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Research

Anti-Urolithiatic Activity Of Ethanolic Root Extract Of Plectranthus Vettiveroides In Wistar Rats

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Abstract

The root of *Plectranthus vettiveroides* (Family: Lamiaceae), 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 *P. vettiveroides* 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.

Keywords: *Plectranthus vettiveroides* , Ethylene glycol, Hyperoxaluria, Lipid peroxidation, Urolithiasis

INTRODUCTION

Urolithiasis has become the third most common affliction of the urinary tract affecting approximately 12% of world's population with an increasingly high recurrence rate in males than in females [1]. Aetiology is multifactorial and is strongly influenced by biochemical, epidemiological, and genetic risk factors [2]. Calcium oxalate (CaOx) is the predominant component of most stones accounting for more than 80% of stones. Remaining 20% are mainly composed of struvite, cysteine, and uric acid [3]. The pathogenesis of urolithiasis is a multistep process that results from a number of physicochemical events. The crystallization of CaOx begins with an increased urinary supersaturation, with the subsequent formation of solid crystalline particles within the urinary tract. It is followed by nucleation, by which stone-forming salts in the supersaturated urinary solution coalesce into clusters that then increase in size by the addition of new constituents [4]. These crystals then grow and

aggregate with other crystals in solution and are ultimately retained and accumulated in the urinary tract. Renal injury promotes crystal retention and the development of a stone nidus on the renal papillary surfaces and further supports crystal nucleation at lower supersaturation level [2]. Supersaturation of urine with mineral salts is the major cause of urolithiasis. The level of urinary supersaturation correlate with the type of stone formed. Therefore, the reduction of supersaturation is effective in the prevention of stone recurrence [3].

Management of urolithiasis depends on the size and location of the stones. Thiazide diuretics and alkali citrate are used commonly in the prevention of recurrence of urolithiasis. Stones larger than 5 mm or stones that fail to pass through should be treated using interventional procedures, such as endoscopic stone removal, extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL). These procedures are very expensive for many patients and are associated with recurrence of kidney stones, which is often up to 60%. They also require careful follow-up for many years for possible complications such as acute renal injury [5]. Therefore, in many countries, including Sri Lanka, phytotherapeutic agents are widely used as complementary and alternative therapies for the management of urolithiasis.

In contrast, traditional medicines have offered a substitute for many diseases and also have provided some supplementary information about the pathogenesis of diseases. Plectranthus vettiveroides, often known as Coleus vettiveroides, is a fragrant herbaceous plant in the Lamiaceae family. The plant, which is native to South India, is no longer found in the wild. There are several factors that have led to its extinction in the wild. The root is bitter and has trichogenous, diuretic, and antipyretic properties. As an antibiotic, deodorant, and cooling agent, it has also been used to treat a variety of conditions, including giddiness, insanity, ulcers, vomiting, nausea, skin conditions, genitourinary disorders, diarrhoea, fever intrinsic haemorrhage, hyperdipsia, stranguriy, leprosy, and eye burning. Doctors appreciate the essential oil that is derived from the root as a natural way to sanitise their hands. [5-8] The aim of the present study was to investigate the anti-urolithiatic activity of ethanolic root extract of *Plectranthus vettiveroides* in wistar rats

MATERIALS AND METHODS

Plant material and extraction procedure

In April 2024, roots of *Plectranthus vettiveroides* 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 inbreed 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±2°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 malondialdeide (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 H2O2 made up the reaction mixture. The addition of 2.0 ml of the dichromate-acetic acid reagent halted the reaction. Micromoles of H2O2 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 µmol/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 *Plectranthus vettiveroides* 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 *P.Vettiveroides* ethanolic root extract (EEPV - 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 *P.Vettiveroides* (EEPV - 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 *Plectranthus vettiveroides* 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 *Plectranthus vettiveroides* (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 *Plectranthus vettiveroides* (ethanolic root extract, EEPV – II) significantly ($P < 0.001$) maintained the high level of CAT. [Table 1-7] [Figure 1-6]

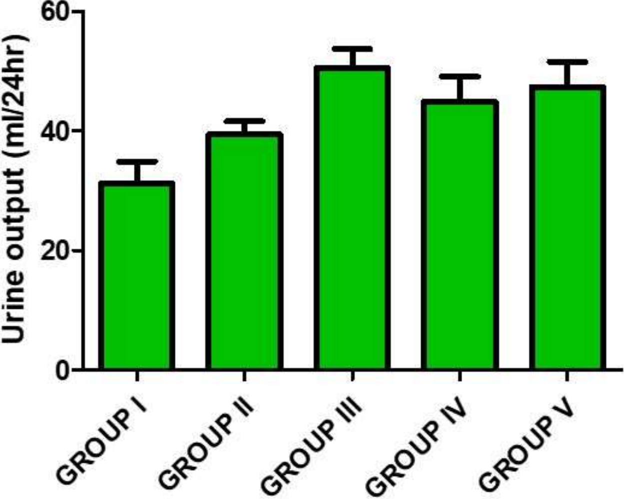

Fig 1: Effect of ethanolic root extract of *P.Vettiveroides* on urine output (ml/24hr)

Table 1: Effect of ethanolic root extract of *P.Vettiveroides* on urinary pH

GROUPS	Urinary pH
GROUP I (CONTROL)	7.22 ± 1.212
GROUP II (NEGATIVE CONTROL)	10.63 ± 1.121****
GROUP III (POSITIVE CONTROL)	8.24 ± 1.212**
GROUP IV (250 mg/kg EEPV)	8.47 ± 2.121****
GROUP V (500 mg/kg EEPV)	7.24 ± 0.112^{ns}

*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).*

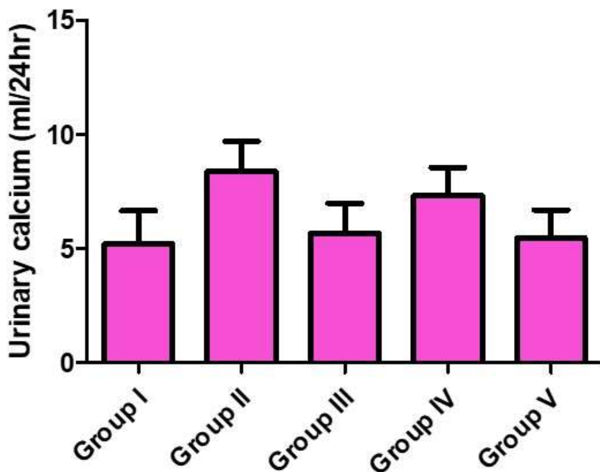

Fig 2: Effect of ethanolic root extract of *P.Vettiveroides* on urinary calcium (ml/24hr)

Table 2: Effect of ethanolic root extract of *P.Vettiveroides* on urinary phosphate (ml/24hr)

GROUPS	Urinary phosphate (ml/24hr)
GROUP I (CONTROL)	7.61 ± 2.14
GROUP II (NEGATIVE CONTROL)	11.84 ± 1.85****
GROUP III (POSITIVE CONTROL)	7.48 ± 1.68**
GROUP IV (250 mg/kg EEPV)	9.88 ± 1.22****
GROUP V (500 mg/kg EEPV)	8.14 ± 1.21***

*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).*

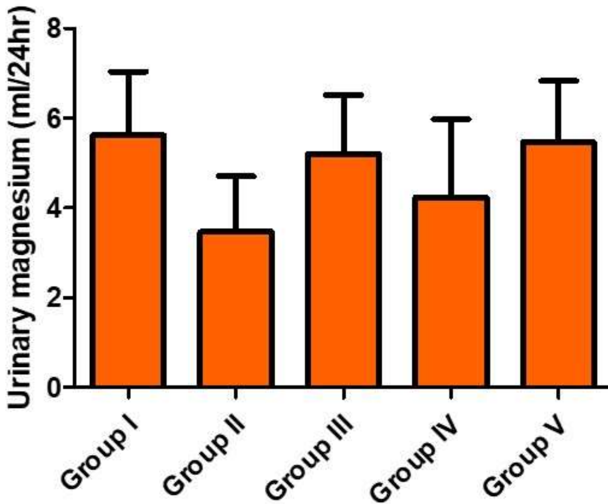

Fig 3: Effect of ethanolic root extract of *P.Vettiveroides* on urinary magnesium (ml/24hr)

Table 3: Effect of ethanolic root extract of *P.Vettiveroides* on serum creatinine (mg/dL)

GROUPS	Creatinine (mg/dL)
GROUP I (CONTROL)	2.16 ± 1.32
GROUP II (NEGATIVE CONTROL)	5.62 ± 1.22****

GROUP III (POSITIVE CONTROL)	2.44 ± 1.25***
GROUP IV (250 mg/kg EEPV)	3.41 ± 1.13****
GROUP V (500 mg/kg EEPV)	2.33 ± 1.22*

*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).*

Table 4: Effect of ethanolic root extract of *P.Vettiveroides*on serum urea (mg/dL)

GROUPS	Urea (mg/dL)
GROUP I (CONTROL)	31.26 ± 1.61
GROUP II (NEGATIVE CONTROL)	80.24 ± 1.84**
GROUP III (POSITIVE CONTROL)	26.74 ± 0.36*
GROUP IV (250 mg/kg EEPV)	57.21 ± 1.26*
GROUP V (500 mg/kg EEPV)	40.27 ± 1.33**

*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).*

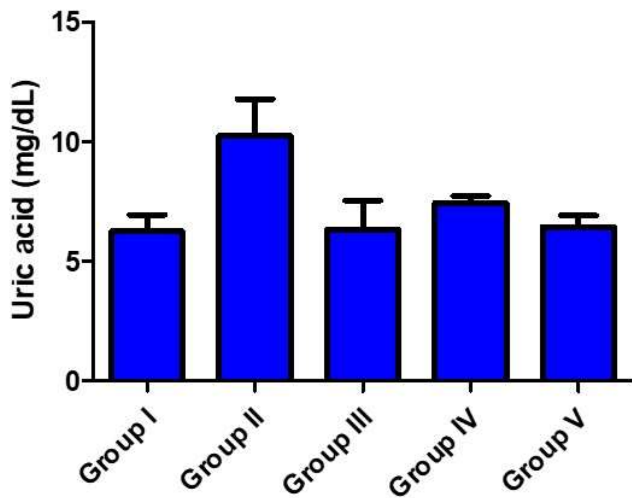

Fig 4: Effect of ethanolic root extract of *P.Vettiveroides* on serum uric acid (mg/dL)

Table 5: Effect of ethanolic root extract of *P.Vettiveroides* on serum BUN (mg/dL)

GROUPS	BUN (mg/dL)
GROUP I (CONTROL)	13.87 ± 0.41
GROUP II (NEGATIVE CONTROL)	36.15 ± 1.24****
GROUP III (POSITIVE CONTROL)	13.84 ± 0.42^{ns}
GROUP IV (250 mg/kg EEPV)	32.74 ± 0.17****
GROUP V (500 mg/kg EEPV)	17.68 ± 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).*

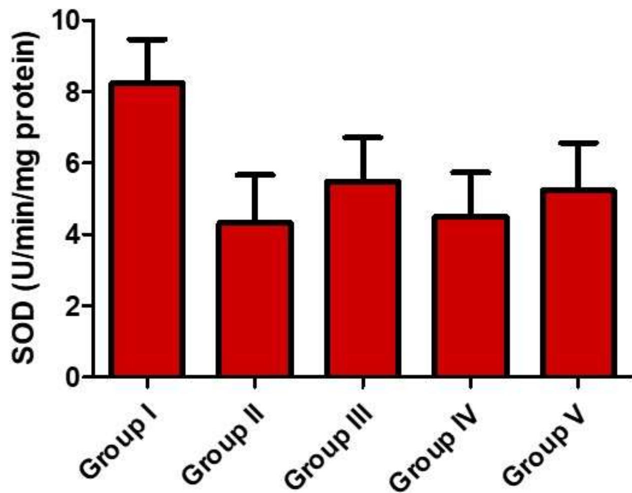

Fig 5: Effect of ethanolic root extract of *P.Vettiveroides* on Superoxide dismutase of tissue homogenate

Table 6: Effect of ethanolic root extract of *P.Vettiveroides* on Catalase of tissue homogenate

GROUPS	Catalase (µM/H_2O_2/min/mg protein)
GROUP I (CONTROL)	4.74 ± 0.23
GROUP II (NEGATIVE CONTROL)	1.97 ± 1.22****
GROUP III (POSITIVE CONTROL)	4.11 ± 1.36*
GROUP IV (250 mg/kg EEPV)	2.41 ± 1.36****
GROUP V (500 mg/kg EEPV)	3.64 ± 0.25***

*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).*

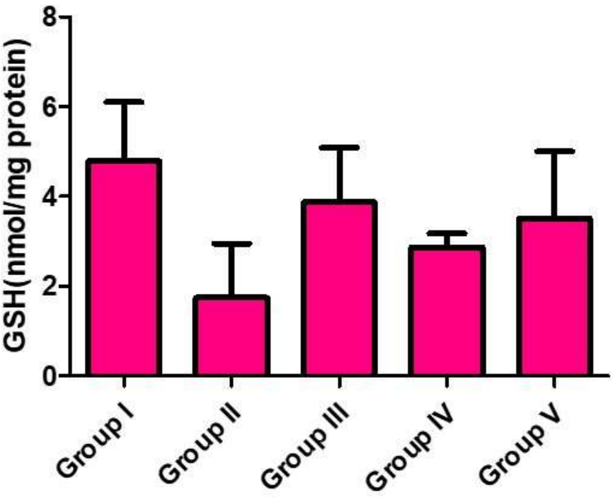

Fig 6: Effect of ethanolic root extract of *P.Vettiveroides* on Glutathione reductase of tissue homogenate

Table 7: Effect of ethanolic root extract of *P.Vettiveroides* on Malonaldehyde of tissue homogenate

GROUPS	MDA (nmol/mg protein)
GROUP I (CONTROL)	4.68 ± 1.32
GROUP II (NEGATIVE CONTROL)	10.33 ± 0.17****
GROUP III (POSITIVE CONTROL)	5.47 ± 0.21*
GROUP IV (250 mg/kg EEPV)	9.36 ± 0.54****
GROUP V (500 mg/kg EEPV)	6.54 ± 0.47****

*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

Because conventional treatments are frequently administered orally, rats were used to investigate the preventative efficacy of an ethanolic root extract of P. vitetiveroides against ethylene glycol-induced urolithiasis. The utilisation of these remedies as alternative or supplementary therapy for the management of urinary stone disease has evolved dramatically since the therapeutic properties of these herbal medicines were discovered. 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 extended intravenous administration of 0.75% (v/v) ethylene glycol led to hyperoxaluria, a condition that increased the excretion of phosphate, calcium, and magnesium. The P.Vettiveroides ethanolic root extract (EEPV - II) significantly (P < 0.001) decreased the elevated levels of calcium, magnesium, and phosphate. There were greater amounts of calcium, phosphate, and magnesium deposits in the renal tissues of the rats (Group II) that produced stones. Under both regimens (Groups IV and V), the Ethanolic root extract of P.Vettiveroides (EEPV - II) treatment significantly (P < 0.001) reduced the renal concentration of stone-forming components. 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 *Plectranthus vettiveroides* in Groups IV and V.

When compared to normal animals [group II], ethylene glycol administration significantly (P < 0.05) increased MDA levels and decreased SOD, CAT, and GSH levels in calculi-induced mice. When P.Vettiveroides' ethanolic root extract (250 and 500 mg/kg) was administered to groups II, III, and V, there was a significant (P < 0.0001) decrease in MDA and a significant increase (P < 0.001) in the amount of antioxidant enzymes such as SOD. P.Vettiveroides (ethanolic root extract, EEPV – II) significantly (P < 0.001) maintained the high level of CAT in both curative and preventive treatments. [23-25]

CONCLUSION

The results showed that the uric acid levels in the blood and urine of the ethylene glycol control group were substantially higher than those of the normal control group. Following treatment with EEPV-I & II and cystone, uric acid levels decreased, hastening the disintegration of existent stones and inhibiting the formation of new ones in the urinary system.The EEPV I & II treatment provided defence against changes brought on by oxidative stress. A multitude of studies have shown that the formation of crystals damages cells and separates them from the basement membrane, and that the breakdown chemicals released also promote the nucleation of new crystals. The administration of *P.Vettiveroides's* ethanolic root extract (EEPV-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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