Journal of Amran University 04 (2024) p. 279-290

مجلة جامعة عمران

journal homepage: www.auy.edu.ye

Anti-urolithiatic Effect of Psiadia Punctulata Leaves Ethanolic Extracts Against Sodium Oxalate-Induced Urolithiasis in Rats

Mohammed Sadeg A. Al-Awar
Department of Biology, Faculty of Applied Science, Amran University, Yemen

Received 19 May 2024; accepted in final form 6 July 2024.

Abstract

Kidney stones are rigid mineral and acidic salt deposits that accumulate in concentrated urine, leading to urolithiasis, the third most common urinary tract disorder. Current treatments for urolithiasis have limitations and frequent recurrences. This study aimed to investigate the anti-urolithiatic effects of ethanolic extracts from Psiadia Punctulata leaves on sodium oxalate-induced urolithiasis in rats. Rats were administered orally Psiadia Punctulata leaves ethanolic extract (PPEE) at 200 and 400 mg/kg doses for 14 days to assess its effectiveness against urolithiasis induced by sodium oxalate. Cystone (500 mg/kg) was used as a positive control. The effects of PPEE on body and kidney weights, urinary output and pH levels, urine and serum parameters, renal oxidative and antioxidative markers, and histopathological evaluations were examined. Oral administration of PPEE at 200 and 400 mg/kg doses showed a significant dose-dependent ($P<0.01$) antiurolithiatic effect, reversing sodium oxalate-induced ion excretion and urinary calcium oxalate concentration. These findings support the traditional use of PPEE for renal calculi management, possibly due to its antioxidant properties identified through phytochemical screening. However, further research is needed to understand the exact mechanism of action.

Keywords: *Psiadia punctulata*, Sodium oxalate, cysteine, Urolithiasis

الملخص: حصوات الكلى عبارة عن رواسب ملحية معدنية وحمضية صلبة تتراكم في البول المركز، مما يؤدي إلى تحص بولي، وهو ثالث أكثر اضطرابات المسالك البولية شيوعًا. العلاجات الحالية لتحصي البول لها قيود وتكرار متكرر. تهدف هذه الدراسة إلى دراسة التأثيرات المضادة لتحصي البول للمستخلصات الإيثانولية لأوراق نبات الفتح Psiadia Punchulata على التحصي البولي المستحث بأكسالات الصوديوم في الجرذان. تم إعطاء الفئران عن طريق الفم PPEE بجرعات 200 و400 ملغم / كغم لمدة 14 يومًا لتقييم فعاليتها ضد التحصي البولي المستحث بأكسالات الصوديوم. تم استخدام السيستون (500 مجم/كجم) كعنصر تحكم إيجابي. تم فحص آثار PPEE على أوزان الجسم والكلى، وإنتاج البول ومستويات الرقم الهيدروجيني، ومؤشرات البول والمصل، وعلامات الأكسدة ومضادات الأكسدة الكلوية، والتقييمات النسيجية المرضية. أظهرت النتائج بأن PPEE كان له تأثيرًا كبيرًا مضادًا للتحصي يعتمد على الجرعة ($P <0.01$)، مما يعكس إفراز الأيونات الناجم عن أكسالات الصوديوم وتركيز أكسالات الكالسيوم في البول. هذه النتائج تكشف لأول مرة عن تبرير الاستخدام التقليدي لـ PPEE في علاج حصوات الكلى. قد يكون هذا بسبب قوته المضادة للأكسدة التي تنشأ من مكوناته الكيميائية النباتية والتي كشف الفحص الكيميائي النباتي عن وجودها في PPEE في هذه الدراسة. ومع ذلك، هناك حاجة إلى مزيد من البحث لتحديد الآلية الدقيقة لهذا السلوك.

Introduction

Urolithiasis, a prevalent urological condition, impacts 5%–15% of the global population [1]. This disease not only jeopardizes individuals' well-being and causes both physical and psychological suffering to patients but also presents a substantial challenge to the healthcare system [2]. The presence of severe urolithiasis can potentially result in the development of chronic kidney disease and, in severe instances, end-stage renal failure (uremia). The recurrent nature of urolithiasis is of

Corresponding Author: Emails: momed.sadeg@gmail.com or momed.sadeg@amu.edu.ye

particular concern, with recurrence rates climbing to as high as 50% within a 5-year timeframe [3, 4]. Each recurrence instance signifies a bleaker prognosis and amplifies the likelihood of future stone formation [3, 5].

Numerous ethnic remedies have demonstrated efficacy in treating urolithiasis [6, 7]. Herbal therapy, as an alternative to current surgical and pharmaceutical interventions, is considered a viable choice that may enhance patient outcomes and exhibit promise in preventing stone reoccurrence with a reduced incidence of adverse effects [8]. The World Health Organization reports that 80% of individuals seek traditional medicine due to its notable clinical effectiveness and cost-effectiveness [9].

Several recent studies have indicated that phytochemical constituents rich in saponins, alkaloids, flavonoids, steroids, triterpenoids, phenolic compounds, tannins, and flavonoids have the potential to be advantageous in the prevention of urolithiasis formation [10–13]. Traditional herbal and natural remedies have been utilized for generations across various global societies. Many medicinal plants containing antispasmodic, diuretic, and antioxidant properties can impede crystallization, nucleation, and aggregation, thus effectively managing urolithiasis [14].

The plant Psiadia Jacq. belongs to the Asteraceae family and is distributed across various African countries and Yemen [15]. Within the Psiadia genus are several species [16], three of which are present in Yemen: Psiadia incanao, Psaidia punctulata, and Psiadia schweinfurthii [17]. Analysis of the leaf exudate of P. punctulata revealed the presence of alkaloids, saponins, polyphenols, flavonoids, tannins, steroids, carbohydrates, bitters, sterols, mucilage, and gum [18,19]. Many investigations have documented P. punctulata's many biological activities. It has been demonstrated, for example, to exhibit cytotoxic activity against many cancer cell lines, such as those from the cervix, bladder, hepatic, and breast cancers.

Antifungal, anti-malarial, anti-Leishmanial, and antioxidant properties were also noted [20,21]. The plant is conventionally utilized for various medicinal purposes in the Arab Peninsula. Indigenous people employ it for the treatment of fractured bones. Furthermore, the extracts derived from the leaves and stems are utilized to alleviate pain and accelerate the healing process for foot injuries among villagers who frequently traverse barefoot. Within East Africa, particularly in Kenya, the decoction of the leaves provides numerous advantages, such as managing common colds and fevers and safeguarding cattle from ectoparasites [15]. Additionally, the plant is recognized for its analgesic properties, especially in addressing abdominal pain [22]. In Yemen, the species have been identified in Hajja and are predominantly utilized for their anti-urolithiatic properties.

No data related to the anti-urolithiasis activity of psiadia punctulata were available in the literature. Therefore, the present study was the first to investigate the anti-urolithiasis activity of psiadia punctulata ethanolic extracts in sodium oxalate-induced urolithiasis in rats' models.

Materials and Methods

Plant collection and extract preparation

P. punctulata leaves were collected from Bani Al-Harith district in Afalh AlSham, Hajjah, Yemen, from May to June 2023 and identified by Hassan M.H. Ibrahim, Assistant Professor of Plant Taxonomy, University of Sanaa. The leaves were cleaned, cut, and weighed, then dried in ethanol for three days at room temperature [23]. The filtered extracts were evaporated using a rotating evaporator below 45°C. The resulting extracts were stored in closed containers at room temperature.

Chemicals

sodium oxalate (HiMedia, India) and Cystone (Himalaya Herbal Healthcare, India) were utilized in the study, alongside other high-purity commercially available chemicals and reagents.

Acute Oral toxicity study (LD_{50})

An acute toxicity test was conducted on male rats using OECD guidelines 423 to determine the LD50 of the P. punctulata extract. The purpose of determining the LD50 of the extract was to ensure in-vivo safety and establish the therapeutic index of a specific drug.

Phytochemical analysis

The preliminary plant-chemical examination of the extracts of P. punctulata leaves was carried out using the methods described [24]. The active components of carbohydrates, alkalis, flavonoids, fixed oils/fats, glycosides, polyphenols, tannins, sterols, peptides/proteins, and saponins are detected.

Animals

Male rats aged 4-5 months (250-300 grams) were obtained from the Sanaa-Jemen Zoo and housed in stainless steel cages at Al-Razi University's animal house. They received a standard diet and water ad libitum in controlled environmental conditions. All procedures followed the NIH Laboratory Care and Use Guide (1978).

NaOx-induced urolithiasis in rats

A 70 mg/kg intraperitoneal dose of NaOx causes lithiasis in 7 days [25]. NaOx, dissolved in physiological saline, is administered alone for lithiasis induction.

Experimental design

The rats were assigned into six groups, with six in each group. The same treatment was repeated for up to 14 days [25].

Group I: The control group received 5 ml/kg of the vehicle only
Group II: The lithiatic group received NaOx 70 mg/kg, i.p.
Group III: Received NaOx 70 mg/kg, i.p., and PPEE 200 mg/kg, p.o.
Group IV: Received NaOx 70 mg/kg, i.p., and PPEE 400 mg/kg, p.o.
Group V: Positive control, received Cystone (500 mg/kg, p.o.) and NaOx 70 mg/kg, i.p.

Urine analysis

Animals were placed in metabolic cages on day fourteen, and urine samples were collected the next day. Urine pH, volume, and biochemical parameters (calcium, uric acid, creatinine, phosphorous, sodium, potassium, and magnesium) were measured using a clinical semi-autoanalyzer with thymol as a preservative, following the instructions provided with the diagnostic kit.

Serum analysis

Blood samples were collected via capillary puncture from the eye for serum analysis. The samples were centrifuged at 3500 rpm for 20 minutes. The serum was separated to analyze sodium, potassium, uric acid, and creatinine levels using diagnostic kits from Stanbio (San Antonio, USA) following the manufacturer's instructions.

Kidney homogenate study

Rats were euthanized to conduct a study on kidney homogenate. The process involved the extraction of kidneys from the rats for subsequent analysis. Utilizing a 10 percent Tris–HCl buffer (0.1M, pH 7.4), the left kidney was minced and homogenized. Subsequently, the resulting homogenate underwent 20-minute centrifugation at 22,000×g at 4°C to isolate the post-mitochondrial supernatant and remove nuclear debris. The renal supernatant was then subjected to lipid peroxidation (LPO) analysis as well as assessments of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione reduced (GSH) as per standard protocols.

Kidney histopathological study

Each animal's right kidney was fixed in 10% neutral buffered formalin for further histological examination to confirm the presence of lithiasis. Histological sections of the kidney were stained with

hematoxylin-eosin and examined under a binocular microscope to identify calcium oxalate crystals, renal cellular and tubular necrosis, and any histopathological abnormalities in the kidney structure.

Statistical Analysis

Data for each group (n = = 6) were reported as mean±SEM. Statistical analysis was performed using ANOVA followed by Duncan's test in SPSS software (version 15.0, SPSS Inc., Chicago, Illinois, USA). Group differences were considered significant at $P<0.01$.

Results

Acute toxicity test

The study's findings indicate that PPEE is non-toxic, based on acute toxicity tests conducted on rats. Even when administered at high doses of up to 5000 mg/kg, rats showed no toxicity or adverse effects. No physical, neurological, or psychological abnormalities were observed throughout the initial fortnight after oral extract intake. No noticeable alterations in the animals' external appearance, skin or fur condition, salivation, bowel movements, or sleep patterns indicated their physical well-being. Additionally, there were no instances of comatose states, neurological irregularities, changes in behavior, or fatalities recorded. These results highlight the safety profile of the substance Punctata in biological settings, with an LD50 exceeding 5000 mg/kg in rats for the methanolic extract, as illustrated in Table 1.

Table 1. LD_{50} of *P. punctulate* ethanolic extract

Dose	No. of rats	No. of Death of rats
100 mg/dl	4	0
1000 mg/dl	4	0
2500 mg/dl	4	0
4000 mg/dl	4	0
5000 mg/dl	4	0

LD_{50} should should be higher than 5000 mg/kg b.w.

Phytochemical screening

Table 2 presents the outcomes of the initial phytochemical screening conducted on ethanolic extracts of P. punctulata. The analysis revealed that despite fixed oils, fats, and proteins yielding negative results, carbohydrates, gum and mucilage, saponins, alkaloids, flavonoids, steroids, triterpenoids, phenolic compounds, and tannins exhibited positive reactions.

Table 2. Result of Phytochemical analysis of *P.* ParticParticulate EEthylatedylated extract

Test	Positive or Negative
Carbohydrate	+
Gum and Mucilage	+
Alkaloids	+
Steroids	+
Triterpenoids	+
Saponins	+
Proteins	-
Fixed oils/fats	-
Tannins	+
Phenolic compounds	+
Flavonoids	+

Impact of the PPEE on body weight and kidney weights

Rats in the regular, extract, and standard treatment groups showed significant increases ($p < 0.01$) in body and kidney weights compared to the Lithiatic group. Kidney weights in the disease control group significantly decreased ($p < 0.01$) compared to the standard control (Table 3).

Table 3. Impact of the PPEE on body weight and kidney weights

Parameters	Groups				
	Normal	Lithiatic	Lithiatic+PPEE (200 mg/kg b.w.)	Lithiatic+PPEE (400 mg/kg b.w.)	Lithiatic+Cystone
Body weight (g)	270 ± 0.13^{a}	319 ± 0.87^{b}	280 ± 0.43^{c}	289.36 ± 0.37^{d}	$272.58\pm0.87^{a,d}$
Kidney weight (g)	13.07 ± 0.04^{a}	16.06 ± 0.05^{b}	11.32 ± 0.29^{c}	12.31 ± 0.19^{d}	$12.41\pm0.17^{a,d}$

Means±S.E.M. values (n = 6 per group) were compared using ANOVA with Duncan's test. Significant differences ($P<0.01$) were observed between values marked with different superscript letters within the same treatment regimen. PPEE stands for Psiadia punctulata ethanolic extract.

Impact of PPEE on the urinary output and pH levels in lithiatic rats.

In comparison between NaOx-treated control and normal rats, a notable ($p < 0.01$) reduction in urine volume was observed, with no significant alterations in pH levels. Rats that received cysteine exhibited significantly ($p < 0.01$) higher urine volumes than the lithiasis control group—upon evaluation against control rats treated with NaOx, rats administered with PPEE at 200 and 400 mg/kg body weight doses displayed a noteworthy ($p < 0.01$) escalation in urine volume, while pH levels remained unaffected (Table 4).

Table 4. *Impact of PPEE on the urinary output and pH levels in lithiatic rats.*

Parameters	Groups				
	Normal	Lithiatic	Lithiatic+PPEE (200 mg/kg b.w.)	Lithiatic+PPEE (400 mg/kg b.w.)	Lithiatic+Cystone
Urine output ml/24hrs	10.54 ± 0.58^{a}	6.37 ± 0.65^{b}	13.08 ± 0.76^{c}	$13.75\pm0.77^{*c,d}$	$10.68\pm0.82^{a,e}$
Urine pH	6.57 ± 0.04^{a}	7.79 ± 0.06^{b}	$7.06\pm0.05^{a,c}$	$6.89\pm0/04^{a,c}$	$6.74\pm0.04^{a,c}$

Means±S.E.M. values (n = 6 per group) were compared using ANOVA with Duncan's test. Significant differences ($P<0.01$) were observed between values marked with different superscript letters within the same treatment regimen. PPEE stands for Psiadia punctulata ethanolic extract.

Impact of the PPEE on the Urine Parameters

Comparing NaOx-treated control rats to normal rats revealed a significant ($p < 0.01$) decrease in urine volume but no significant changes in pH. Rats treated with cystone had significantly ($p < 0.05$) higher urine volumes than the lithiatic control group. Compared to control rats treated with NaOx, rats treated with PPEE at dosages of 200 and 400 mg/kg body weight showed a significant ($p < 0.05$ to 0.01) increase in urine volume but no significant change in pH (Table 5).

Table 5. *Impact* of the PPEE on the Urine Parameters

Parameters	Groups				
	Normal	Lithiatic	Lithiatic+PPEE (200 mg/kg b.w.)	Lithiatic+PPEE (400 mg/kg b.w.)	Lithiatic+Cystone
Oxalate (mg/dl)	0.74 ± 0.03^{a}	1.84 ± 0.03^{b}	$0.84\pm0.04^{a,c}$	$0.72\pm0.01^{a,c}$	$0.75\pm0.01^{a,c}$
Creatinine (mg/dL)	1.57 ± 0.04^{a}	3.60 ± 0.15^{b}	$1.95 \pm 0.03^{a,c}$	$1.80\pm0.04^{a,c}$	$1.67 \pm 0.07^{a,c}$
Uric acid (mg/dL)	0.17 ± 0.006^{a}	2.23 ± 0.002^{b}	1.86 ± 0.001^{c}	$1.65\pm0.001^{c,d}$	$1.64\pm0.002^{c,d}$
Phosphorus (mg/L)	44.08 ± 0.08^{a}	51.10 ± 0.06^{b}	40.07 ± 0.07^{c}	$41.07\pm^{c,d}$	$42.08\pm0.05^{d,e}$
Urea (mg/dL)	1.06 ± 0.02^{a}	8.05 ± 0.02^{b}	3.07 ± 0.02^{c}	2.17 ± 0.03^{d}	1.10 ± 0.02^{d}
Magnesium (mg/dL)	2.06 ± 0.03^{a}	1.05 ± 0.05^{b}	2.04 ± 0.07^{c}	$2.85\pm0.08^{a,d}$	$2.03\pm0.03^{c,e}$

Means±S.E.M. values (n = 6 per group) were compared using ANOVA with Duncan's test. Significant differences ($P<0.01$) were observed between values marked with different superscript letters within the same treatment regimen. PPEE stands for Psiadia punctulata ethanolic extract.

Impact of the PPEE on the Serum Parameters

The renal function was compromised due to the induction of kidney stones by NaOx treatment, leading to elevated levels of urea, creatinine, uric acid, and magnesium in the serum, which are indicators of damage to the glomerular and tubular areas. Nonetheless, when comparing the control group with the NaOx-treated group, it was observed that the continuous administration of PPEE at doses of 200 and 400 mg/kg body weight and Cystone at 500 mg/kg for 14 days effectively prevented these changes (Table 6).

Table 6. *Impact* of the PPEE on the Serum Parameters

Parameters	Groups				
	Normal	Lithiatic	Lithiatic+PPEE (200 mg/kg b.w.)	Lithiatic+PPEE (400 mg/kg b.w.)	Lithiatic+Cystone
Creatinine (mg/dL)	0.52 ± 0.01^{a}	1.41 ± 0.01^{b}	$0.60\pm0.01^{a,c}$	$0.60\pm0.01^{a,c}$	$0.54\pm 0.01^{a,c}$
Phosphorus (mg/dL))	3.08 ± 0.32^{a}	5.00 ± 0.57^{b}	3.67 ± 0.36^{c}	3.03 ± 0.30^{d}	$3.0\pm0.42^{d,e}$
Magnesium (mg/dL)	1.23 ± 0.01^{a}	0.48 ± 0.01^{b}	0.84 ± 0.01^{c}	1.05 ± 0.01^{cd}	1.19 ± 0.01^{cd}
Uric acid (mg/dL)	2.49 ± 0.01^{a}	4.70 ± 0.01^{b}	$2.64 \pm 0.01^{a,c}$	$2.61 \pm 0.01^{a,c}$	$2.53 \pm 0.01^{a,c}$
Urea (mg/dL)	12.18 ± 0.03^{a}	15.59 ± 0.04^{b}	13.34 ± 0.03^{c}	$11.23\pm0.03^{a,d}$	$11.21\pm0.03^{a,d}$

Means±S.E.M. values (n = 6 per group) were compared using ANOVA with Duncan's test. Significant differences ($P<0.01$) were observed between values marked with different superscript letters within the same treatment regimen. PPEE stands for Psiadia punctulata ethanolic extract.

Impact of the PPEE on the renal oxidative and antioxidative markers

In comparison to the standard control rats, the rats subjected to sodium oxalate treatment and developing lithiasis exhibited notably elevated levels of LPO and MDA, alongside diminished levels of GSH and impaired CAT and SOD enzyme activities ($p < 0.01$). Nonetheless, upon contrasting the treated rats with lithiatic control rats, those administered with a 500 mg/kg dosage of Cystone for 14 days manifested a remarkable reduction in MDA and LPO levels, coupled with an elevation in GSH concentration, CAT, and SOD functionalities ($p < 0.01$). Conversely, in the scenario where the rats were juxtaposed with lithiasis control rats treated solely with NaOx, successive administration of PPEE at 200 and 400 mg/kg doses over 14 days significantly decreased LPO and MDA levels and enhanced GSH concentration, CAT, and SOD activities in a manner dependent on the dosage ($p < 0.01$) (Table 7).

Table 7. *Impact* of the PPEE on renal oxidative and antioxidative markers

Parameters	Groups				
	Normal	Lithiatic	Lithiatic+PPEE (200 mg/kg b.w.)	Lithiatic+PPEE (400 mg/kg b.w.)	Lithiatic+Cystone
LPO (nmol/mg. tissue)	20.72±1.41	49.31±1.98	35.53±2.01	30.11±2.67	24.22±1.99
MDA (nmol/mg. tissue)	3.65±0.09	8.65±0.07	6.23±0.08	4.54±0.13	4.26±0.09
SOD (U/mg. tissue)	8.91±0.16	3.97±0.10	5.89±0.08	6.87±0.05	7.23±0.05
CAT (U/mg. tissue)	3.45±0.06	0.91±0.05	2.31±0.07	3.12±0.03	3.32±0.03
GSH (nmol/mg. tissue)	3.89±0.07	0.79±0.03	2.02±0.07	2.99±0.04	3.35±0.05

Means±S.E.M. values (n = 6 per group) were compared using ANOVA with Duncan's test. Significant differences ($P<0.01$) were observed between values marked with different superscript letters within the same treatment regimen. PPEE stands for Psiadia punctulata ethanolic extract.

Histopathological observation of kidney

The NaOx treatment-induced renal stone resulted in notable histological changes such as glomerular shrinkage and degeneration, tubular degeneration, hemorrhage, and calcium oxalate crystal deposits in the kidneys. However, throughout the 14 days, the quantity of calcium oxalate deposits and other irregularities in the renal tubules notably decreased with the repeated administration of PPEE at doses of 200 and 200 mg/kg body weight and Cystone at 500 mg/kg in a manner dependent on the dosage (Fig. 1).

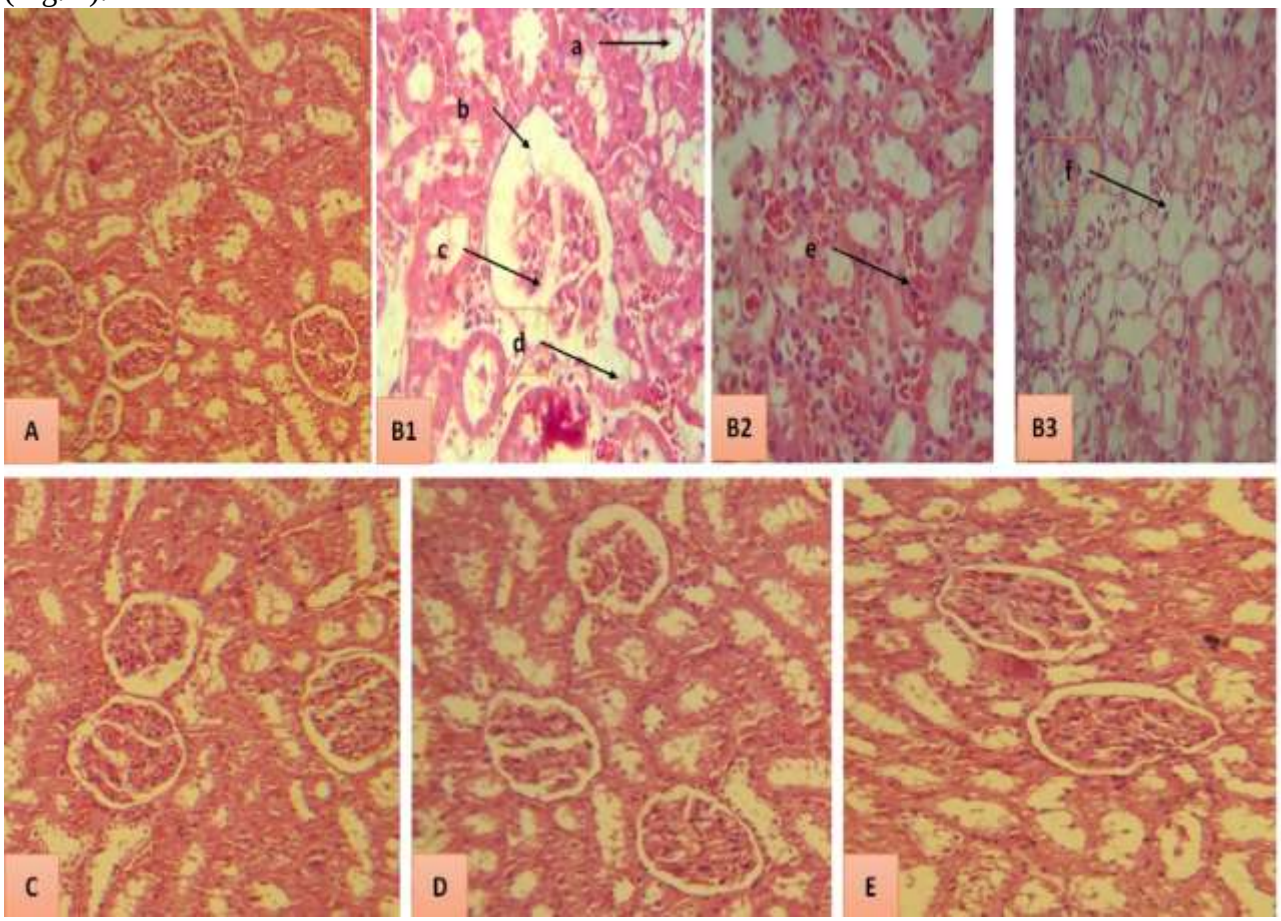

Fig. 1 Illustrates the histopathological analysis of kidney tissue in various experimental groups. The standard control group (A) displayed typical histology, while the Na oxalate-induced calculi group (B) exhibited severe alterations such as calcium oxalate crystal deposits, glomerular shrinkage, glomerular degeneration, calcium oxalate crystals, hemorrhage, and tubular degeneration. The cysteine-treated group (E) and the prophylactic treatment groups with PPEE at 200 and 200 mg/kg doses (C and D) showed moderate histological changes. The microscopic magnification used for analysis was 40.

DISCUSSION

In a rat model, Psiadia punctulata ethanolic extract (PPEE) was assessed against urolithiasis as the fastest way to screen for new antiurolithiatic medications for the most common and prevalent stones [10]. Even though P. punctulata as a whole has no significant harmful effects; this study looks into the safety of PPEE to determine its safety before use. As per the acute oral toxicity test (OECD standards, 423), no significant symptoms or behavioral changes were observed in PPEE, and there were no recorded deaths up to a dosage of 5000 mg/kg b.wt. As a result, PPEE can be classified as softly safe because compounds with LD50 values greater than 50 mg/kg are non-toxic [26]. These results are comparable to those reported by [18].

The results indicated that PPEE's phytochemical screening identified components (such as proteins and amino acids, glycosides, alkaloids, flavonoids, sterols, triterpenes, and phenolic compounds) that may be involved in the plant's purported medical properties. These results are comparable to those found by [18, 19]. Most of these compounds have been reported to exhibit antilithiatic activity in various forms, which may cause antilithiatisis-related activities [10, 12, 13].

The current investigation evaluated the antilithiatic effect of the sodium oxalate-induced hyperoxaluria model in Wistar rats [27]. Hormone oxalate (70 mg/kg, i.p.) was given to male Wistar rats over an extended period, causing hypercalciuria. The Group II animals induced with calculi had higher phosphate, calcium, and oxalate excretions. The process's biochemical mechanism is linked to increased oxalate concentration in the urine. Hippocampaluria, which results in increased renal retention and oxalate excretion, is the source of stone formation in animals injected with sodium oxalate [10]. In this investigation, the body and kidney weight of urolithiasis rats exhibit a discernible rise in comparison to the body weight of control rats. This may indicate fluid and crystal formation accumulation in the renal tissues supported by the histological examination, which aligns with the data [11, 28].

Examining urine compositions is crucial for detecting the presence of crystals and understanding the minerals responsible for lithiasis [29]. NaOx is known to cause the formation of CaOx crystals, with intraperitoneal injection of oxalate and elevated blood and urine calcium levels playing a significant role in this process [10, 30]. The alkaline pH of urine in lithiatic groups promotes the formation of CaOx crystals, while reduced urine production leads to oxalate supersaturation and crystal formation [29, 31]. High phosphate content in urine, combined with oxalate, creates an environment favorable for producing calcium phosphate crystals and stone formation [32]. Lower serum and urine magnesium levels in lithiatic groups contribute to urine supersaturation and stone formation, as magnesium deficiency accelerates CaOx crystal deposition [33]. Elevated urine uric acid levels also encourage crystal formation and reduce the effectiveness of stone inhibitors [34]. The study suggests that reduced magnesium excretion, increased calcium excretion, and low urine output contribute to lithiasis [33]. Renal stones obstruct urine flow, leading to renal failure and the blood accumulation of urea, uric acid, and creatinine [34]. Histological evidence supports the presence of intratubular crystal deposition in the kidneys, confirming the formation of renal calculi [10].

A critical mediator in the development of lithiasis is thought to be oxidative stress [35, 36]. Oxidative stress in renal tissue is linked to the deposition of calcium oxalate crystals [37]. According to the current study, oxidative damage was triggered by a NaOx injection, as evidenced by elevated LPO and decreased levels of GSH, GST, SOD, and CAT. The current findings are consistent with those from the past [10, 11, 13, 36]. Because renal epithelial injury reveals a variety of crystal adhesion particles on epithelial surfaces, it promotes crystal retention. One potential cause of lithiasis is the involvement of crystals in renal tubular tissue [38]. The deterioration of renal tissue caused by oxalate, which reacts with polyunsaturated fatty acids (PUFA) in cellular membranes to produce lipid peroxidation, makes this oxidant/antioxidant regularity change feasible [39]. Furthermore, reduced renal glutathione content favors lipid peroxidation and keeps the kidneys' calcium and oxalate levels stable, according to research by Sayed et al. [39].

The gold standard drug for comparing the antilithiatic effects of plant extracts is often cystone [25]. To assess the degree of PPEE's ameliorative effect, the current study contrasts the effects of PPEE

with cystone. It is interesting to note that PPEE had a beneficial effect in preventing the creation of new stones and aiding in the treatment of existing ones. The work results were consistent with earlier studies from [10, 11–13]. According to the paper, phytochemicals such as flavonoids, phenolic compounds, and saponins are responsible for anti-lithiatic activity.

A recent study comparing PPEE (an extract) to a group of individuals with untreated lithiasis (stone formation) found that PPEE led to significantly higher levels of magnesium and urine volume, which are known to inhibit stone formation. PPEE exhibited diuretic action, increasing renal fluid volume and facilitating the removal of deposits. Rats treated with PPEE showed increased urine production, resulting in diluted urine electrolyte levels and confirming the diuretic effect of PPEE. This activity led to a noticeable decrease in calcium and phosphate levels in the urine, preventing the components that contribute to stone formation. The diuretic impact of PPEE is attributed to its flavonoid concentration [40], as flavonoids and their derivatives are believed to be antagonists of the adenosine A1 receptor (AA1R), resulting in diuresis [32]. As a result, there is a reduced likelihood of precipitation and a defect in stone formation. The antilithiatic efficacy of natural agents, including PPEE, is due to their dual impact of increasing stone inhibitors and decreasing stone promoters in urine [41].

Additionally, PPEE treatment improved renal functions and reduced oxidative damage caused by hyperoxaluria in lithiatic rats. This was evident through decreased renal lipid peroxidation (LPO) levels and enhanced antioxidant activity. PPEE's antioxidant properties may attenuate the formation of calcium oxalate (CaOx) crystals by reducing hyperoxaluria-induced peroxidation of the renal tubular membrane. Histological evidence confirmed that PPEE treatment resulted in fewer or no crystals in the renal tissue of lithiatic rats, similar to the effects observed with cystone, a common antilithiatic medication. Both PPEE and cystone prevented the formation of CaOx crystals, potentially by accelerating the breakdown of existing stones and inhibiting the formation of new crystals, supporting the effectiveness of PPEE as a treatment or preventive measure for lithiasis.

Finally, the current research implies that PPEE could prevent crystal formation by reducing the size of crystal development and aggregation. This idea is consistent with [10], which found that antioxidant phytochemicals such as flavonoids and saponins limit the formation and aggregation of crystals in vitro. These findings contributed to the antilithiatic effect by dissolving stones and acting as an antioxidant.

Furthermore, the antioxidant power of PPEE, which comes from its phytochemical ingredients, such as phenolic compounds, may be the cause of its effects [11]. According to Al-Mahbashi et al. [18], P. punctulata's ethanolic extract has a significant phenolic content. Bawari et al. [42] stated that flavonoids and saponins can dissolve and disintegrate CaOx crystals, suggesting that TAEE may contain significant levels of flavonoids, alkaloids, and tannins in addition to terpenoids that can inhibit the formation of lithiasis. Additionally, tannins hinder the production of CaOx crystals by forming calcium complexation, which is an anti-crystallization effect of tannins. Renal calculi can be prevented or disintegrated by PPEE, thanks to all of the previously listed characteristics.

CONCLUSION

The study provides evidence of the anti-urolithiasis (anti-urinary stone formation) effect of an ethanolic extract derived from P. punctulata leaves in rats with Na-oxalate-induced lithiasis. This effect is likely due to the presence of potent antioxidants in the extract. The extract inhibits peroxidative damage caused by hyperoxaluria to the renal tubular membrane, which can lead to the attachment of calcium oxalate crystals and the formation of kidney stones. As a result, the extract helps prevent the deposition of calcium oxalate crystals in the kidneys. The study also demonstrates that the ethanolic extract of P. punctulata leaves can prevent and reduce the development of urinary stones. This effect may be attributed to a decrease in the components responsible for stone formation in urine and the nephroprotective properties of antioxidants. However, further investigation is needed to determine the exact mechanism underlying this effect.

REFERENCES

[1] Qian, X., Wan, J., Xu, J., Liu, C., Zhong, M., Zhang, J., ... & Wang, S. (2022). Epidemiological Trends of Urolithiasis at the Global, Regional, and National Levels: A Population-Based Study. *International Journal of Clinical Practice*, (1), 6807203 (1-12).

[2] Jahrreiss, V., Seitz, C., & Quhal, F. (2024). Medical management of urolithiasis: Great efforts and limited progress. Asian Journal of Urology, 11(2), 149-155.

[3] Abufaraj, M., Al Karmi, J., & Yang, L. (2022). Prevalence and trends of urolithiasis among adults. Current Opinion in Urology, 32(4), 425-432.

[4] Thakore P, Liang TH. Urolithiasis. 2023 Jun 5. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 32644527.

[5] Sattar, A., Raza, S. M. F., Sarfraz, S., Shahzad, M., Farooq, M. U., & Shabbir, A. (2024). Phytochemical Analysis and Antimicrobial Activity of Pongamia Pinnata: A Comprehensive Study. Journal of Health and Rehabilitation Research, 4(1), 196-202.

[7] Kasote, D. M., Jagtap, S. D., Thapa, D., Khyade, M. S., & Russell, W. R. (2017). Herbal remedies for urinary stones used in India and China: A review. *Journal of ethnopharmacology*, *203*, 55-68.

[7] Khan, A., Bashir, S., & Khan, S. R. (2021). Antiurolithic effects of medicinal plants: results of in vivo studies in rat models of calcium oxalate nephrolithiasis—a systematic review. *Urolithiasis*, *49*(2), 95-122.

[8] Zeng, L., Tang, G., Wang, J., Zhong, J., Xia, Z., Li, J., ... & Guo, J. (2019). Safety and efficacy of herbal medicine for acute intracerebral hemorrhage (CRRICH): a multicentre randomised controlled trial. *BMJ open*, *9*(5), e024932 (1-9).

[9] Tleubayeva, M. I., Datkhayev, U. M., Alimzhanova, M., Ishmuratova, M. Y., Korotetskaya, N. V., Abdullabekova, R. M., ... & Gemejiyeva, N. G. (2021). Component composition and antimicrobial activity of CO2 extract of Portulaca oleracea, growing in the territory of Kazakhstan. *The Scientific World Journal*, 22(1), 5434525 (1-10).

[10] Pandhare, R. B., Shende, R. R., Avhad, M. S., Deshmukh, V. K., Mohite, P. B., Sangameswaran, B., & Daude, R. B. (2021). Anti-urolithiatic activity of Bryophyllum pinnatum Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats. *Journal of Traditional and Complementary Medicine*, *11*(6), 545-551.

[11] Sayed, A. A. (2023). Antilithiatic effect of Triticum aestivum against sodium oxalate-induced lithiasis in rat model. *The Journal of Basic and Applied Zoology*, *84*(1), 2-13.

[12] Raj, S., Rajan, M. S. G. S., Ramasamy, S., Goldy, R. I. R. S., Ariyamuthu, R., Sudhagar, M., ... & Gurusamy, M. (2024). An in vitro Anti-urolithiasis Activity of a Herbal Formulation: Spinacia oleracea L. and Coriandrum sativum L. *Clinical Complementary Medicine and Pharmacology*, *4*(1), 100124 (1-9).

[13] Mammate, N., El Oumari, F. E., Imtara, H., Belchkar, S., Mothana, R. A., Fatemi, H. E., ... & Houssaini, T. S. (2024). The Anti-urolithiatic effect of the roots of Saussurea costus (falc) Lipsch agonist ethylene glycol and magnesium oxide induced urolithiasis in rats. Saudi Pharmaceutical Journal, 32(3), 101967 (1-16).

[14] Yadav, A., Das, R., & Mehta, D. K. (2021). Benefaction of Herbals in the Management of Urolithiasis. *Current Traditional Medicine*, *7*(4), 541-551.

[15] Midiwo, J. O., Owuor, F. A. O., Juma, B. F., & Waterman, P. G. (1997). Diterpenes from the leaf exudate of Psiadia punctulata. *Phytochemistry*, *45*(1), 117-120.

[16] Mabberley, D. J. (1997). The plant-book: a portable dictionary of the vascular plants. 1st, Cambridge university press. pp123.

[17] Al-Khulaidi, A. A. (2013). Flora of Yemen. Sustainable Natural Resource. *Management Project (SNRMP) II, Sana'a, Yemen*,pp 97.

[18] Al-Mahbashi, H., Moharram, B. A., & Al-Maqtari, T. (2020). Phytochemical, anti-inflammatory, analgesic, antipyretic and acute toxicity of Psiadia punctulata growing in Yemen. *Univers J Pharm Res*, *5*, 61-66.

[19] Dal Piaz, F., Bader, A., Malafronte, N., D'Ambola, M., Petrone, A. M., Porta, A., and Severino, L. (2018). Phytochemistry of compounds isolated from the leaf-surface extract of Psiadia punctulata (DC.) Vatke growing in Saudi Arabia. *Phytochemistry*, *155*, 191-202.
[20] Mothana, R. A., Kriegisch, S., Harms, M., Wende, K., & Lindequist, U. (2011). Assessment of selected Yemeni medicinal plants for their in vitro antimicrobial, anticancer, and antioxidant activities. *Pharmaceutical Biology*, *49*(2), 200-210.
[21] Koch, A., Tamez, P., Pezzuto, J., & Soejarto, D. (2005). Evaluation of plants used for antimalarial treatment by the Maasai of Kenya. *Journal of ethnopharmacology*, *101*(1-3), 95-99.
[22] Mulwa, L. S. (2012). *Phytochemical investigation of Psiadia punctulata for analgesic agents*. Doctoral dissertation, University of Nairobi, Kenya.
[23] Juma, B. F., Yenesew, A., Midiwo, J. O., & Waterman, P. G. (2001). Flavones and phenylpropenoids in the surface exudate of Psiadia punctulata. *Phytochemistry*, *57*(4), 571-574.
[24] Harbourne, J. B., 1984. Phytochemical Methods. A guide to modern techniques of plant analysis. (2nd ed.) Chapman and Hall, London: 282.
[25] Takawale, R. V., Mali, V. R., Kapase, C. U., & Bodhankar, S. L. (2012). Effect of Lagenaria siceraria fruit powder on sodium oxalate induced urolithiasis in Wistar rats. *Journal of Ayurveda and integrative medicine*, *3*(2), 75-79.
[26] Buck, W., Osweiter, G., Van Gelder, A., 1976. Clinical and Diagnostic Veterinary Toxicology. 2nd Edn., Kendall/Hunt Publishing Co., Iowa, USA., ISBN-13: 9780840307200, Pages: 380.
[27] Mitra, S. K., Gopumadhavan, S., Venkataranganna, M. V., & Sundaram, R. (1998). Effect of cystone, a herbal formulation, on glycolic acid-induced urolithiasis in rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 12(5), 372-374.
[28] Saleem, U., Ahmad, N., Shah, M. A., Anwar, F., & Ahmad, B. (2020). Anti-urolithiatic activity of Salvia hispanica L. seeds in ethylene glycol induced urolithiasis rat's model. *Anais da Academia Brasileira de Ciências*, *92*(4), e20200067 (1-13).
[29] Manissorn, J., Fong-Ngern, K., Peerapen, P., & Thongboonkerd, V. (2017). Systematic evaluation for effects of urine pH on calcium oxalate crystallization, crystal-cell adhesion and internalization into renal tubular cells. Scientific reports, 7(1), 1798 (1-11).
[30] Mandavia, D. R., Patel, M. K., Patel, J. C., Anovadiya, A. P., Baxi, S. N., & Tripathi, C. R. (2013). Anti-urolithiatic effect of ethanolic extract of Pedalium murex linn. fruits on ethylene glycol-induced renal calculi. Urology journal, 10(3), 946-952.
[31] Patel, V. B., & Acharya, N. (2020). Effect of Macrotyloma uniflorum in ethylene glycol induced urolithiasis in rats. Heliyon, 6(6), e04253 (1-7).
[32] Ramalingam, M., Ramesh, T., & Begum, H. (2006). Effect of Aerva lanata on calcium oxalate urolithiasis in rats. Indian journal of experimental biology, 44, 981-986.
[33] Shah, R., Shidham, G., Agarwal, A., Albawardi, A., & Nadasdy, T. (2011). Diagnostic utility of kidney biopsy in patients with sarcoidosis and acute kidney injury. International journal of nephrology and renovascular disease, 4, 131-136.
[34] Ahmed, O. M., Ebaid, H., El-Nahass, E. S., Ragab, M., & Alhazza, I. M. (2020). Nephroprotective effect of pleurotus ostreatus and agaricus bisporus extracts and carvedilol on ethylene glycol-induced urolithiasis: roles of NF-κB, p53, bcl-2, bax and bak. Biomolecules, 10(9), 1317 (1-37).
[35] Devkar, R. A., Chaudhary, S., Adepu, S., Xavier, S. K., Chandrashekar, K. S., & Setty, M. M. (2016). Evaluation of antiurolithiatic and antioxidant potential of Lepidagathis prostrata: A Pashanbhed plant. Pharmaceutical biology, 54(7), 1237-1245.
[36] Sayed, A. A., Fahmy, S. R., Soliman, A. M., & Mohamed, D. M. (2020). Antinephrolithiatic activity of Ananas comosus extract against experimentally induced renal calculi in rats. Pakistan Journal of Pharmaceutical Sciences, 33(4), 1679–1688.

[37] Peng, Z., Chen, W., Wang, L., Ye, Z., Gao, S., Sun, X., & Guo, Z. (2015). Inhalation of hydrogen gas ameliorates glyoxylate-induced calcium oxalate deposition and renal oxidative stress in mice. International journal of clinical and experimental pathology, 8(3), 2680–2689.
[38] Touhami, M., Laroubi, A., Elhabazi, K., Loubna, F., Zrara, I., Eljahiri, Y., ... & Chait, A. (2007). Lemon juice has protective activity in a rat urolithiasis model. BMC urology, 7, 1-10.
[39] Karadi, R. V., Gadge, N. B., Alagawadi, K. R., & Savadi, R. V. (2006). Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats. Journal of ethnopharmacology, 105(1-2), 306-311.
[40] Dhaliwal, J., Leach, S., Katz, T., Nahidi, L., Pang, T., Lee, J. M., ... & Ooi, C. Y. (2015). Intestinal inflammation and impact on growth in children with cystic fibrosis. Journal of pediatric gastroenterology and nutrition, 60(4), 521-526.
[41] Dodoala, S., Diviti, R., Koganti, B., & Prasad, K. (2009). Effect of ethanolic extract of Phyla nodiflora (Linn.) Greene against calculi producing diet induced urolithiasis. Indian Journal of Natural Products and Resources, 1(3), 314–321.
[42] Bawari, S., Sah, A. N., & Tewari, D. (2020). Anticalcifying effect of Daucus carota in experimental urolithiasis in Wistar rats. Journal of Ayurveda and integrative medicine, 11(3), 308-315.