https://doi.org/10.52973/rcfcv-e35502 Revista Científica, FCV-LUZ / Vol. XXXV

Antioxidant and anti–inflammatory effects of *Helichrysum plicatum* DC. subsp. *plicatum* extract in an experimental model of acute urolithiasis

Efectos antioxidantes y antiinflamatorios del extracto de *Helichrysum plicatum* DC. subsp. *plicatum*, en un modelo experimental de urolitiasis aguda

Selvinaz Yakan[1]*, Kıvılcım Eren Erdoğan[2], Yusuf Kenan Dağlıoğlu[3], Tuba Aydın[4], Ahmet Çakır[5]

[1]Ağrı İbrahim Çeçen University, Eleşkirt Celal Oruç School of Animal Production, Animal Health Department. Ağrı, Türkiye.
[2]Çukurova University, Faculty of Medicine, Department of Pathology. Adana, Türkiye.
[3]Kirşehir Ahi Evran University, Faculty of Medicine, Department of Microbiology. Kırşehir, Türkiye.
[4]Ağrı İbrahim Çeçen University, Faculty of Pharmacy. Ağrı, Türkiye.
[5]Kilis 7 Aralık University, Faculty of Science and Literature, Department of Chemistry. Kilis, Türkiye.
**Corresponding author: syakan@gmail.com*

ABSTRACT

This study aimed to view the antioxidant and anti–inflammatory properties of *Helichrysum plicatum* DC. subsp. *plicatum* (HP) methanol extract on the urinary tract using an experimentally induced urolithiasis (U) model. The study included four groups: Group 1 was given a standard diet, Group 2 was given a diet added with HP, Group 3 was given a standard diet with induced urolithiasis, and Group 4 received an HP–supplemented diet with induced urolithiasis. Rats in Groups 2 and 4 were administered 500 mg·kg^{-1}·day^{-1} of HP via gavage feeding for 21 days. Urolithiasis was induced in Groups 3 and 4 by administering 1% ethylene glycol and 1% ammonium chloride in their swig water for 21 days to create a calcium oxalate (CaOx) urolithiasis model. The study analyzed plasma concentrations of thiobarbituric acid reactive substances (TBARS), an indicator of serum oxidative stress (OS), HP, and HP levels. Additionally, oxalate (Ox), urea, calcium, and creatinine clearance levels were measured in both blood and urine, and routine histological evaluations were conducted. The results indicated important higher concentrations of HP in the groups given HP (P<0.001), while plasma TBARS concentrations were lower in Group 4 compared to Group 3 (P=0.001). The findings suggest that HP reduces OS by lowering plasma TBARS levels induced by CaOx, due to its antioxidant and anti–inflammatory properties. Furthermore, the measured biochemical measurements supported the anti–urolithiasis effects of HP. In results, this study supports the hypothesis that HP's antioxidative and anti–inflammatory properties help prevent OS, which is a factor in stone formation, thereby preventing acute renal damage and stone formation.

Key words: Calcium oxalate crystals; urolithiasis; rat; thiobarbituric acid reactive substance (TBARS); *Helichrysum plicatum* DC. subsp. *plicatum*

RESUMEN

Este estudio tuvo como objetivo evaluar las propiedades antioxidantes y antiinflamatorias del extracto de metanol de *Helichrysum plicatum* DC. subsp. *plicatum* (EP) en el tracto urinario, utilizando un modelo de urolitiasis (U) inducido experimentalmente. El estudio incluyó cuatro grupos: al Grupo 1 se le administró una dieta estándar convencional, al Grupo 2 se le administró una dieta complementada con EP, al Grupo 3 se le administró una dieta estándar con urolitiasis inducida y el Grupo 4 recibió una dieta complementada con EP y con urolitiasis inducida. A las ratas de los Grupos 2 y 4 se les administró 500 mg·kg^{-1}·dia^{-1} de EP mediante alimentación por sonda durante 21 dias. La urolitiasis se indujo en los Grupos 3 y 4 mediante la administración de 1 % de etilenglicol y 1 % de cloruro de amonio en su agua potable durante 21 dias para crear un modelo de urolitiasis por oxalato de calcio (CaOx). El estudio analizó las concentraciones plasmáticas de sustancias reactivas al ácido tiobarbitúrico (TBARS), un indicador de estrés oxidativo (EO), así como los niveles de EP en el suero. Además, se midieron los niveles de oxalato (Ox), urea, calcio y la depuración de creatinina en sangre y orina, y se realizaron evaluaciones histológicas de rutina. Los resultados indicaron concentraciones significativamente más altas de EP en los grupos que recibieron EP (P<0,001), mientras que las concentraciones plasmáticas de TBARS fueron más bajas en el Grupo 4 en comparación con el Grupo 3 (P=0,001). Los hallazgos sugieren que EP reduce el EO al disminuir los niveles plasmáticos de TBARS inducidos por CaOx, debido a sus propiedades antioxidantes y antiinflamatorias. Además, las mediciones bioquímicas realizadas respaldaron los efectos antiurolíticos de EP. En resumen, este estudio respalda la hipótesis de que las propiedades antioxidantes y antiinflamatorias de EP ayudan a prevenir el EO, que es un factor en la formación de cálculos, previniendo así el daño renal agudo y la formación de cálculos.

Palabras clave: Cristales de oxalato de calcio; urolitiasis; rata; sustancia reactiva al ácido tiobarbitúrico (TBARS); *Helichrysum plicatum* DC. subsp. *plicatum*

Received: 19/08/2024 Accepted: 04/11/2024 Published: 20/01/2025

INTRODUCTION

Urolithiasis (U) is a disease caused by the development of stones within the urinary system. About 80 percent of these stones consist of calcium oxalate (CaOx). Besides CaOx stones, other types can result from infections, uric acid, cystine, and calcium phosphate [1, 2, 3, 4, 5]. Subjecting renal cells to oxalate (Ox) and/or CaOx crystals results in the formation of free oxygen radicals, leading to oxidative stress (OS) and consequent damage and suppuration. Kidney damage and inflammation are key factors in the formation of stones [6, 7, 8, 9, 10, 11]. Researchers have shown that treatments involving antioxidants and free radical sweepers can decrease the harm caused by Ox/CaOx crystals. Furthermore, the aggregation of CaOx crystals in the kidneys is largely reduced by treatments with antioxidants and free radical sweepers, providing evidence for the efficacy of antioxidants [11, 12, 13, 14, 15]. Developing treatments to prevent OS could be an alternative method that may raise the achievement ratio in avoiding and intercepting future occurrences of kidney stones [12]. Since OS occurs due to various sources and lanes, it is important to determine all enzymes and lanes involved in the formation of free radicals caused by Ox and/or CaOx crystals [6]. It can be argued that flavonoids can play an effective role in protecting against many illnesses that may occur as a result of damage caused by free radicals [16, 17].

Helichrysum species, which are rich in flavonoids, are used in traditional medicine worldwide. This plant, which encompasses hundreds of species, has been utilized in folk medicine for treating wounds, and infections, and as a diuretic since ancient times. Türkiye alone has about 20 varieties of *Helichrysum,* which are used in Turkish traditional medicine for treating wounds, and burns, reducing kidney stones, as diuretics, and for relieving ear pain. Previous research has revealed that this plant possesses antimicrobial, antioxidant, anti–inflammatory, antidiabetic, and anti–mitotic properties [18, 19, 20, 21, 22, 23, 24]. The medicinal features of *Helichrysum* are believed to be attributed to flavonoids and other metabolites. In a previous study, β–sitosterol, apigenin, nonacosanoic acid, astragalin, β–sitosterol-3-O–β–D–glucopyranoside, helichrysin A, helichrysin B, and isosalipurposide were isolated from the HP. It has been observed that secondary metabolites extracted from HP methanol have potent inhibitory effects, particularly on human carbonic anhydrase I (hCAI) and II (hCAII) [24]. Although Helichrysum species are extensively used in Turkish traditional medicine, the lack of scientific evidence regarding their specific clinical effects, activity, and side effects has prevented the clear definition of their potential use as an alternative or complement to conventional treatment. Therefore, further scientific research is necessary to define the exact mechanism of the effect of these traditional remedies [12].

In this study, the antioxidant and anti–inflammatory action of *Helichrysum plicatum* DC. subsp. *plicatum* (HP) methanol extract was investigated in the urinary tract using an experimentally excited urolithiasis model.

MATERIALS AND METHODS

Animals

Twenty–eight male Wistar albino rats (*Rattus norvegicus*) weight 140 ± 10 g and aged 10–12 weeks were used in the research. The animals were housed in lattices in a controlled room at a constant temperature of 24 ± 1°C and were provided with a twelve hour (12 h) dark/light cycle. Prior to the study, the rats were consent to acclimate to the perimeter for 10 days (d) without any interventions. The Wistar albino rats were given access to feed (pellets) and water *ad libitum*. The research was applied at Çukurova University Health Sciences Experimental Research and Application Center (SABİDAM) following ethical guidelines for experimental animal studies, and confirmed by the Çukurova University Experimental Animals Ethics Committee (confirmation no: 2019/75).

Experimental protocols

Twenty–eight male rats intended for use in the experiment were divided into four equal groups. To induce the formation of CaOx crystals, a mixture of 1% ethylene glycol (EG) and 1% ammonium chloride (AC) was added to the swig water of the animals for 21 d.

The control and research groups were designed in the following manner:

- Group 1 (control, n:7): In rats in the control group, 1 mL of water was given by gastric gavage method for 21 d.
- Group 2 (HP only, n:7): The rats in this group received 500 $mg \cdot kg^{-1} \cdot day^{-1}$ of HP via gavage feeding each morning for 21 d, along with their regular daily nutrients, under the same laboratory conditions.
- Group 3 (urolithiasis only, n:7): In the urolithiasis group, 1% EG and 1% AC were additional to the rats' swig water daily for 21 d to stimulate urolithiasis.
- Group 4 (urolithiasis–HP, n:7): The animals in this group were administered 500 $mg \cdot kg^{-1} \cdot day^{-1}$ of HP via gavage feeding each morning for 21 d, in addition to their daily nutrients. They were also given 1% EG and 1% AC in their swig water. The doses of HP and the urolithiasis model were chosen based on previous studies.

In the experiment, all animals were anesthetized with ether using a mask with an inhalation anesthesia device (Harvard Apparatus, Anesthesia Machine, USA). Once they reached deep anesthesia, and then immolate by taking approximately 3 mL blood samples from their hearts for biochemical analysis. After the rats had died, their abdominal cavities were immediately opened for histopathological examination and the kidneys were quickly removed.

Biochemical measurements

Blood samples taken from rat hearts were centrifuged (Eppendorf, 5424R, Germany) at 3000 G for 10 min to obtain serum, which was then preserved at -80 degrees until analyzed. The samples taken from -80°C on the study day were thawed at room temperature.

High–performance Liquid Chromatography (HPLC) analysis

Measurement of HP in serum was conducted utilizing high–performance liquid chromatography (HPLC) (Agilent Technologies, 1260 Infinity II, USA). The analysis of HP serum concentration by HPLC was carried out according to the methods outlined in the literature [25, 26]. According to this, a C30 phase column

separation system was used to isolate HP in serum. 1 mL of serum was homogenized in a 3 mL salt/ethanol mix (1:2, weight: volume). Then, 5 mL of a hexane/ether mixture (1:1, volume: volume) was incorporated, and the mix was vortexed for 3 min before being centrifuged at 4°C at a speed of 2000 G for 10 min. The upper layer was accumulated, and HP was separated from the samples. The samples were extracted three times and treated with 100 mL of an ethanol/ether mixture (2:1, volume: volume) and then evaporated under nitrogen gas. The concentration of HP was measured by injecting 50 mL of the final sample into the instrument.

Determination of antioxidant and anti–inflammatory activity

Blood samples were taken on 9th days in group 1 and on 1, 9, 17, and 21 d in the other groups. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) were measurement with spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000, USA). The change in TBARS aimed to identify the antioxidant and anti–inflammatory.

After the rats were sedated with ether, 1 mL of blood was taken from their tails. Plasma TBARS levels, which are composed of two molecules of Thiobarbituric acid (TBA) and malondialdehyde (MDA) and serve as an indicator of oxidative membrane damage, were determined following the fluorometric method described in the literature [27, 28]. A solution of 29 mmol·L^{-1} TBA (Sigma, T5500), 5 mol·L^{-1} hydrochloric acid (HCI) (Sigma, H–7020), and n–butanol (prepared in 8.75 M Acetic acid (Sigma, A-6283)) was used to measure plasma TBARS levels. Standard solutions of MDA bis (dimethyl acetal) (Sigma, T–1642) were prepared at concentrations of 0; 0.5; 1; 2.5; 3.5; 5; 7; 8; 9; 10 µmol·L^{-1}.

The plasma samples taken in anticoagulant tubes were centrifuged at 3000 G for 10 min, aliquoted, and stored at -80°C (Thermo Fisher Scientific, Forma 900 Series, USA) up to the study day. On the day of the study, samples were thawed at room temperature. For testing, 10 mL tubes each containing 1 mL of distilled water were prepared for each blood specimen and 50 µl of plasma was added. Then, 1 mL of the 29 mmol·L^{-1} TBA solution prepared in acetic acid was added to each tube and mixed. The tubes were tightly closed and placed in a 100°C boiling water bath for 1 h. After cooling the tubes under tap water, the pH was adjusted to 1.6–1.7 by adding 25 µL of 5 mol·L^{-1} HCl solution to each tube. 3.5 mL of n–butanol was supplemented to the reaction concoction, which was vortexed for 5 min.

Subsequently, the fluorescences of the upper phase divided by centrifugation at 1500 G for 10 min were read using a fluorometer (Thermo Fisher Scientific, Fluoroskan FL, Finland) at wavelengths of 525 nm (excitation) and 547 nm (emission). The MDA contents of the blood samples were calculated in µmol·L^{-1} based on the standard solutions.

Assays in blood and urine

Serum parameters: Oxalate levels were measured using the Trinity Biotech diagnostic kit with spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000, USA). Concentrations of creatinine and urea in the serum were determined using autoanalyzer diagnostic (Siemens Healthineers, ADVIA 1800, Germany) kits with spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000, USA).

Urine parameters: On the 20th day of the experiment, two rats from each group were placed in metabolic cages for 24 h to collect their urine. The total urine volume was measured with a measuring cylinder and recorded in milliliters. Ox levels were determined using the Trinity Biotech diagnostic kit with a spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000, USA). Levels of creatinine (measured with Jaffe's method) and calcium (measured with the Schwarzenbach and o–Cresolphthalein method) were determined in the urine samples using an autoanalyzer (Siemens Healthineers, ADVIA 1800, Germany). Creatinine clearance was calculated according to the methods described in the literature [29].

Histopathological evaluation

The kidney tissues were fixed in 10% formaldehyde for 3 h after the cassette process was completed and sample numbers were assigned. Subsequently, overnight tissue detection and tracking were carried out on the fully automated device. Following tissue fixation, paraffin embedding and blocking were performed. The prepared paraffin blocks were sampled with 5 µm sections. The parts obtained were stained with hematoxylin–eosin using a fully automatic staining device. The kidney tissue was examined under a microscope (Olympus, BX53, Japan) for the presence of crystals and inflammation. Crystals were scored based on their density under a polarized lens. Mean group scores were determined based on the scores of rats in the same group.

According to this scoring system: Score 0: no stones and clumps of CaOx crystals; score 1: 1–5 stones, CaOx crystals; score 2: 5–10 stones, CaOx crystals; score 3: more than 10 stones, CaOx crystals.

Statistical analysis

The SPSS 22.0 package program was using for statistical analysis. The data is adherence to a normal distribution was evaluated through the Shapiro–Wilk test. Data that met the criteria for a normal dispersion were analyzed usage analysis of variance and student *t*–tests for group comparisons, as well as *t*–tests for dependent variables for intra–group comparisons. Post–hoc comparisons were conducted utilizing the Scheffe test. Data that did not meet the criteria for a normal distribution were compared using Kruskal–Wallis variance analysis and the Mann–Whitney U test.

RESULTS AND DISCUSSIONS

Biochemical findings

Serum HP values

After administering HP to rats for 21 d, the average ± SD HP value was found to be 340.5 ± 66.4 nmol·L^{-1} in the HP group and 120.3 ± 19 nmol·L^{-1} in the U–HP group. These values were found to be statistically significantly higher (P<0.001) compared to the group 1 (< 0.000 + 0) and U (< 0.000 + 0) groups where no HP was given. When comparing the groups that received HP (HP, U–HP) with those that did not (Control, U), the HP concentration in the HP groups was statistically higher (P<0.001), indicating successful administration via. Furthermore, when comparing the HP and U–HP groups, the HP concentration was found to be significantly lower in the U–HP group (P<0.001). This difference was attributed to increased antioxidant need in the U–HP group, leading to lower HP levels.

Plasma TBARS levels

Plasma levels of TBARS decreased significantly between 9 and 17 d in the HP group (P=0.01). There was an important reduction in TBARS concentrations between 17 and 21 d in the U–HP group (P=0.003). TBARS levels increased significantly from 9 to 17 d in the U group (P=0.004). The TBARS level was significantly riser compared to the initial level (P=0.006) on 21 d in the U group. On 17 d, TBARS levels were under in the HP group compared to the U–HP group (P=0.044). Additionally, the TBARS level was significantly lower on 17 d (P=0.001) and 21 d (P=0.001) in the U–HP group compared to the U group. The average TBARS levels for all rats can be found in TABLE I.

TABLE I
The average of plasma TBARS changes in groups*

	TBARS (nmol·L^{-1})			
Days	**Group 1 (Control)**	**Group 2 (HP)**	**Group 3 (U)**	**Group 4 (U+HP)**
1	–	0.026 ± 0.020	–	0.026 ± 0.020
9	0.026 ± 0.020	0.025 ± 0.010	0.021 ± 0.010	0.022 ± 0.001
17	–	0.020 ± 0.002	0.180 ± 0.240	0.025 ± 0.002
21	–	0.024 ± 0.001	0.121± 0.003	0.013± 0.003

*: Abbreviations are as follows; TBARS: thiobarbituric acid reactive substance, HP: *Helichrysum plicatum*, U: urolithiasis

Assays in blood and urine

The serum oxalate concentration in the U–HP group was higher than in the control group but lower than in the U group, although this increase was not statistically significant. Even though the serum Ox level was riser in group U than in other groups, there was no important distinction in serum Ox levels between the groups (P>0.05). The serum urea level was found to be significantly higher in the U group compared to the other groups in the group comparisons (P<0.05). Urine Ox concentrations were significantly riser in the U group compared to the other groups (P<0.001). In the U–HP group, urine Ox level was showed to be significantly lower compared to the U group (P<0.05). Urine calcium concentrations were significantly higher in the U group compared to the control, HP, and U–HP groups (P<0.05). There was no important difference in the comparisons between the control, HP, and U–HP groups (P>0.05). Creatinine clearance was significantly riser in the U group compared to other groups (P<0.05). There was no important distinction in creatinine clearance levels between the control, HP, and U–HP groups (P>0.05). Biochemical measurement outcomes of blood and urine samples are provided based on the groups in TABLE II.

Histopathological results

For histopathological examination, the stone creation density of kidney tissues was scored between 0 and 3 after examining all kidney sections. Mean group scores were then determined (see TABLE II). In FIG. 1, photomicrographs of kidney sections demonstrate diverse group scores with varying kidney stone intensity. No abnormal histological formations were found in the control and HP groups during the histological examination of

TABLE II
Average values and standard deviation ($\bar{x}$ ± SD) of blood and urine, stone formation score by groups[1]

Parameters	Group 1 (Control)	Group 2 (HP)	Group 3 (U)	Group 4 (U–HP)
Serum oxalate (µmol·L^{-1})	30.16 ± 15.24^{a}	34.79 ± 9.08^{a}	74.86 ± 60.81^{a}	36.76 ± 8.04^{a}
Serum urea (mg·dL^{-1})	40.35 ± 3.27^{b}	40.14 ± 13.08^{b}	84.78 ± 60.34^{a}*	41.36 ± 15.06^{b}
Urine oxalate (µmol·24 h^{-1})	2.67 ± 0.46^{c}	2.65 ± 0.28^{c}	10.11 ± 5.12^{a}**	7.04 ± 3.01^{b}*
Urine calcium (mg·dL^{-1})	6.60 ± 3.11^{b}	7.48 ± 4.31^{b}	12.89 ± 4.26^{a}*	7.66 ± 2.13^{b}
Creatinine clearance (mL·min^{-1})	0.22 ± 0.06^{b}	0.26 ± 0.16^{b}	0.55 ± 0.27^{a}*	0.29 ±0.13^{b}
Stone formation score	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	2.56 ± 0.48^{a}**	0.21 ± 0.69^{b}*

[1]: Abbreviations are as follows; HP: *Helichrysum plicatum,* U: urolithiasis. ^{a,b,c}: There is a statistically significant difference between values with different letters in the same row (*P<0.05, **P<0.001)

kidney tissue sections. However, the U group animals showed large quantities of CaOx crystals deposited in masses. Stone creation and accumulation were as expected in group U, with a stone creation score of 2.56. The healing effect of HP extract on stone creation was evident, as the stone creation score of the U–HP group was lower (0.21) compared to the U group (2.56). Within the interstices

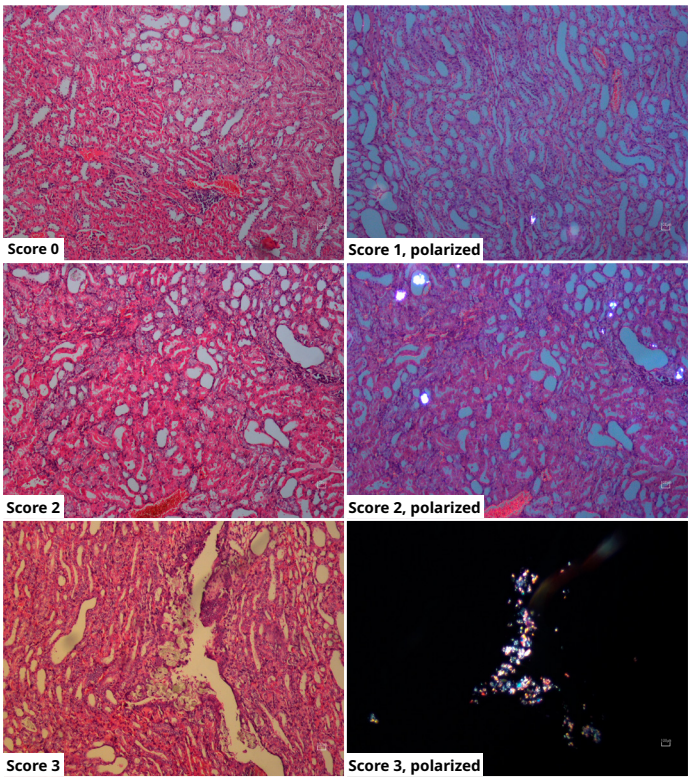

FIGURE 1. Photomicrography of kidney divisions illustrates varying group scores based on stone density and calcium oxalate deposits. Score 0: Absence of stones and deposits; Score 1: 1-5 stones with calcium oxalate crystal deposits; Score 2: 5-10 stones with calcium oxalate crystal deposits; Score 3: Over 10 stones with calcium oxalate crystal deposits

deposited mononuclear inflammatory cells were usually present in the U group, less so in the U–HP group. Additionally, capsular inflammatory lesions were detected in the U group.

Previous researches have demonstrated that *Helichrysum* extracts are beneficial for treating urolithiasis, a condition long recognized in traditional medicine [30, 31]. This research aims to contribute to a better understanding of the mechanism of the HP effect in future studies.

Oxalate is a natural by–yield of metabolism and is excreted without causing harm in healthy individuals. However, hyperoxaluria, which is characterized by improved urinary excretion of oxalate, can lead to crystal formation at normal pH levels and may result in the accumulation of calcium oxalate crystals in the kidneys. Oxalate has a stimulating effect on renal epithelial cells at low concentrations, but at high concentrations, it can be toxic by causing the creation of calcium oxalate crystals. The collection of calcium oxalate crystals in the kidneys can trigger the production of certain macromolecules that can cause the formation of pus and lead to fibrosis. Studies on animals and renal epithelial cell cultures have indicated that hyperoxaluria and the formation of calcium oxalate crystals can lead to the generation of free radicals [32, 33, 34, 35, 36, 37, 38].

In a study made by Huang *et al*. [39], it was found that levels of alpha glutathione S transferase, MDA, and TBARS in the urine of CaOx kidney stone patients were elevated, indicating oxidative stress in the kidneys. The study also revealed increased urinary excretions of beta–galactosidase and N–acetyl–beta glucosaminidase (NAG), which are indicators of renal epithelial injury. Tungsanga *et al*. [40] observed in their studies that stone patients exhibited higher levels of oxidative stress and renal tubular cell injury. Urolithiasis patients were also found to have higher plasma MDA, urinary MDA, and urinary NAG activity compared to normal controls. Furthermore, decreased levels of glutathione and cellular glutathione peroxidase activity, protein thiol, and vitamin E were reported in urolithiasis patients. A study investigating the oxidant and antioxidant status of blood in urolithiasis patients before and after surgery reported a reduce in oxidant enzymes and an rise in antioxidant status following surgical treatment [41]. In another study, plasma TBARS levels were used to assess membrane damage caused by oxygen radicals in renal tissue affected by urolithiasis. The study suggested that higher plasma TBARS levels in the group receiving HP (a treatment) compared to the non–HP group may be due to the expected antioxidant and anti–inflammatory properties of HP. While there was a slight increase in plasma TBARS levels in HP patients, this increase was not statistically significant. The findings indicated that HP supplementation improved oxidative stress and reduced lipid peroxidation products, supporting the antioxidant activity of HP in the treatment of kidney stones.

In this study, biochemical parameters were evaluated the level of creatinine clearance was found to be an important riser in the U group according to the other groups due to damage to the kidneys. The decrease in calcium ratios in the U–HP group according to the U group in 24-h urine shows that hypercalciuria decreased with the treatment we applied. In the amount of oxalate studied in the urine, a very high amount of Ox was found in the U group compared to the other groups. We found that HP suppressed urinary Ox levels in the U–HP group. These findings can be said to be one of the preventive effects of HP stone creation.

With this study, observed that the concentration of creatinine clearance was significantly higher in the U group compared to the other groups, due to kidney damage. The decrease in calcium ratios in the U–HP group, compared to the U group, in 24-h urine samples, indicates that hypercalciuria decreased with the treatment we administered. We observed a higher amount of Oxalate in the urine of the U group compared to the other groups. However, we found that HP reduced urinary Oxalate levels in the U–HP group. These findings indicate HP may have a preventive effect on kidney stone creation.

CONCLUSIONS

In conclusion, we support the hypothesis that HP prevents the formation of stones by correcting acute kidney damage through its antioxidant and anti–inflammatory effects, thus preventing oxidative stress.

Financial support

This study was not financially supported by any institution or organization.

Conflict of interest statement

The authors state that they have no conflicts of interest.

BIBLIOGRAPHIC REFERENCES

[1] Ilhan M, Ergene B, Süntar I, Özbilgin S, Saltan-Çitoğlu G, Demirel MA, Keleş H, Altun L, Küpeli-Akkol E. Preclinical evaluation of antiurolithiatic activity of *Viburnum opulus* L. on sodium oxalate-induced urolithiasis rat model. Evid. Based Compl. Alternat. Med. [Internet]. 2014; 2014:578103. doi: https://doi.org/f6dsz4

[2] Araújo Viel T, Diogo Domingos C, da Silva Monteiro AP, Riggio Lima-Landman MT, Lapa AJ, Souccar C. Evaluation of the antiurolithiatic activity of the extract of *Costus spiralis* Roscoe in rats. J. Ethnopharmacol. [Internet]. 1994; 66(2):193–198. doi: https://doi.org/d8zr4v

[3] Atmani F, Farell G, Lieske JC. Extract from *Herniaria hirsute* coats calcium oxalate monohydrate crystals and blocks their adhesion to renal epithelial cells. J. Urol. [Internet]. 2004; 172(4):1510–1514. doi: https://doi.org/c4hzm3

[4] Ramello A, Vitale C, Marangella M. Epidemiology of nephrolithiasis. J. Nephrol. 2000; 13(Suppl. 3):s45–s50. PMID:11132032

[5] Sohgaura A, Bigoniya P. A Review on epidemiology and etiology of renal stone. Am. J. Drug Discov. Dev. [Internet]. 2017; 7(2):54-62. doi: https://doi.org/g8z65g

[6] Khan SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol. Res. [Internet]. 2005; 33:349–357. doi: https://doi.org/bpzzn3

[7] Rodgers AL. Physicochemical mechanisms of stone formation. Urolithiasis [Internet]. 2017; 45:27–32. doi: https://doi.org/g8z65h

[8] Hackett RL, Shevock PN, Khan SR. Madin-Darby canine kidney cells are injured by exposure to oxalate and to calcium oxalate crystals. Urol. Res. [Internet]. 1994; 22:197–203. doi: https://doi.org/cbzm6d

[9] Khan SR. Role of renal epithelial cells in the initiation of calcium oxalate stones. Nephron Exp. Nephrol. [Internet]. 2004; 98(2):e55–e60. doi: https://doi.org/cxq54x

[10] Khan SR. Pathogenesis of oxalate urolithiasis: Lessons from experimental studies with rats. Am. J. Kidney Dis. [Internet]. 1991; 17(4):398–401. doi: https://doi.org/g8z65j

[11] Khan SR. Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue culture studies. J. Clin. Exp. Nephrol. [Internet]. 2004; 8:75–88. doi: https://doi.org/bmhhn9

[12] Gurocak S, Kupeli B. Consumption of historical and current phytother- apeutic agents for urolithiasis: a critical review. J. Urol. [Internet]. 2006; 176(2):450–455. doi: https://doi.org/dfjnzm

[13] Grases F, Melero G, Costa-Bauza A, Prieto R, March JG. Urolithiasis and phytotherapy. Int. Urol. Nephrol. [Internet]. 1994; 26(5):507–511. doi: https://doi.org/fr97nt

[14] Khan SR, Kok DJ. Modulators of urinary stone formation. Front. Biosci. [Internet]. 2004; 9:1450–1482. doi: https://doi.org/bzvj7b

[15] Yasui T, Okada A, Hamamoto S, Ando R, Taguchi K, Tozawa K, Kohri K. Pathophysiology-based treatment of urolithiasis. Int. J. Urol. [Internet]. 2017; 24(1):32–38. doi: https://doi.org/f9ndhj

[16] Zeng X, Xi Y, Jiang W. Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: A review. Crit. Rev. Food Sci. Nutr. [Internet]. 2018; 59(13):2125–2135. doi: https://doi.org/g8z65k

[17] Facino RM, Carini M, Franzoi L, Pirola O, Bosisio E. Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (Compositae). Pharm. Res. [Internet]. 1990; 22(6):709–721. doi: https://doi.org/bcd6fd

[18] Binu TV, Vijayakumari B. Herbal plants as a remedy for urolithiasis - A review. Int. J. Pharm. Res. Dev. 2014; 6(6):35–39.

[19] Altundag E, Ozturk M. Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. Procedia Soc. Behav. Sci. [Internet]. 2011; 19:756–777. doi: https://doi.org/b79m79

[20] Aslan M, Orhan DD, Orhan N, Sezik E, Yesilada E. *In vivo* antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *plicatum* capitulums in streptozotocin-induced-diabetic rats. J. Ethnopharmacol. [Internet]. 2007; 109(1):54–59. doi: https://doi.org/bjfw45

[21] Ozbek T, Gulluce M, Adiguzel A, Ozkan H, Sahin F, Orhan F. Antimutagenic activity of the methanol extract of *Helichrysum plicatum* ssp *plicatum*. Asian J. Chem. [Internet]. 2009 [cited 12 Jul. 2024]; 21(4):2705–2710. Available in: https://goo.su/JsLi

[22] Demir A, Mercanoglu Taban B, Aslan M, Yesilada E, Aytac SA. Antimicrobial effect of *Helichrysum plicatum* subsp *plicatum*. Pharm. Biol. [Internet]. 2009; 47(4):289–297. doi: https://doi.org/cgkwwp

[23] Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P, Rios JL. Antioxidant activity of anti-inflammatory plant extracts. Life Sci. [Internet]. 2002; 70(9):1023–1033. doi: https://doi.org/fjm45w

[24] Aydin T. Secondary metabolites of *Helichrysum plicatum* DC. subsp. *plicatum* flowers as strong carbonic anhydrase, cholinesterase and α-glycosidase inhibitors. Z. Naturforsch. C. [Internet]. 2020; 75(5-6):153–159. doi: https://doi.org/g8z65m

[25] Sahin K, Tuzcu M, Sahin N, Akdemir F, Ozercan I, Bayraktar S, Kucuk O. Inhibitory effects of combination of lycopene and genistein on 7,12- dimethyl benz(a)anthracene-induced breast cancer in rats. Nutr. Cancer. [Internet]. 2011; 63(8):1279–1286. doi: https://doi.org/bmjrkd

[26] Coskun H, Andic F, Daglioglu YK, Doran F, Sahin K, Tunali C, Kucuk O. Lycopene in the prevention of radiation-induced esophagitis. Nutr. Cancer. [Internet]. 2017; 69(2):319–329. doi: https://doi.org/g8z65n

[27] Wazowicz W, Nève J, Peretz A. Optimized steps in fluorometric determination of acid-thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. Clin. Chem. [Internet]. 1993; 39(12):2522–2526. doi: https://doi.org/g8z65p

[28] Andic F, Garipagaoglu M, Yurdakonar E, Yurdakonar E, Tuncel M, Kucuk O. Lycopene in the prevention of gastrointestinal toxicity of radiotherapy. Nutr. Cancer. [Internet]. 2009; 61(6):784-788. doi: https://doi.org/bdx9xb

[29] Oksay T, Yunusoğlu S, Calapoğlu M, Candan IA, Onaran I, Ergün O, Özorak A. Protective impact of resveratrol in experimental rat model of hyperoxaluria. Int. Urol. Nephrol. [Internet]. 2017; 49:769–775. doi: https://doi.org/f96tmc

[30] Bayir Y, Halıcı Z, Keles MS, Colak S, Cakır A, Kaya Y, Akcay F. *Helichrysum plicatum* DC. subsp. *plicatum* extract as a preventive agent in experimentally induced urolithiasis model. J. Ethnopharmacol. [Internet]. 2011; 138(2):408–414. doi: https://doi.org/cq2wcw

[31] Onaran M, Orhan N, Farahvash A, Ekin HN, Kocabiyik M, Gönül II, Şen I, Aslan M. Successful treatment of sodium oxalate ınduced urolithiasis with *Helichrysum* flowers. J. Ethnopharmacol. [Internet]. 2016; 186:322–328. doi: https://doi.org/g8z65q

[32] Koul H, Kennington L, Nair G, Honeyman T, Menon M, Scheid C. Oxalate induced initiation of DNA synthesis in LLC-PK1 cells, a line of renal epithelial cells. Biochem. Biophys. Res. Comm. [Internet]. 1994; 205(3):1632–1637. doi: https://doi.org/ft9s4s

[33] Scheid C, Koul H, Hill WA, Luber-Narod J, Kennington L, Honeyman T, Jonassen J, Menon M. Oxalate toxicity in LLC-PK_1 cells: role of free radicals. Kidney Int. [Internet]. 1996; 49(2):413–419. doi: https://doi.org/bnqd3p

[34] Thamilselvan S, Hackett RL, Khan SR. Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. J. Urol. [Internet]. 1997; 157(3):1059–1063. doi: https://doi.org/b2k2vm

[35] Thamilselvan S, Byer KJ, Hackett RL, Khan SR. Free radical scavengers catalase and superoxide dismutase provide protection from oxalate associated injury to LLC-PK_1 and MDCK cells. J. Urol. [Internet]. 2000; 164(1):224–229. doi: https://doi.org/ftzghm

[36] Thamilselvan S, Khan SR, Menon M. Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. Urol. Res. [Internet]. 2003; 31:3–9. doi: https://doi.org/b4n7ff

[37] Selvam R. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. Urol. Res. [Internet]. 2002; 30:35–47.doi: https://doi.org/c9b73x

[38] Ilbey YO, Ozbek E, Simsek A, Cekmen M, Somay A, Tasci AI. Effects of pomegranate juice on hyperoxaluria-induced oxidative stress in the rat kidneys. Ren. Fail. [Internet]. 2009; 31(6):522–531. doi: https://doi.org/bxm8cr

[39] Huang HS, Ma MC, Chen CF, Chen J. Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology [Internet]. 2003; 62(6):1123–1128. doi: https://doi.org/fqqkg5

[40] Tungsanga K, Sriboonlue P, Futrakul P, Yachantha C, Tosukhowong P. Renal tubular cell damage and oxidative stress in renal stone patients and the effect of potassium citrate treatment. Uro. Res. [Internet]. 2005; 33:65–69. doi: https://doi.org/br57zv

[41] Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. J. Med. Life. [Internet]. 2016 [cited 18 Jul. 2024]; 9(3):259–262. PMID: 27974930. Available in: https://goo.su/5XI7n