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Research Article

PROTECTIVE EFFECT OF *HYPTIS SUAVEOLENS* AGAINST ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN RATS

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ABSTRACT

Urolithiasis is a common disease in which renal stones has been observed. Modern treatment of urolithiasis is either expensiv with side effects. Therefore, traditionally reported more effective and safer anti urolithiatic medicinal plants need to be stu Thus, this study was aimed to evaluate the anti urolithiatic effect of ethanolic extract from the seeds of *Hyptis suaveolens* induce urolithiasis, 0.75 %v/v ethylene glycol + 1%w/v ammonium chloride was administered orally to the rats for 21 days studied the impact of ethanolic extract of *Hyptis suaveolens* (EEHS) low dose (100 mg/kg; p.o) and high dose (200 mg/kg; Cystone (750 mg/kg; p.o) was taken as the standard. The urine samples were collected by metabolic cage and were anal under light microscopy for determination of calcium oxalate crystals. Besides, blood samples were drawn by cardiac pun under anesthesia and serum was centrifuged for biochemical analysis using standard diagnostic kits. Biochemical estimatic blood and urine sample reveals that treatment groups lower the urine calcium levels, serum creatinine, serum uric acid levels shows improvement in urinary pH, diuresis and body weight. Thus, ethanolic extract of *Hyptis suaveolens* seeds has proved a good traditional medicine to treat urolithiasis.

Keywords: *Hyptis suaveolens*, Urolithiasis, Ethanolic extract, Wistar rats, Quercetin.

INTRODUCTION

A condition referred to as urolithiasis, or kidney stones, is the formation of solid mineral deposits within the kidneys, ureters, bladder, and urethra primarily composed of calcium, uric acid, cystine, or struvite, the stones may be small crystals or large, obstructing masses (Wong *et al*, 2015). A common disorder that has been steadily rising in frequency around the globe, urolithiasis occurs in individuals of all ages, though it is more prevalent in men between the ages of 20 and 50 (Stamatelou *et al*, 2003). Urolithiasis possesses a complex and multivariate etiology that consists of metabolic, environmental, and genetic factors. From the accidental discovery of asymptomatic cases through to intense pain, the clinical presentation may differ (Yasui *et al*, 2017). The management of urolithiasis can include extracorporeal shock wave lithotripsy, dietary modification, medical therapy, or surgery, according to the size, location, and composition of the stone (Finlayson *et al*, 1989). Due to the potential for recurrent stone formation, extended treatment and prevention are key factors. This dissertation examines the pathogenesis, risk factors, and treatment options of urolithiasis (Thompson *et al*, 2020). The main mechanisms behind this process include supersaturation, where the concentration of substances like calcium and oxalate exceeds their solubility in urine, causing crystals to form. These crystals can grow and aggregate into larger stones over time. Factors like urine pH, the presence of substances that promote or inhibit crystal growth, and metabolic disorders (such as high calcium or oxalate levels in urine) can all influence stone formation (Pak *et al*, 2020).

Additionally, nucleation the process where crystals begin to form can either be homogeneous (from a single substance) or heterogeneous (from multiple substances), further contributing to stone development (Bishop *et al*, 2019). Urolithiasis, or the formation of stones in the urinary system, can be classified based on the location, chemical composition, and etiology of the stones. The primary classifications include nephrolithiasis (renal),

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ureterolithiasis (ureteral), and cystolithiasis (bladder) (Srinivas*et al*, 2012). Risk factors are genetic predisposition, dietary habits, obesity, dehydration, and some medical conditions such as gout and hyperparathyroidism (Trinchieri *et al*, 2008). The chemical composition of the stone's further divides urolithiasis into calcium oxalate, calcium phosphate, uric acid, struvite (magnesium ammonium phosphate), and cystine stones, each being associated with certain metabolic disorders or infections. Calcium-containing stones are most prevalent and are usually associated with hypercalciuria or hyperoxaluria, while uric acid stones are most commonly associated with low urine pH and hyperuricemia. Struvite stones most commonly form as a result of urinary tract infections by bacteria that are urease producer (Vijaya *et al*, 2013). Acknowledging these classifications is essential for effective prevention and treatment strategies in the medical context.

Different allopathic drugs are commonly employed in the management of urolithiasis, including potassium citrate, thiazide diuretics (e.g., hydrochlorothiazide), and allopurinol (Arumuham *et al*, 2019). While these drugs help to dissolve or inhibit the formation of kidney stones by altering urinary pH, reducing calcium excretion, or reducing uric acid levels, they are usually accompanied by side effects like gastrointestinal disturbances, hypotension, electrolyte disturbances, and hypersensitivity reactions (Spernat *et al*, 2011). Its limitations have created growing interest in plant-derived alternatives. The main problem with surgery for kidney stones is that the stones often come back, but plant-based products and their main compounds might help lower the chances of this happening again (Yadav *et al*, 2011). *Hyptis suaveolens* (L.) Poit., commonly known as the "bush mint," is a fast-growing, herbaceous plant in the Lamiaceae family, native to tropical and subtropical regions of the Americas but now widely distributed in parts of Africa, Asia, and Australia (Singh, 2017). This species is noted for its aromatic leaves and distinctive purple flowers. It has been traditionally used for various medicinal purposes, including as an antimicrobial, anti-inflammatory, and antispasmodic agent (Bohlmann *et al*, 2003). In addition to its medicinal applications, *H. suaveolens* is considered an invasive weed in many regions due to its rapid growth and ability to outcompete native vegetation, which presents challenges for agricultural systems (Mukherjee *et al*, 2016). The plant's chemical composition includes a variety of bioactive compounds such as essential oils, flavonoids, and terpenes, which contribute to its therapeutic properties (Ncube *et al*, 2013). Studies have highlighted the plant's potential in controlling various pests, making it a candidate for sustainable agricultural practices (Rahman *et al*, 2018). Despite its promising applications, the environmental impact and management of its invasive nature require further investigation to balance its uses and ecological concerns (Nerium *et al*, 2020). The present studies show evaluation of natural compound *Hyptis suaveolens* seed extract, which is assessed for its antiurolithiatic properties. Its phytochemical constituents, including flavonoids and terpenoids, could offer a safer and perhaps superior method of urolithiasis treatment to conventional synthetic medication (Mishra *et al*, 2021).

MATERIALS AND METHODS

Plant material

Fresh seeds (250 gm) of *Hyptis suaveolens* (Lamiaceae) seeds have been purchased from local market in Guntur in the state of Andhra Pradesh. The plant material was taxonomically identified and authenticated by Dr. P. Satyanarayana Raju garu M.Sc., M.Phil., Ph.D from Department of Botany and Microbiology, Acharya Nagarjuna University, Nagurjuna Nagar, AP. The freshly collected seeds were broken into small pieces and dried under shade. The completely dried stuff was ground to coarse powder using mechanical grinder and subsequently passed through 40 mesh sieves in order to obtain uniform powder and stored in an air tight container (Smith *et al*, 2020).

Preparation of the extract

The ethanolic extract of *Hyptis suaveolens* seeds was prepared by using Maceration, at room temperature. powdered dried seeds of *Hyptis suaveolens*(150g) were extracted with ethanol (EtOH) for 7 days with occasional stirring. The extract was filtered using muslin cloth, pour into a China dish and evaporate. The resultant extract was used for further studies (Islam *et al*,2010).

Chemicals and reagents

Cystone was obtained from Himalaya wellness company. Ethylene glycol, ammonium chloride and other chemicals and reagents are procured from chalapathi drug testing laboratory, lam, Guntur, Andhra Pradesh.

Preliminary phytochemical screening

The prepared extract was analysed for the presence of phytochemicals The phytochemical screening shows the presence and absence of phytoconstituents present in EEHS viz carbohydrates (Molisch's test), proteins (Millon's test), alkaloids (Mayer's test), cardiac glycosides (Legal's test), flavonoids (Alkaline reagent test), tannins (Lead acetate test), steroids (Salkowski test)(Khandelwal, K. R., 2008).

Experimental Animals

Healthy adult Albino wistar rats of either sex weighing 150-200g were used to evaluate anti-urolithiatic activity in-vivo. The animals are given free access to food and water and are kept in a natural 12-hour light and dark cycle. Rats were housed in the animal house seven days prior to the commencement of the experiment to acclimatize to the environment (Deacon, R. M., 2006) The experimental

protocol was approved by the institutional animal ethics committee through certified project proposal number 10/IAEC/CLPT/2024-25. Laboratory animals were maintained and cared for according to guidelines of the Committee for control and supervision of experiments on animals (CCSEA).

Acute toxicity

Oral administration of ethanolic extract of *Hyptis suaveolens* upto 2000mg/kg did not produce any toxic effects. No mortality was observed and it was found to be safe at given doses. Dose was selected according to OECD 423 guidelines (Narumalla *et al*, 2023).

Experimental induction of urolithiasis in rats

Rats received ethylene glycol (0.75% v/v) and ammonium chloride (1% w/v) in the drinking water during the initial three days, with subsequent administration of ethylene glycol (0.75% v/v) alone for eighteen days to develop urolithiasis.

Study protocol

Thirty rats were divided into five groups (n = 6). Group-I was represented as Control and administered only saline. Group II was represented as a disease control group and given ethylene glycol (0.75% v/v). Group-III was represented as the standard and administered Cystone (750 mg/kg) orally for three weeks. Groups IV-V were administered extract of EEHS at different doses of 100 and 200 mg/kg, orally, for 21 days. Urine samples were collected on the 22nd day for the study biochemical analysis, such as oxalate, phosphate, magnesium, sodium, and calcium, which were analyzed using standard diagnostic kits.

Figure 1. Extraction procedure of *Hyptis suaveolens*seeds.

Urine collection and urine parameters measurement

On the 22nd day of the experiment, urine samples were obtained for biochemical analysis to estimate parameters like oxalate, phosphate, magnesium, sodium, and calcium, which were determined by using standard diagnostic kits (Saleem *et al*, 2020) and analysed through light microscopy (Smith *et al*, 2021).

Determination of the serum parameters

Blood was collected through cardiac puncture under anesthesia on day 22 of the study, and serum was isolated for biochemical measurement (serum creatinine, serum uric acid, blood urea nitrogen, serum protein, and serum albumin) with standard diagnostic kits (Saleem *et al*, 2020).

RESULTS AND DISCUSSION

Preliminary phytochemical screening shows the presence of carbohydrates, proteins, flavonoids, alkaloids, cardiac glycosides, tannins, steroids by using standard methods. EEHS administered orally at a dose of 200mg/kg for 21 days significantly lowered the levels of serum creatinine and uric acid, with effects similar to those of the standard drug cystone (750 mg/kg). As compared to this, the disease control group presented with increased levels of these markers, reflecting extreme kidney damage induced by ethylene glycol (EG) administration. Therapy with EEHS and cystone significantly reduced these increased levels, indicating renoprotective activity. The decrease in uric acid is due to the flavonoids present in the plant extract, as evidenced by the work.

Table 1. Preliminary Phytochemical Screening of Ethanolic extract of *Hyptis suaveolens.*

Chemical constituent	Presence or Absence
Carbohydrates	+
Proteins	+
Alkaloids	+
Anthraquinone glycosides	-
Cardiac glycosides	+
Saponin glycosides	-
Amino acids	+
Flavonoids	+
Tannins & polyphenolic compounds	+
Steroids	+

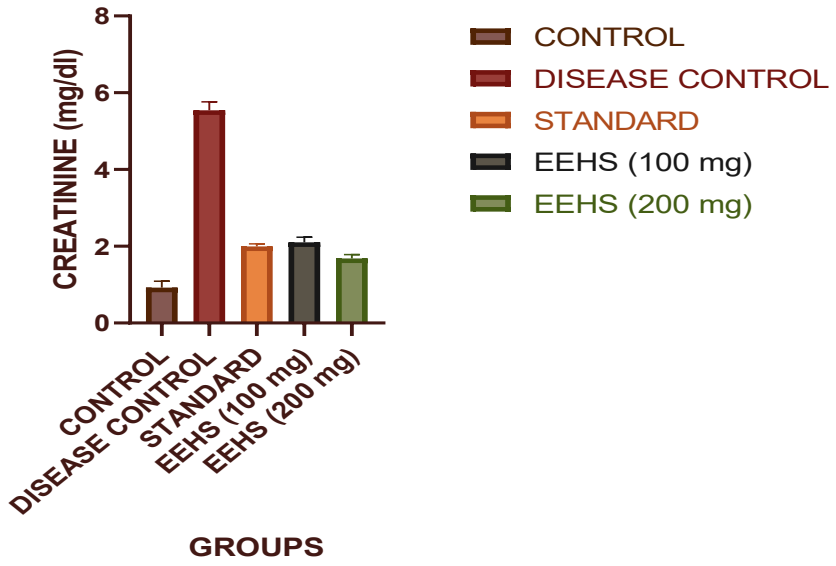

Figure 2. Effect of EEHS on serum creatinine levels.

All values expressed as mean ± SEM; n=6 rats in each group, by one-way ANOVA, Tukey's Multiple Comparison Test; ####: P≤0.0001 (EEHS 200 mg corelated with disease control group), and **: P= 0.0098 (EEHS 200 mg corelated with standard group). The disease control group had a dramatic rise in the level of serum uric acid when compared with the normal group, reflecting great kidney damage in EG-induced rats. Nevertheless, treatment with EEHS and cystone significantly brought down the high levels of uric acid and creatinine as compared to the disease control group, as evident from the table. The fall in the levels of uric acid is credited to the flavonoids present in the plant extract (Doe *et al*, 2021).

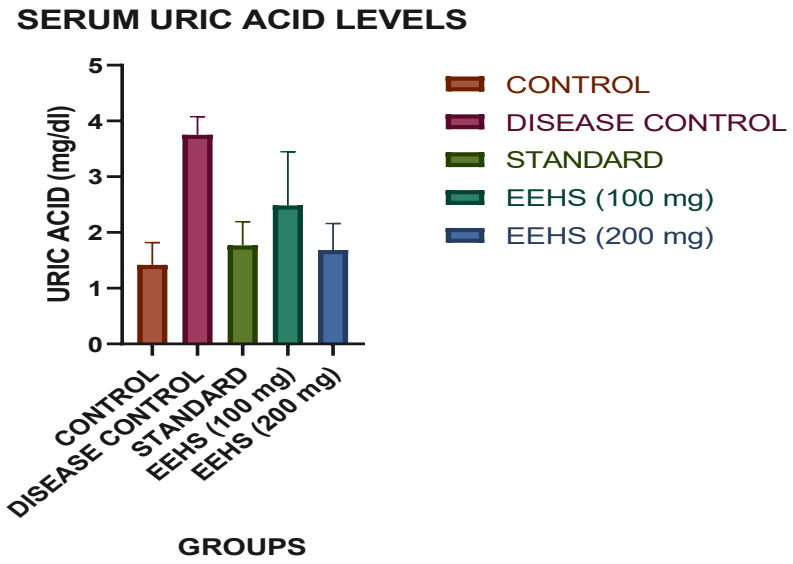

Figure 3. Effect of EEHS on serum uric acid levels.

All values expressed as mean ± SEM; n=6 rats in each group, by one-way ANOVA, Tukey's Multiple Comparison Test; ****: P=0.0006 (EEHS 200 mg corelated with standard group). Decreased or increased levels of calcium are observed in renal failure, the degree of calcium excreted in urine represents intestinal absorption, skeletal resorption and renal tubular filtration and reabsorption (Johnson *et al*, 2019). EEHS 200mg per kg oral administration for 21 days had revealed significant effect on calcium levels equivalent to standard cystone (750mg per kg). Calcium levels were significantly increased in disease control group as compared to normal group. Elevated calcium levels is a factor favoring the nucleation and precipitation which caused the crystal growth (Williams *et al*, 2020). But EEHS and cystone treated groups decrease the level of calcium, thereby decreased the risk of stone formation .

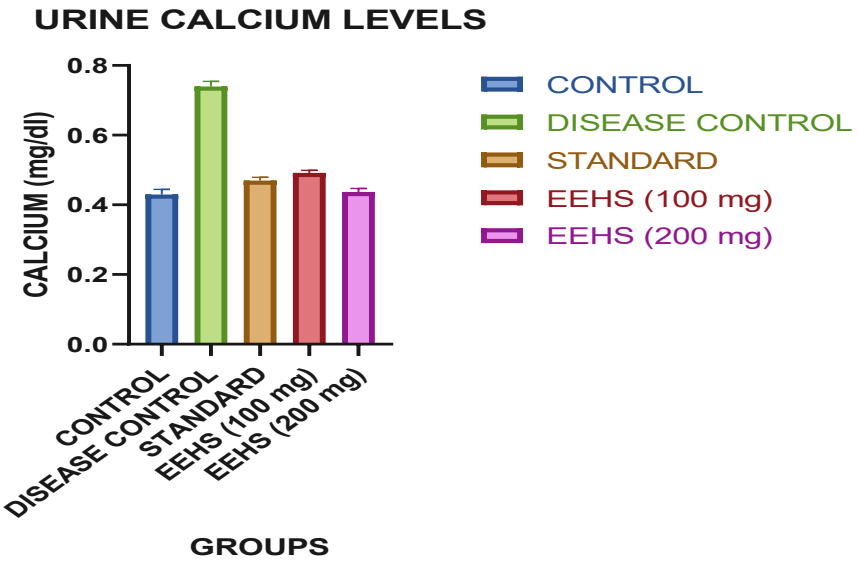

Figure 4. Effect of EEHS on urine calcium levels.

All values expressed as mean ± SEM; n=6 rats in each group, by one-way ANOVA, Tukey's Multiple Comparison Test; ####: P≤0.0001 (corelated with disease control group), and ***: P = 0.0003 (corelated with standard group) Body weight was drastically decreased in the disease control group when compared to the normal group, reflecting extensive renal damage induced by ethylene glycol in rats. Treatment with EEHS and Cystone, however, significantly restored body weight when compared to the disease control group.

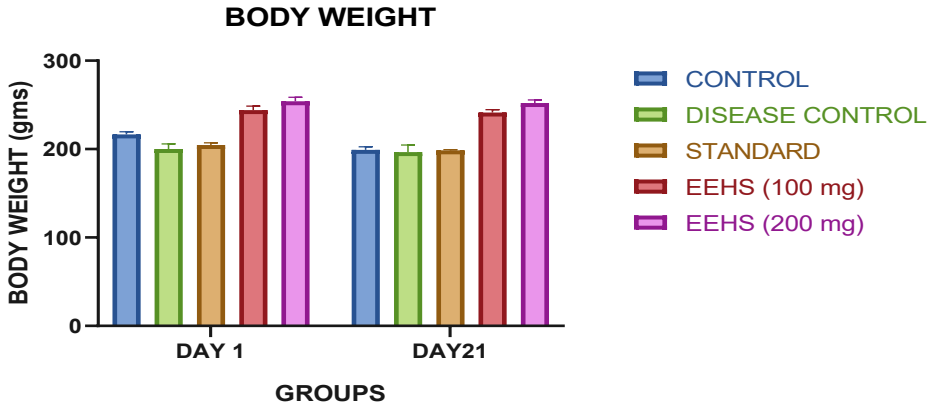

Figure 5. Effect of EEHS on Body weight.

All values expressed as mean ± SEM; n=6 rats in each group, by one-way ANOVA, Tukey's Multiple Comparison Test; **: P = 0.0013 (corelated with disease control group), and **: P = 0.0014 (corelated with standard group) The urinary pH was increased in the disease control group versus the normal control group. Oral administration of EEHS at a dose of 200 mg/kg for 21 days markedly affected urinary pH and showed an activity comparable to that of the reference drug cystone at 750 mg/kg.

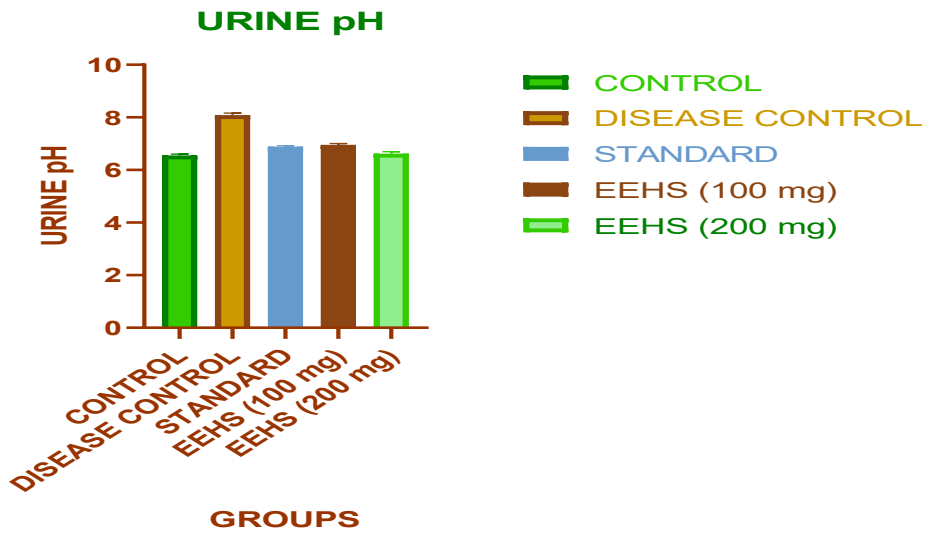

Figure 6. Effect of EEHS on urine pH.

All values expressed as mean ± SEM; n=6 rats in each group, by one-way ANOVA, Tukey's Multiple Comparison Test; ####: P≤0.0001 (corelated with disease control group), and ****: P≤0.001**** (corelated with standard group) Volume of urine was considerably higher in both the EEHS- and cystone-treated groups when compared to the disease control group. Though cystone showed stronger efficacy, oral EEHS at 200 mg/kg for 21 days also had a significant increase in urine output, with results similar to that of the cystone-treated group (750 mg/kg).

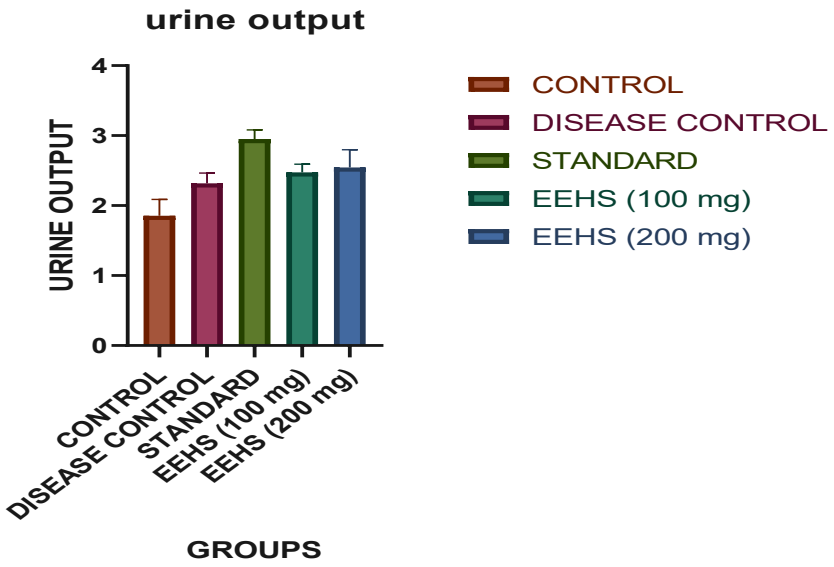

Figure 7. Effect of EEHS on urine output.

All values expressed as mean ± SEM; n=6 rats in each group, by one-way ANOVA, Tukey's Multiple Comparison Test; **: P=0.0062 (EEHS 200 mg corelated with standard group).

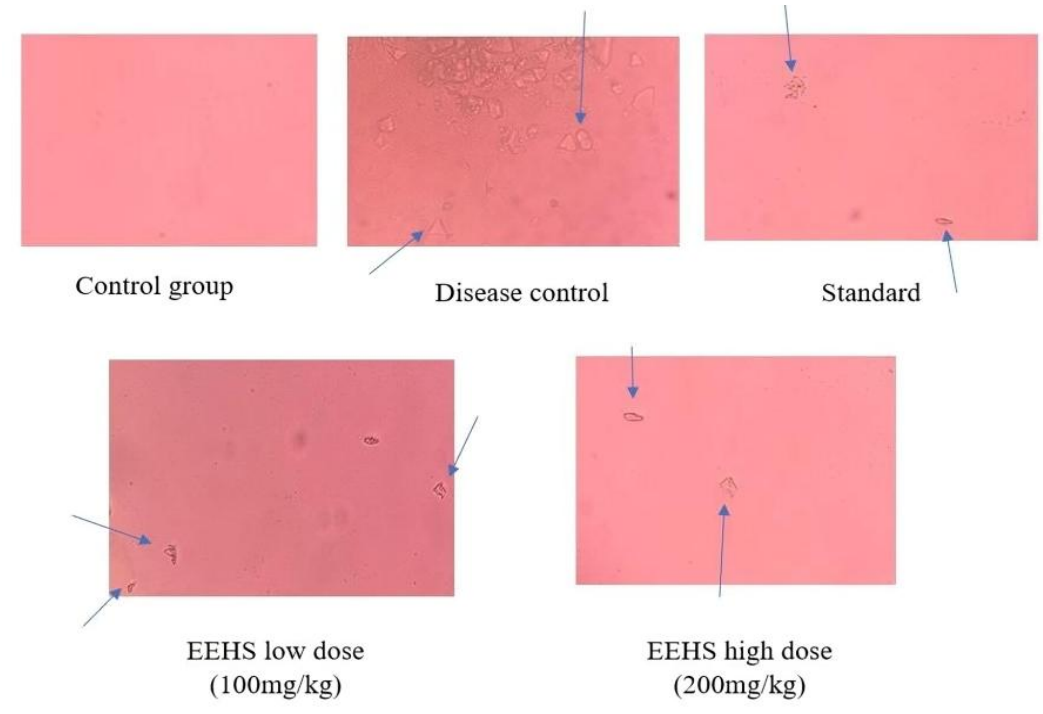

Figure 8. Microscopical examination of urine.

Microscopic examination of urine from normal control animals revealed no presence of renal calculi. In contrast, the disease control group exhibited large, rectangular crystals indicative of stone formation. Animals treated with cystone showed a near-complete dissolution of stones. Treatment with EEHS at 200 mg/kg resulted in a reduced number of urinary crystals than EEHS 100mg/kg, which is similar to standard cystone, indicating a more effective preventive action against stone formation. The present study illustrates the fact that ethanolic extract of *Hyptis suaveolens* seed (EEHS) possesses strong anti-urolithiatic activity in ethylene glycol-induced urolithiasis in rats. Administration of EEHS, especially at a dose of 200 mg/kg, resulted in considerable decreases in serum creatinine, uric acid, and urinary calcium levels, which reflect renal protection and prevention of stone formation. The extract also normalized urinary pH, enhanced urine output, and promoted body weight, indicating systemic recovery. The effects noted are probably attributed to the bioactive components including flavonoids and tannins that have antioxidant and anti-inflammatory activities. Microscopic examination also verified decreased crystal formation in treated groups, further indicating the efficacy of the extract. In general, EEHS had similar results to the reference drug Cystone, indicating its potential as a safe, herbal alternative for the treatment of urolithiasis. Isolation of active constituents and further studies are recommended to elucidate the mechanisms involved.

CONCLUSION

The research determined that EEHS enhanced kidney function by reducing levels of creatinine, uric acid, and calcium to normal. This is possibly because growth of urinary stones induced by ethylene glycol was reduced, microscopical examination of urine samples affirmed this. The dose of EEHS200mg/kg body weight was effective in normalizing creatinine, uricacid, pH, and calcium levels. The EEHS can influence the level of stone-forming elements and urolithiasis- promoting substances in urine, which makes it a potential source of ancient urolithiasis therapy. Further research of the extract may reveal the underlying mechanisms. The study confirms the classic use of *Hyptis suaveolens* to treat urinary aliaments and calls for further pharmacological and phytochemical research. Future studies should be directed towards isolating the active principles responsible for the anti-urolithiatic activity and exploring their mechanism of action to establish its clinical relevance. In conclusion, *Hyptis suaveolens*seed extract demonstrates promising anti-urolithiasis activity and can potentially act as a natural remedy for prevention and treatment of kidney stones.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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