Superoxide Dismutase

100 μL of epinephrine (3 mM) and 0.8 ml of carbonate buffer (100 mM, pH 10.2) were combined with

the 500 μL of supernatant. Then, for two minutes at intervals of fifteen seconds, the change in absorbance of each sample was recorded at 480 nm in a spectrophotometer. To determine SOD activity, parallel runs of the standard and blank were conducted. The quantity of enzyme needed to cause 50% inhibition of epinephrine auto-oxidation is known as one unit of SOD. Shortly before obtaining spectrophotometer readings, the reaction mixtures are diluted 1/10.

Estimation of Catalase

As stated by Sinha, a colorimetric test was used to measure catalase (CAT).[88] One milliliter of pH 7.0 phosphate buffer, one milliliter of tissue homogenate (supernatant), and four milliliters of 0.2 M H_2O_2 made up the reaction mixture. The addition of 2.0 ml of the dichromate-acetic acid reagent halted the reaction. Micromoles of H_2O_2 consumed/min/mg protein was the unit used to represent CAT activity, and color intensity was evaluated colorimetrically at 620 nm.

Estimation of Reduced Glutathione

To each tube containing 0.5ml of Trichloroacetic acid (TCA 10%), 0.5ml of homogenate was added. After gently shaking the test tube every ten minutes for ten minutes, it was centrifuged at 3000 rpm for five minutes at room temperature. In separate test tubes, precisely 0.1 ml of the clear supernatant that resulted was combined with 1.8 ml of the phosphate buffer (0.1M, pH 8). For every sample, at least one duplicate was created. Each tube received 0.1 ml of Ellman's reagent (0.39%), and the optical density was measured at 412 nm against a reagent blank after 5 minutes. The information was given as $\mu\text{mol/g}$ of tissue.

Estimation of Malonaldehyde

In glacial acetic acid, a standard stock solution of MDA (1 mM) was made. Following precise weighing, 31.35 mg of MDA were dissolved in 100 mL of solvent. Various concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM were produced from the stock solution. The concentration range of 0.1 to 1.0 mM was used to generate the calibration curve. In a 10 mL test tube, 1 mL of the standard MDA solution was combined with 1 mL of TBA. For sixty minutes, the mixture was cooked to 95°C in a boiling water bath. A UV-visible spectrophotometer was used to detect the absorbance at 532 nm after the test tubes had been cooled to room temperature. As per the aforementioned protocol, each calibration standard was performed three times ($n = 3$). Five blank samples were repeated, with acetic acid or water used in place of the standard or sample. Protein was represented as nmol/mg in the data.[18-22]

Statistical Analysis

One-way ANOVA was used for the statistical analysis, and the Dunnett multiple comparison test was used after. P values less than 0.05 were deemed statistically significant, and Graph Pad Prism Version 8 was utilized to compute them.

RESULTS

Preliminary Phytochemical Analysis:

The phytochemical examination of the ethanolic root extract of *Pisonia alba* explores the presence of Alkaloids, Flavonoids, Carbohydrates, Tannins, Phytosterols etc.

Ethylene Glycol-Induced Urolithiasis in Rats

The prolonged injection of 0.75% (v/v) ethylene glycol resulted in hyperoxaluria, which elevated the excretion of calcium, magnesium, and phosphate. The increased levels of calcium, magnesium, and phosphate were dramatically ($P < 0.001$) reduced by the *Pisonia alba* ethanolic root extract (EEPA - II). The rats that developed stones (Group II) had higher levels of calcium, phosphate, and magnesium deposits in their renal tissues.

In both regimens (Groups IV and V), the renal concentration of stone-forming components was considerably ($P < 0.001$) decreased by the Ethanolic root extract of *Pisonia alba* (EEPA - II) therapy. In comparison to the control group, the calculi-induced animals (Group II) exhibited substantial increases in blood creatinine, urea, uric acid, and BUN. Additionally, the higher serum creatinine in Group II indicated considerable kidney impairment. Nonetheless, the higher blood levels of creatinine, urea, uric acid, and BUN were considerably ($P < 0.001$) reduced by the ethanolic root extract of *Pisonia alba* in Groups IV and V.

In calculi-induced mice, ethylene glycol treatment significantly ($P < 0.05$) raised MDA levels and lowered SOD, CAT, and GSH levels in comparison to normal animals [group II]. Comparing group II, group III, and group V to the treatment with ethanolic root extract of *Pisonia alba* (250 and 500 mg/kg), there was a substantial ($P < 0.0001$) drop in MDA and a significant improvement in the level of antioxidant enzymes like SOD ($P < 0.001$). Both curative and preventative treatments with *Pisonia alba* (ethanolic root extract, EEPA – II) significantly ($P < 0.001$) maintained the high level of CAT. [Table 1-7] [Figure 1-6]

Table 1: Effect of ethanolic root extract of *Pisonia alba* on urine output (ml/24hr)

GROUPS	Urine output (ml/24hr)
Group I (Control)	29.36 ± 4.79
Group II (Negative Control)	36.96 ± 3.04**
Group III (Positive Control)	47.98 ± 3.12****
Group IV (250 Mg/Kg EEPA)	41.64 ± 3.02****
Group V (500 Mg/Kg EEPA)	44.94 ± 3.06****

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns – not significant).

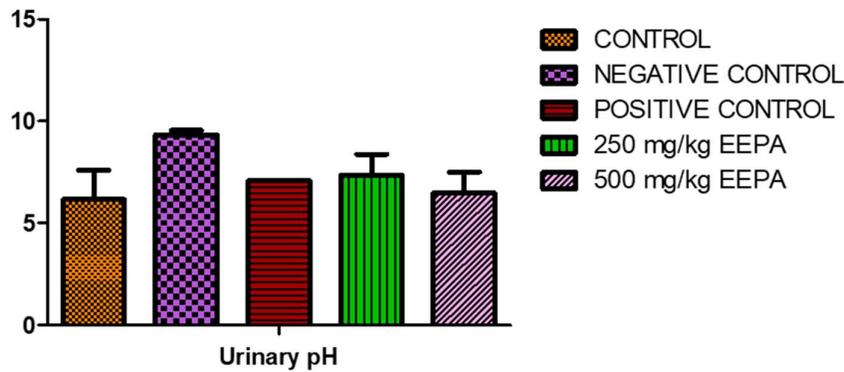


Fig 1: Effect of ethanolic root extract of *Pisonia alba* on urinary pH

Table 2: Effect of ethanolic root extract of *Pisonia alba* on urinary calcium (ml/24hr)

GROUPS	Urinary calcium (ml/24hr)
Group I (Control)	4.04 ± 0.31
Group II (Negative Control)	7.11 ± 0.21****
Group III (Positive Control)	4.89 ± 0.20**
Group IV (250 Mg/Kg EEPA)	6.21 ± 0.12****
Group V (500 Mg/Kg EEPA)	4.95 ± 0.07****

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns – not significant).

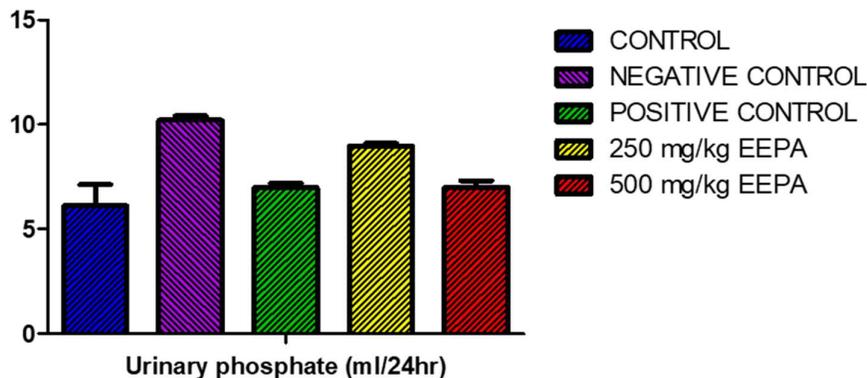


Fig 2: Effect of ethanolic root extract of *Pisonia alba* on urinary phosphate (ml/24hr)

Table 3: Effect of ethanolic root extract of *Pisonia alba* on urinary magnesium (ml/24hr)

GROUPS	Urinary magnesium (ml/24hr)
Group I (Control)	4.28 ± 0.12
Group II (Negative Control)	2.59 ± 0.13****
Group III (Positive Control)	4.34 ± 0.12 ^{ns}
Group IV (250 Mg/Kg EEPA)	3.41 ± 0.36****
Group V (500 Mg/Kg EEPA)	4.54 ± 0.40*

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (*^{ns}p < 0.0001, ****p < 0.001, **p < 0.01, *p < 0.05, ns – not significant).

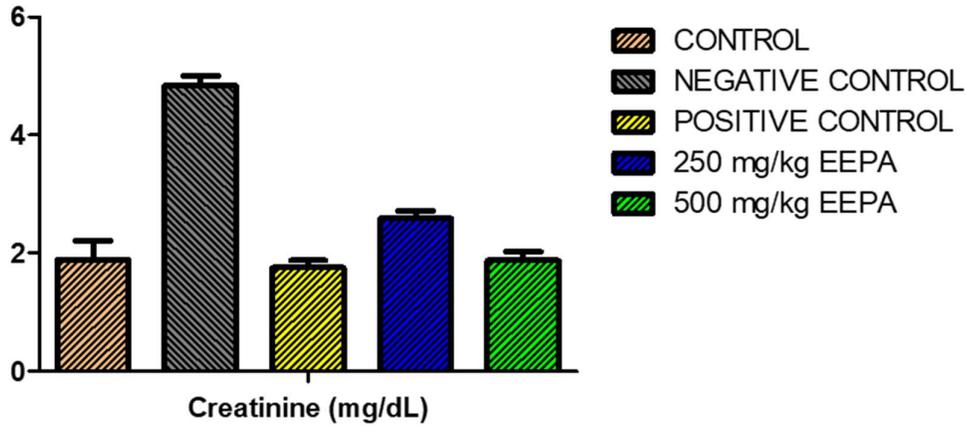


Fig 3: Effect of ethanolic root extract of *Pisonia alba* on serum creatinine (mg/dL)

Table 4: Effect of ethanolic root extract of *Pisonia alba* on serum urea (mg/dL)

GROUPS	Urea (mg/dL)
Group I (Control)	29.51 ± 0.72
Group II (Negative Control)	78.71 ± 0.34**
Group III (Positive Control)	24.74 ± 0.25*
Group IV (250 Mg/Kg EEPA)	55.72 ± 0.17*
Group V (500 Mg/Kg EEPA)	38.97 ± 0.21**

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (p < 0.0001, ****p < 0.001, **p < 0.01, *p < 0.05, ns – not significant).

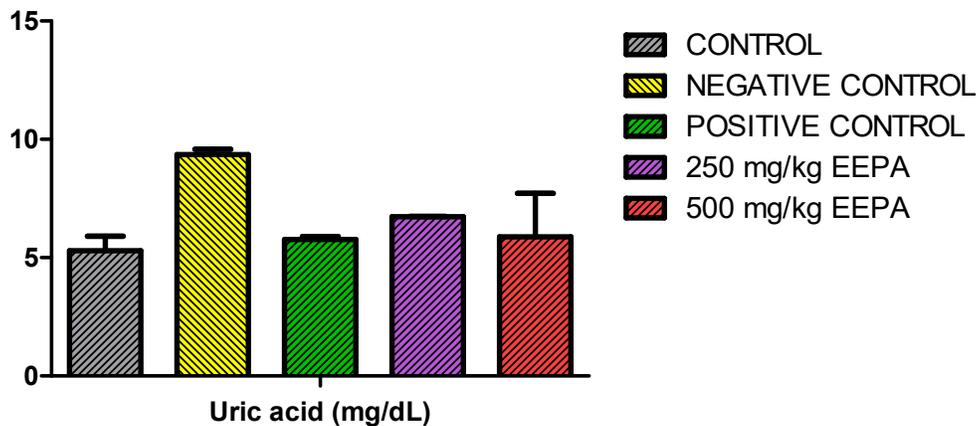


Fig 4: Effect of ethanolic root extract of *Pisonia alba* on serum uric acid (mg/dL)

Table 5: Effect of ethanolic root extract of *Pisonia alba* on serum BUN (mg/dL)

GROUPS	BUN (mg/dL)
Group I (Control)	14.99 ± 0.55
Group II (Negative Control)	37.15 ± 4.19****
Group III (Positive Control)	14.54 ± 0.12 ^{ns}
Group IV (250 Mg/Kg EEPA)	33.45 ± 0.12****
Group V (500 Mg/Kg EEPA)	18.78 ± 0.18**

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (*^{****}p < 0.0001, ^{***}p < 0.001, ^{**}p < 0.01, ^{*}p < 0.05, ns – not significant).

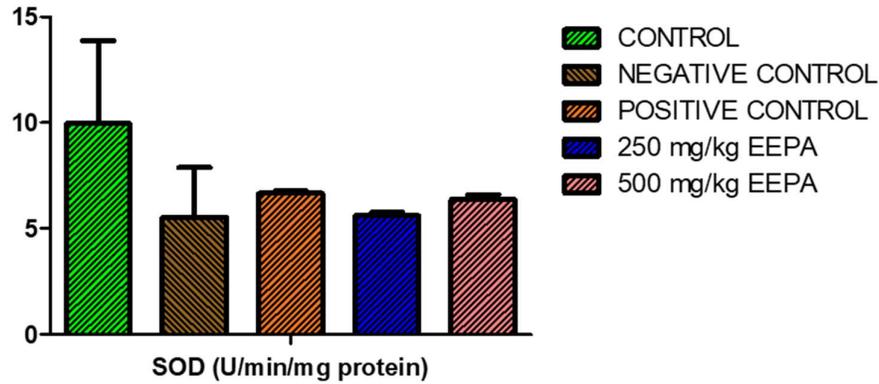


Fig 5: Effect of ethanolic root extract of *Pisonia alba* on Superoxide dismutase of tissue homogenate

Table 6: Effect of ethanolic root extract of *Pisonia alba* on Catalase of tissue homogenate

GROUPS	Catalase (µM/H ₂ O ₂ /min/mg protein)
Group I (Control)	4.92 ± 1.25
Group II (Negative Control)	1.87 ± 0.12****
Group III (Positive Control)	4.00 ± 0.28*
Group IV (250 Mg/Kg EEPA)	2.35 ± 0.25****
Group V (500 Mg/Kg EEPA)	3.57 ± 0.33***

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (*^{****}p < 0.0001, ^{***}p < 0.001, ^{**}p < 0.01, ^{*}p < 0.05, ns – not significant).

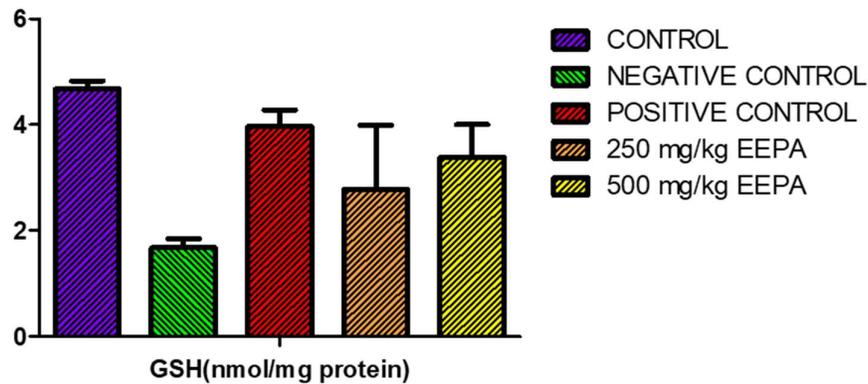


Fig 6: Effect of ethanolic root extract of *Pisonia alba* on Glutathione reductase of tissue homogenate

Table 7: Effect of ethanolic root extract of *Pisonia alba* on Malonaldehyde of tissue homogenate

GROUPS	MDA (nmol/mg protein)
Group I (Control)	4.83 ± 0.27
Group II (Negative Control)	10.26 ± 0.12****
Group III (Positive Control)	5.38 ± 0.11*
Group IV (250 Mg/Kg EEPA)	9.34 ± 0.12****
Group V (500 Mg/Kg EEPA)	6.44 ± 0.86****

Values are expressed as mean ± SD (n=6). Values comparison were made between Group I Vs Group II, III, IV (***) $p < 0.0001$, **** $p < 0.001$, * $p < 0.01$, $p < 0.05$, ns – not significant).

DISCUSSION

Rats were chosen to test the preventive effect of an ethanolic root extract of *Pisonia alba* against ethylene glycol-induced urolithiasis, as traditional medications are often given orally. The identification of the therapeutic functions of these herbal medicines has significantly advanced the use of these treatments as adjunctive or alternative therapies for the management of urinary stone disease. A complex interplay of biological processes, mostly initiated by genetic vulnerability in conjunction with dietary variables and lifestyle choices, leads to the development of kidney stones. Because male rats' urinary systems are similar to those of humans and because previous research has demonstrated that female rats exhibit noticeably less stone deposition, male rats were used to induce urolithiasis in this investigation.

The prolonged injection of 0.75% (v/v) ethylene glycol resulted in hyperoxaluria, which elevated the excretion of calcium, magnesium, and phosphate. The increased levels of calcium, magnesium, and phosphate were dramatically ($P < 0.001$) reduced by the *Pisonia alba* ethanolic root extract (EEPA - II). The rats that developed stones (Group II) had higher levels of calcium, phosphate, and magnesium deposits in their renal tissues. In both regimens (Groups IV and V), the renal concentration of stone-forming components was considerably ($P < 0.001$) decreased by the Ethanolic root extract of *Pisonia alba* (EEPA - II) therapy. In comparison to the control group, the calculi-induced animals (Group II) exhibited substantial increases in blood creatinine, urea, uric acid, and BUN. Additionally, the higher serum creatinine in Group II indicated considerable kidney impairment. Nonetheless, the higher blood levels of creatinine, urea, uric acid, and BUN were considerably ($P < 0.001$) reduced by the ethanolic root extract of *Pisonia alba* in Groups IV and V.

In calculi-induced mice, ethylene glycol treatment significantly ($P < 0.05$) raised MDA levels and lowered SOD, CAT, and GSH levels in comparison to normal animals [group II]. Comparing group II, group III, and group V to the treatment with ethanolic root extract of *Pisonia alba* (250 and 500 mg/kg), there was a substantial ($P < 0.0001$) drop in MDA and a significant improvement in the level of antioxidant enzymes like SOD ($P < 0.001$). Both curative and preventative treatments with *Pisonia alba* (ethanolic root extract, EEPA – II) significantly ($P < 0.001$) maintained the high level of CAT. [23-29]

CONCLUSION

The ethylene glycol control group's blood and urine levels of uric acid were significantly higher than those of the normal control group, according to the data. After receiving therapy with EEPA-I & II and cystone, the levels of uric acid dropped, which accelerated the breakdown of preexisting stones and prevented the creation of new ones in the urinary tract. The EEPA I & II therapy offered protection from oxidative stress-related alterations. Numerous investigations have demonstrated that the development of crystals causes harm to cells and their separation from the basement membrane and that the breakdown products that are emitted also encourage the nucleation of new crystals. The administration of *Pisonia alba*'s ethanolic root extract (EEPA-II) considerably inhibited the development of urinary stones, according to the results. Additionally, it appears that the therapeutic impact outweighs the prevention effect. Its diuretic action, antioxidant capacity, nephroprotective quality, and ability to reduce the concentration of components that cause urinary stones might be the fundamental mechanism. To clarify the chemical components of the extract and the mechanism or mechanisms behind the pharmacological actions, more experimental and clinical research is needed.

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