The effect of ethanolic extract of Halfa-bar (Cymbopogon Proximus) and parsley (Petroselinum sativum) as an Anti-urolithiasis

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ABSTRACT:

In order to investigate the anti-urolithic effects of *Cymbopogon proximus* (halfa-bar) *and Petroselinum sativum* (parsley) extracts, ethylene glycol-induced renal stones were assessed in male albino rats at a dose of 500 mg/kg body weight/day and compared with a dose of 750 mg/kg body weight/day of cystone as a reference drug. Ethylene glycol (0.75% v/v) and ammonium chloride (1% w/v) in drinking water can trigger urolithiasis. Animals were divided into six groups: normal control, positive control, standard control, halfa-bar group, parsley group, and plants mixture (1:1). Plant extract therapy changed all of the increased biochemical indicators, including serum (creatinine, urea, and uric acid). In addition, the plant extract groups normalized urine pH, provided a closer look at calcium oxalate crystals under a microscope, and more effectively supported kidney function. Microscopic and histopathological results confirmed and showed that the potential preventive and therapeutic plant extracts inhibit kidney stones. Finally, the plant extracts have a combination of phytochemicals, antioxidant, diuretic, urine alkalinizing, and calcium-lowering actions that act as an anti-urolithiasis impact against calcium oxalate stones. The results confirmed that a mixture of the two plants was superior in potency, followed by *C. proximus* and then *P. sativum*.

Keywords: Renal stones; Renal function; Phytochemical; *petroselinum sativum* and *cymbopogon proximus*.

INTRODUCTION

Herbs have a long history of medicinal efficacy with low toxicity and few side effects (Liu *et al.,* 2023). Kidney disease is a major public health problem affecting a lot of people over the world, and the main limitations of traditional therapies are adverse effects on human health and high drug costs. therefore, new therapeutic strategies that is cheaper and has fewer side effects need to be developed (Noureddine *et al.*, 2022) medicinal plants can be used as an alternative therapy in the treatment of various diseases, including kidney disease, due to their natural constituents.

Castaneda *et al.* (2023) reported that kidney disease is now one of the main causes of death worldwide and that its frequency has risen quickly in recent years. For those with kidney stones, complementary and alternative medicine (CAM) is frequently employed. It is made up of preparations with a variety of substances, like herbs and vitamins (Cupisti *et al.,* 2023).

One of the most prevalent illnesses of the urinary system is kidney stone disease. Super saturation of stone-forming solutes in urine, which results in the nucleation, crystallization, and aggregation of minerals and salts, primarily calcium oxalate (CaOx), is the primary cause of kidney stone formation (Li *et al.,* 2022).

Kumar *et al.,* (2016); Niharika *et al.,* (2018); Nirumand *et al.,* (2018) and Agawane *et al.,* (2019) reported that Plant extracts have been used in the prevention and treatment of many diseases, especially kidney disease and kidney stones.

Especially for kidney illness and kidney stones, plant extracts have been employed in the prevention and treatment of various ailments. A diverse plant genus known as halfa-bar is extensively found in tropical and subtropical areas of Asia, Africa, and America (Avoseh *et al.,* 2015). The use of *Cymbopogon* in traditional medicine is well documented, and different species of *Cymbopogon* around the world have been reported to treat coughs, fevers, infections, cancer, and digestive disorders (Dutta *et al.,* 2016).

In vivo and in vitro studies demonstrate the beneficial pharmacological effects of *Cymbopogon spp*. According to Ekpenyong *et al.,* (2014) and Khan *et al.,* (2018), displays include anticancer, cardioprotective, anti-inflammatory, antioxidant, antidiabetic, anticholinesterase, antibacterial,

and antifungal properties. *Cymbopogon proximus* is an herbaceous plant. It belongs to the Umbelliferae family (Eltahir and Abuereish 2010). According to Selim (2011), *C. proximus* is also found in the Egyptian desert and the sandy shores of the Red Sea in central and northern Sudan and on the southern border of Egypt.

The presence of saponins, flavonoids, glycosides, and tannins in *Cymbopogon proximus*, as determined by phytochemical assays, may be the basis for this plant's usage in therapeutic formulations. Lower levels of serum calcium, serum blood urea nitrogen (BUN), and renal calcium were treated with *Cymbopogon proximus*. Additionally, it significantly reduces the risk of renal stones in rats brought on by ethylene glycol (Warrag *et al.,* 2014).

Petroselinum sativum. Commonly called "parsley" in English, it belongs to the Umbelliferae family (Agyare *et al.,* 2017). Parsley is native to the Mediterranean region and is now grown worldwide (Farzaei *et al.,* 2013). *Petroselinum sativum*. Has multiple beneficial activities, mainly antioxidant, analgesic, antispasmodic, antidiabetic, immunomodulatory, and gastrointestinal effects (Farzaei *et al.,* 2013).

Petroselinum sativum has been popular since ancient times for its ability to enhance flavour and aroma, due to its presence of vitamins, minerals, and other bioactive compounds such as furanocoumarins, essential oils, flavonoids, carotenoids, oleoresins, tannins, glycosides, and fatty acids (Chauhan and Aishwarya 2018).The aim of this work is investigate the *in-vivo* anti urolithiatic effect of extract of *C.proximus, P.sativum* and the mixture of two plants in Ethylene glycol induced urolithiasis.

MATERIALS AND METHODS

Collection of Plant Material:

Plants used in this study were collected from different regions in (Egypt; April 2022). Halfa-bar samples were obtained from Halayeb and Wadi Alaba, Aswan, Egypt. While the parsley samples were collected from a Calendula farm in Fayoum province for the production of medicinal and aromatic plants, the plant extracts were kept in a refrigerator until use.

Animals:

Thirty-six Wistar albino rats (180-200 g) used in this study were obtained from the Animal House, Faculty of Pharmacology, Al-Azhar University, Cairo, Egypt. They were housed under standard temperature, humidity and light conditions (12 hrs dark, 12 hrs light) and fed a commercially available pelleted diet with free access to water.

Methods:

Extraction of plant material:

Fresh plant leaves of *Cymbopogon proximus* (Cp) and *Petroselinum sativum* (Ps) were used for extraction according to (Ferrigni *et al*., 1982). The leaves of each plant were washed with water and cut into pieces. Five grams (5g) were extracted with 50 ml of 70% ethanol (v/v) and hot water (30 min. at 80^{o}C) by grinding and socked overnight. After socking the sample were filtered using Whitman No.1 filter papers. There the filtrated were centrifuged at 4000 rpm for 10 min. then kept at 4^{o}C until use.

Phytochemical screening of extracts:

The phytochemical screening of plants ethanol extract were tested according to El-khamissi *et al.,* (2019) to detect the secondary metabolites (tannins, saponins, alkaloids, terpenoids, flavonoids, phenols, phytosterols and steroids).

Identification of phenolic compounds by HPLC:

HPLC system consisting E-Chrom Tech Model LC 1620 A Liquid chromatography equipped with a UV detector at wavelength 280 nm. The analysis was achieved on Column C 18: Shodex C18-120-5 4 E (250*4.6 mm), Pump: P 1620A Pump, Software: PA Station 2015 ChemStation Version 2.0, Flow Rate:1ml/minute, Eluent: Methanol: water: tetrahydrofuran: acetic acid (23: 75: 1: 1), respectively. Total phenolic compounds were extracted and subjected to HPLC analysis according to El-Hamamsy & El-Khamissi (2020).

In vivo anti-urolithiasis activity of various extracts:

The anti-urolithiasis activity of *Cymbopogon proximus* and *Petroselinum sativum* extracts was evaluated in vivo in urolithiasis affected Wistar rats. Urolithiasis was induced by oral administration of ethylene glycol (0.75% v/v)

and 1% ammonium chloride in drinking water (Karadi *et al.,* 2006).

Ethylene glycol plus ammonium chloride model was used to induce urolithiasis. Kidney stone formation was induced in rats by ad libitum administration of 0.75% v/v ethylene glycol and 1% w/v ammonium chloride in drinking water for 3 days. Treatment was then switched to only 0.75% ethylene glycol for 25 days. The anti-urolithiasis effect of plant extracts was compared with the standard drug Cystone (Mahmud *et al.,* 2021).

Cystone: Each tablet contains 223 mg of Cystone – (Multipharma 6C Taksim Asmaa Fahmy Heliopolis, Cairo, Egypt) as a standard anti-urolithiasis drug. Cystone drug dissolved in distilled water at a dose of 750 mg/kg body weight of rat using a stomach tube (Mitra *et al.,* 1998). After a week of acclimatization, the rats were divided into six groups containing six animals in each.

Experiment design:

Urine analysis (urine pH):

The acidity of the urine was tested using pH meter according to Wagner and mohebbi, (2010).

Microscopic evaluation of calcium oxalate crystallization in urine:

Microscopic examination of calcium oxalate crystals was also performed on this specimen and urolithiasis was confirmed. Urine samples were collected and separated by centrifugation at 10,000 rpm for 10 minutes. The formed precipitate was examined using the objective lens (40 xs) on a microscope provided with ProView software (B-3W Windows tablet PC with B3 camera, Italy).

Stone analysis:

Spot test analysis for qualitative testing of urinary stone components was performed according to the kit instructions (Kit for the analysis of urinary stones, DiaSys, Diagnostic System GmbH, Holzheim, Germany). This method allows detecting the presence of cystine and following ions usually present in urinary calculi: carbonate, calcium, oxalate, ammonium, phosphate, magnesium, and urate. The assay consists of the addition of chemical reagents labeled R1 to R15 dropwise to the finely pulverized sample and placed into a vessel with 50 mL of distilled water. Then the appearance of certain colors, precipitates, or air bubbles would indicate positive results for one of the ions and cysteine (Shabsoug, 2003).

Biochemical assay in serum:

Collection of blood samples:

At the end of the experiment, retro-orbital blood samples were withdrawn from the orbital plexus under anesthesia, using heparinized micro-capillaries (Opti lab, Berlin, Germany).

Separation of serum samples:

Serum was separated by blood centrifugation at 4000 rpm for 10 min at – 4°C (Heraeus Biofuge, Loughborough, UK). The serum supernatant was collected and then used for the determination of serum constituents like creatinine, uric acid and urea.

Kidney Function Test

***Determination of urea*:**

When exposed to water and urease, urea breaks down into ammonia and carbon dioxide. The resulting ammonia ions then engage with hypochlorite and salicylate to generate a vibrant green indophenol dye. In line with the Fawcett and Scott (1960) method, the saturation of color is directly linked to the level of urea concentration.

Determination of Creatinine:

Schirmeister *et al.,* (1964) discovered that creatinine produces a colored complex when combined with picrate in an alkaline solution, using the above-mentioned method.

Determination of Uric acid:

Burtis (1999) technique of Uricase/Pap methodology is employed to assess uric acid levels in serum.

Histopathological examination of kidney sections:

To prepare Kidney Tissue samples for analysis, they were flushed and fixed in 10% neutral buffered formalin for 72 hours. After trimming, the samples underwent processing in successive grades of ethanol's and were ultimately cleared in Xylene. To facilitate embedding, samples were infiltrated with Paraplast tissue embedding media. Using a rotatory microtome, 4µn thick tissue sections were then cut and mounted on glass slides.

For a comprehensive morphological investigation, Hematoxylin and Eosin stained no less than 3 tissue sections per sample. Following staining, an experienced histologist assessed the specimens via light microscope (Leica Microsystems GmbH, Wetzlar, Germany) in a blinded manner. Lesions were then graded as per **(El-**Nabarawy *et al*., 2020) protocol.

Statistical analysis:

Analysis of quantitative and graphical data was conducted with the Microsoft Excel Package. ANOVA analysis was used to determine the mean ± and standard deviation for each series of experiments, which were performed in triplicates (n =3). The device system software was utilized for HPLC analysis, including measurement of peak areas and data processing. **(**Steel and Torrie,1980).

RESULTS AND DISCUSSION

Phytochemical screening of extracts:

Petroselinum sativum contains tannins, saponins, alkaloids, terpenoids, flavonoids, and phenols, according to the phytochemical analysis of the plant extracts shown in Table 2. On the other hand, Steroids and phytosterols are only found in *Cymbopogon proximus* extracts. Alkaloids were detected in both extracts, making them a valuable addition to the field of medicine.

pharmaceutical companies use some plant extracts rich in bioactive phytochemicals as a diuretic and prevent kidney stone around the world and in Egypt for a very long time (Ammar, 2011; Monsef, 2011 and Badr, 2015).

Cymbopogon proximus contains a full complement of bioactive compounds, including saponins, alkaloids, terpenoids, flavonoids, phenols, steroids, and phytosterols agreement with Malin *et al.* (2018) and Abaddi (2019).

Petroselinum sativum's phytochemical composition are agreement with Alqethami and Aldhebiani (2021) and Mohammed and Al-Ibrahemi (2022).

Identification of phenolic compounds by HPLC:

Analysis of ethanolic extract of C.proximus by HPLC:

Data in Table (3) and Figure (1) indicated that the HPLC analysis of the extracts were given five phenolic compounds. The main phenolic compounds were p-coumaric 10.50µg/ml with Pyrogallol 8.29µg/ml. Also, the ethanolic extract of *C. proximus* plant contains of Catechol, Cinnamic and Ferulic were 5.23, 4.89 and 7.30µg/ml, respectively.

Gonçalves *et al.,* (2021) noted that presence of catechol, pyrogallol, and methoxy groups in plant extracts, which provide substantial scavenging, anti-apoptotic, and anti-inflammatory effects, and improve kidney function.

Analysis of ethanolic extract of P. sativum by HPLC:

Data in Table (4) and Figure (2) indicated that the HPLC analysis of the extracts were given six phenolic compounds. The main phenolic compound was Gallic 11.44 µg/ml. Also, the ethanolic extract of *P. sativum* plant contains of Syringic (4.66 µg/ml), Benzoic (5.47 µg/ml), Caffeic (7.60 µg/ml), Pyrogallol (2.19 µg/ml) and Cinnamic (3.69 µg/ml).

Cechinel-Zanchett *et al.,* (2021) found that the Gallic Acid (GA) inhibited about 44-57% of the total CaOx crystals formation invitro:

Analysis of ethanolic extract of mixture of two plants by HPLC:

Data in Table (5) and Figure (3) indicated that the HPLC analysis of the extract was given nine phenolic compounds which have been detected in ethanolic extract obtained from mixture of two plants.

The main phenolic compounds were Gallic (12.18µg/ml) and Cinnamic (10.55µg/ml). The ethanolic extract of mixture of two plants also contains Chlorogenic (6.11µg/ml), Syringenic (2.22µg/ml), P-coumaric (2.36µg/ml), Caffeic (4.22µg/ml), Ferulic (2.17µg/ml), Salicylic (2.09µg/ml) and Benzoic (1.80µg/ml). Babaeenezhad *et al.,* (2021) noted that cinnamic acid reduces induced nephrotoxicity and changes in transaminase activities in rats through its antioxidant activities.

In-Vivo Anti-urolithiatic activity of various extract of C. Proximus and P. Sativum. on ethylene glycol induced in rats:

Urine analysis (pH):

Urinary pH of negative control is neutral, ethylene glycol induced pH is reduced compared with control group. Treatment with

(Cystone 750 mg/kg) was found to increase the urine pH (7.63± 0.03), whereas, group receiving the mix extract also found to increase significant the urinary pH (7.56± 0.02) but *C. proximus* extract and *P. sativum* extract produces increase in pH nearly to that of control ($p<0.05$) (Table 6 and Figure 4); this result is similar to the result Moram (2016) regarding *P. sativum* and Ibrahim and El-Khateeb (2013) regarding *C. proximus.*

Microscopic evaluation of calcium oxalate crystallization in urine:

Microscopic analysis of calcium oxalate precipitation in urine: In this study, it was found that, when compared to healthy control rats, the positive control rats had a high density of calcium oxalate crystals, which were only partially absent in the all-plant extract and cystone-treated groups (Figure 5). The similarity of the outcomes led to the random selection of the images.

Stone analysis:

At the end of the experiment and during the slaughter of one of the rats in the second group (the positive control), we noticed that a large stone had formed in the urinary bladder, and we extracted and analyzed it in order to identify its size and chemical composition by chemical spot testing. As a result of feeding with ethylene glycol and ammonium chloride, the results showed that it is composed of calcium oxalate salts and ammonium salts (Table 7 and Figure 6). Animals given ethylene glycol develop stones because of hyperoxaluria, which increases oxalate excretion and renal retention, according to Bahuguna *et al.* (2009).

Biochemical in serum:

Kidney Function Tests:

The results in table (8) and figure (7) shown that the ethanolic plant extract of mixture and C.proximus (Group 4 and 6) and Cystone (Group3) significantly lowered the elevated serum level uric acid as compared to positive control. *P. sativum* extract also decreased the levels of urea, uric acid, and creatinine in the serum of kidney stone-induced rats (Group 5) and came in last place after the mixture and half a bar; this result is in agreement with ElGhazali *et al.,* (2022).

A decrease in glomerular filtration due to obstruction generated in the kidney causes accumulation of waste products in the blood, and thus the level of waste components like uric acid, urea, and creatinine increases in the blood. Creatinine is a metabolism molecule product that is produced from the muscle metabolism. Creatinine is an important molecule in the process of energy production in the muscle; it is produced from creatine. Every day, about 2% of the creatine in the body is converted into creatinine (Horio, 2014).

These chemicals are carried by blood vessels into the kidneys. The creatinine in the urine is filtered out and eliminated by the kidneys. The creatinine was a trustworthy predictor of renal function. The end result of protein metabolism and nitrogen-containing amino acids is urea. Eliminating these potentially hazardous compounds from the body is one of the major functions of the kidneys. Blood urea nitrogen (BUN) levels rise when renal function declines (Meier *et al.,* 2004).

One indicator of renal health was the BUN level. Rats' blood serum urea levels were measured in order to ascertain the effects of ethylene glycol 0.75% and 1% ammonium chloride on kidney injury, as well as the impact of various ethanol plant extracts.

The higher serum levels of creatinine, uric acid, and urea in calculi-induced rats (Group II positive control) indicated significant kidney injury (Bahuguna *et al.,* 2009). However, the kidney function (as measured by creatinine, uric acid, and urea) was improved by the extract treatment. Similar to positive control rats, the cystone-treated group showed improvements in the preceding serum parameters.

Histopathological examination of kidney sections:

Microscopic examination of kidney samples from different groups revealed; Normal control samples showed almost intact well organized morphological features of renal parenchyma with almost apparent intact renal tubular segments with showing intact tubular epithelium (arrow), minimal records of degenerated tubular epithelial cells, intact renal corpuscles (star) and intact vasculatures (Figure 8).

Positive control samples

wide areas different tubular segments revealed marked tubular necrosis or degenerative changes with many records of

nuclear pyknosis (red arrow), moderate dilatation of Bowman's space as well as tubular dilatations (star) with intraluminal cellular casts (yellow arrow). Abundant records of interstitial mononuclear inflammatory cells infiltrates were showed (black arrow head) accompanied with many congested vasculatures (red arrow head) (Figure 9).

Standard control samples (Cystone)

Showed significant protective efficacy on renal tubular epithelium (black arrow) with persistent mild records of tubular degenerative changes (red arrow), moderate congested interstitial BVs (red arrow head) with mild focal dilatation of renal tubular segments (star). Moreover, minimal records of inflammatory cells infiltrates were observed (Figure 10).

P. sativum extract samples

showed evidence of lesser protective efficacy compared with *C. proximus* treated samples with almost more or less persistent records as model samples (Figure 12). This result agree with Bushuty *et al.,* (2021) who reported that, in relation to the results of histopathological studies, it indicated good effects of *P. sativum* on the kidney and liver.

Mixture Extract samples

demonstrated the best protective efficacy among treatment groups with abundant records of apparent intact tubular segments and lining epithelium (black arrow), sporadic occasional degenerative changes records (red arrow) as well as minimal inflammatory cells (black arrowhead). However, mild to moderate focal records of congested vasculatures were showed (red arrow head) (Figure 13).

CONCLUSION

As a result of physicochemical changes in the urine environment, which cause crystal nucleation, growth aggregation, and retention at particular places inside the kidney, kidney stone formation is a complicated, multifactorial, and multistep process. Herbal remedies that contain phytochemicals with antioxidant qualities can lessen crystallization, which will lessen crystal deposition on the kidney and diminish stone recurrence.

Additionally, by lowering epithelial damage, the treatments may lessen crystal formation, deposition in the terminal collecting ducts, and the creation of new stones. The plants in this study have decreased CaOx deposition, improved renal architecture, increased urine pH, decreased lithogenic components, enhanced renal function, and diuresis as its antiurolithic mechanisms.

C.proximus extract samples showed moderate protective efficacy with alternated higher records of apparent intact tubular segments (black arrow), Fewer focal records of necrotic tubular segments in some tissue sections were showed (red arrow) as well as congested interstitial BVs. Mild persistent interstitial mononuclear inflammatory cells infiltrates (arrow head) were showed (Figure 11). This results agreement with El-Nabtity *et al.,* (2019) who noted that Examined sections from kidney showed mild degenerative changes in the tubular epithelium, ureter and urethra revealed normal histomorphological structures mean while the Urinary bladder revealed focal desquamation of the transitional epithelia, on Sulfadimidine induced urolithiasis in male New Zealand rabbits.

REFERENCES

Abaddi, T.A.J. 2019: *Antimicrobial Activity of Solenostemma argel and Cymbopogon proximus Against Carbapenem Resistant Gram Negative Bacteria Isolated from Different Hospitals in Khartoum State* (Doctoral dissertation, Sudan University of Science & Technology).

Agawane, S.B., Gupta, V.S., Kulkarni, M.J., Bhattacharya, A.K., Koratkar, S.S., Rao, V.K. 2019: Patho-physiological evaluation of Duranta erecta for the treatment of urolithiasis. *Journal of Ayurveda and integrative medicine*, *10*(1), 4-11.

Agyare, C., Appiah, T., Boakye, Y.D., Apenteng, J.A. 2017: Petroselinum crispum: a review. *Medicinal spices and vegetables from Africa*, 527-547.

Alqethami, A., Aldhebiani, A.Y. 2021: Medicinal plants used in Jeddah, Saudi Arabia: phytochemical screening. *Saudi Journal of Biological Sciences*, *28*(1), 805-812.

Ammar, N. 201**1:** *Effect of low protein, phosphorus, 432rabic432um diets and high gum 432rabic content on ratssuffering from chronic renal failure* (Doctoral dissertation, Ph. D. Thesis in Nutrition and Food Science, Faculty of Home Economics, AlAzhar University, Nawag, Tanta, Egypt).

Avoseh, O., Oyedeji, O., Rungqu, P., Nkeh-Chungag, B., Oyedeji, A. 2015**:** Cymbopogon species; ethnopharmacology, phytochemistry

and the pharmacological importance. *Molecules (Basel, Switzerland)*, *20*(5), 7438–7453.

Babaeenezhad, E., Nouryazdan, N., Nasri, M., Ahmadvand, H., Moradi Sarabi, M. 2021: Cinnamic acid ameliorate gentamicin-induced liver dysfunctions and nephrotoxicity in rats through induction of antioxidant activities. Heliyon, 7(7), e07465.

Badr, W. 2015: *The protective effect of black mulberry and plum in rats with induced renal dysfunction* (Doctoral dissertation, Ph. D. Thesis in Home Ecnomics (Nutrition and Food Science), Faculty of Home Economics, Al-Azhar University, Nawag, Tanta, Egypt).

Bahuguna, Y., Rawat, M.S.M., Juyal, V., Gupta, V. 2009: Antilithiatic effect of flowers of Jasminum auriculatum Vahl. International Journal of Green Pharmacy (IJGP), 3(2).

Burtis, C.A. 1999: *Tietz textbook of clinical chemistry*. Saunders.

Bushuty, H.E., Talaat Sahloul, O., Ebrahim Abdel-Halim, E. 2021: Impact of Parsley Extract on Experimental Rats with Kidney Stones and using it to make some food products.*journal of home economics*, *37*(1), 37-58.

Castañeda, R., Cáceres, A., Cruz, S.M., Aceituno, J.A., Marroquín, E.S., Barrios Sosa, A.C., Strangman, W.K., Williamson, R.T. 2023: Nephroprotective plant species used in traditional Mayan Medicine for renal-associated diseases. *Journal of ethnopharmacology*, *301*, 115755.

Cechinel-Zanchett, C.C., Bolda Mariano, L.N., Schlickmann, F., Cechinel-Filho, V., de Souza, P. 2021: In vitro effects of two bioactive compounds, gallic acid and methyl gallate, on urolithiasis. Actas urologicas espanolas, 45(9), 604–608.

Chauhan, E.S., Aishwarya, J. 2018: Nutraceuticals potential of Petroselinum crispum: A Review. *J. Complement. Med. Altern. Healthc*, *7*, 1-6.

Cupisti, A., Giannese, D., D'Alessandro, C., Benedetti, A., Panichi, V., Alfieri, C., Castellano, G., Messa, P. 2023: Kidney Stone Prevention: Is There a Role for Complementary and Alternative Medicine?. *Nutrients*, *15*(4), 877.

Dutta, S., Munda, S., Lal, M., Bhattacharyya, P.R. 2016: A short review on chemical composition, therapeutic use and enzyme inhibition activities of Cymbopogon species. *Indian Journal of Science and Technology*, *9*(46), 1-9.

Ekpenyong, C.E., Davies, K., Antai, E.E. 2014: Cymbopogon citratus Stapf (DC) extract ameliorates atherogenic cardiovascular risk in diabetes-induced dyslipidemia in rats. *British Journal of Medicine and Medical Research*, *4*(28), 4695-4709.

ElGhazali, M.N., Mostafa, U.E.S., Tawfeuk, H.Z., Abd Almaged1, H.A. 2022: Effect of nutrition on halfa barr (cymbopogen proximus) and damsissa (ambrosia maritima) on infected experimental rats of kidney and liver diseases.

El-Hamamsy, S.M.A., El-Khamissi, H.A.Z. 2020: Phytochemicals, Antioxidant Activity and Identification of Phenolic Compounds by HPLC of Pomegranate (Punica granatum L.) Peel Extracts. *Journal of Agricultural Chemistry and Biotechnology*, *11*(4), 79-84.

El-khamissi, H., Saad, Z., Rozan, H. 2019: Phytochemicals screening, antioxidant and anticancer activities of garlic (Allium sativum) extracts. *Journal of Agricultural Chemistry and Biotechnology*, *10*(4), 79-82.

El-Nabarawy, N.A., Gouda, A.S., Khattab, M.A., Rashed, L.A. 2020: Effects of nitrite graded doses on hepatotoxicity and nephrotoxicity, histopathological alterations, and activation of apoptosis in adult rats. *Environmental Science and Pollution Research*, *27*, 14019-14032.

El-Nabtity, S., Abdllatief, S., Al-Attar, A.S.,Taha, S. 2019: Antiurolithiatic effect of Cymbopogon Proximus, Alhagi Maurorum, on Sulfadimidine induced urolithiasis in male New Zealand rabbits. Mansoura Veterinary Medical Journal, 20(1), 14-21.

Eltahir, A.S., AbuEReish, B.I. 2010: Comparative foliar epidermal studies in Cymbopogon citratus and Cymbopogon schoenanthus in Sudan. *Journal of Chemical and Pharmaceutical Research*, *2*(4), 449-455.

Farzaei, M.H., Abbasabadi, Z., Ardekani, M.R.S., Rahimi, R., Farzaei, F. 2013: Parsley: a review of ethnopharmacology, phytochemistry and biological activities. *Journal of traditional Chinese medicine*, *33*(6), 815-826.

Fawcett, J., Scott, J. 1960: A rapid and precise method for the determination of urea. *Journal of clinical pathology*, *13*(2), 156-159.

Ferrigni, N., Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., Melaughlin, J. 1982: Brine shrimp: A convenient general bioassay for active plant constituents. J. Planta Medica., 45: 31-34.

Gonçalves, A.C., Nunes, A.R., Falcão, A., Alves, G., Silva, L.R. 2021: Dietary Effects of Anthocyanins in Human Health: A Comprehensive Review. Pharmaceuticals (Basel, Switzerland), 14(7), 690.

Horio, M. 2014: New topics regarding equations for GFR estimation based on serum creatinine and

cystatin C. *Rinsho byori. The Japanese journal of clinical pathology*, *62*(2), 153-162.

Ibrahim, F.Y., El-Khateeb, A.Y. 2013**:** Effect of herbal beverages of Foeniculum vulgare and Cymbopogon proximus on inhibition of calcium oxalate renal crystals formation in rats. *Annals of Agricultural Sciences*, *58*(2), 221-229.

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.

Khan, S.J., Afroz, S., Khan, R.A. 2018: Antihyperlipidemic and anti-hyperglycemic effects of Cymbopogon jwarancusa in high-fat high-sugar diet model. *Pakistan Journal of Pharmaceutical Sciences*, *31*(4).

Kumar, B.N., Wadud, A., Jahan, N., Sofi, G., Bano, H., Makbul, S.A.A., Husain, S. 2016: Antilithiatic effect of Peucedanum grande CB Clarke in chemically induced urolithiasis in rats. *Journal of ethnopharmacology*, *194*, 1122-1129.

Li, S., Macaringue, E.G.J., Zhou, D., Shi, P., Tang, W., Gong, J. 2022: Discovering inhibitor molecules for pathological crystallization of CaOx kidney stones from natural extracts of medical herbs. *Journal of ethnopharmacology*, *284*, 114733.

Liu, H., Bai, Y., Huang, C., Wang, Y., Ji, Y., Du, Y., Xu, L., Yu, D.G., Bligh, S.W.A. 2023: Recent Progress of Electrospun Herbal Medicine Nanofibers. *Biomolecules*, *13*(1), 184.

Mahmud, S.A., Khdhr, A.R., Taha, A.M., Abdulla, P.M., Chato, K.B. 2021**:** Effects of Allium siculum in ethylene glycol induced kidney stone in male albino rats. *Kuwait Journal of Science*, *48*(3).

Malin, M.A., Ali, M.M., Ramadhani, A.M. 2018: GC-MS analysis and antimicrobial activities of Cymbopogon proximus essential oil and phytochemical screening of its crude extracts. Journal of Medicinal Plants, 6(4), 117-122.

Meier, J.J., Nauck, M.A., Kranz, D., Holst, J.J., Deacon, C.F., Gaeckler, D., Gallwitz, B. 2004: Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes*, *53*(3), 654-662.

Mitra, S.K., Gopumadhavan, S., Venkataranganna, M.V., Sundaram, R. 1998: Effect of cystone, a herbal formulation, on glycolic acid-induced urolithiasis in rats.

Mohammed, R.K., Al-Ibrahemi, N. 2022: Phytochemical and Protective Study for The Petroselinum Sativum L. (Parsley) on The Oxidative Stress and Antioxidant in Rats. *The Egyptian Journal of Hospital Medicine*, *89*(2), 8039-8042.

Monsef, W. 2011: *Toxicological studies of some plant extracts rich with phenolic compounds in vitro* (Doctoral dissertation, M. Sc. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt).

Moram, G.S.E. 2016: Evaluation of anti-urolithiatic effect of aqueous extract of parsley (Petroselinum sativum) using ethylene glycol-induced renal calculi. *Wor J Pharmace Res*, *5*(2), 1721-1735.

Niharika, M., Suchitha, N., Akhila, S., Himabindhu, J., Ramanjaneyulu, K. 2018: Evaluation of in vitro antiurolithiatic activity of Gossypium herbaceum. *Journal of Pharmaceutical Sciences and Research*, *10*(5), 1236-1237.

Nirumand, M.C., Hajialyani, M., Rahimi, R., Farzaei, M.H., Zingue, S., Nabavi, S.M., Bishayee, A. 2018: Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. *International journal of molecular sciences*, *19*(3), 765.

Noureddine, B., Mostafa, E., Mandal, S.C. 2022: Ethnobotanical, pharmacological, phytochemical, and clinical investigations on Moroccan medicinal plants traditionally used for the management of renal dysfunctions. *Journalofethnopharmacology*, *292*, 115178.

Schirmeister, J., Willmann, H., Kiefer, H., Hallauer, W. 1964: Für und wider die Brauchbarkeit der endogenen Kreatininclearance in der funktionellen Nierendiagnostik. *DMW-Deutsche Medizinische Wochenschrift*, *89*(35), 1640-1647.

Selim, S.A. 2011: Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol extract of the Egyptian lemongrass Cymbopogon proximus Stapf. *Grasas y aceites*, *62*(1), 55-61.

Shabsoug, B. 2003: Qualitative analysis of kidney stones by one wet chemical method for patients in Al-Qasseem area in Kingdom of Saudi Arabia. journal-chemical society of pakistan, 25, 334-336.

Steel, R.G.D., Torrie, J.H. 1980**:** *Principles and procedures of statistics, a biometrical approach* (No. Ed. 2). McGraw-Hill Kogakusha, Ltd.

Wagner, C.A., Mohebbi, N. 2010: Urinary pH and stone formation. *Journal of nephrology*, *23*(16), S165-S169.

Warrag, N.M., Tag Eldin, I.M., Ahmed, E.M. 2014: Effect of Cymbopogon proximus (Mahareb) on

ethylene glycol-induced nephrolithiasis in rats. *African J. Pharm. Pharmacol, 8,* 443-450.

Table 1: In vivo anti-urolithiasis experimental design:

Group	Treatment	No.of Animals
1	Normal Control	6
2	Positive Control Ethylene Glycol (0.75% v/v) for 28 days	6
3	Ethylene Glycol (0.75% v/v) + standard drug Cystone 750 mg/kg	6
4	Ethylene Glycol (0.75% v/v) + Ethanolic extract of CP 500mg/kg	6
5	Ethylene Glycol (0.75% v/v) + Ethanolic extract of PS 500mg/kg	6
6	Ethylene Glycol (0.75% v/v) + Ethanolic extract of CP + PS 500mg/kg (1:1)	6

Table 2: phytochemical screening of Plant Extracts:

Compound	*Cymbopogon proximus*	*Petroselinum sativum*
Tannins	++	+++
Saponins	++	++
Alkaloids	+++	++
Terpenoids	+++	++
Flavonoids	++	+
Phenols	++	+
Phytosterols	+	–
Steroids	+	–

Table 3: Analysis of ethanolic extract of *C. proximus* by HPLC:

Retention Time (min)	Compound	Concentration µg/ml
4.0	Catechol	5.23
6.0	p-coumaric	10.50
7.0	Cinnamic	4.89
9.0	Pyrogallol	8.29
11.0	Ferulic	7.30

Table 4: Analysis of ethanolic extract of *P. sativum* by HPLC:

Retention Time (min)	Compound	Concentration µg/ml
5.0	Syringic	4.66
7.0	Benzoic	5.47
8.1	Caffeic	7.60
9.3	Pyrogallol	2.19
10.0	Gallic	11.44
13.0	Cinnamic	3.69

Table 5: Analysis of ethanolic extract of mixture of two plants by HPLC:

Retention Time (min)	Compound	Concentration µg/ml
3.0	Chlorogenic	6.11
4.9	Syringenic	2.22
6.0	P-coumaric	2.36
7.0	Cinnamic	10.55
8.0	Caffeic	4.22
10.0	Gallic	12.18
11.0	Ferulic	2.17
12.0	Salicylic	2.09
15.0	Benzoic	1.80

Table 6: Estimation of urine pH:

Group	pH
Negative control	7.09 ± 0.08
Positive control Induced (Ethylene glycol 0.75%v/v)	6.22 ± 0.04
Standard (Cystone 750mg/kg)	7.63 ± 0.03
Ethanolic *C.proximus* extract (500mg/kg)	7.45 ± 0.03
Ethanolic *P.sativum* extract (500mg/kg)	7.33 ± 0.03
Ethanolic *Mixture* extract (500mg/kg)	7.56 ± 0.02
L.S.D 0.05	1.18

Table 7: stone analysis:

Test	Result
Physical examination	
Shape:	Oval
Size:	0.8 * 0.6 * 0.4 Cm
Colour:	White
Texture:	Mummulated
Consistency:	Formed
Chemical examination	
Urate:	Negative
Magnesium:	Negative
Calcium oxalates:	Positive
Phosphates:	Negative
Uric acid:	Negative
Ammonium ion:	Positive
Cysteine:	Negative

Table 8: Kidney Function Tests:

Group	CREATINE	UREA	URIC ACID
Negative control	0.66±0.02	24.33±1.52	3.63±0.03
Positive control	1.23±0.02	64.66±1.52	5.52±0.03
Standard (Cystone)	0.73±0.02	31.33±1.52	4.82±0.02
Ethanolic *C.proximus*	0.83±0.02	37.00±1.00	5.12±0.03
Ethanolic *P.sativum*	0.95±0.02	40.00±1.00	5.33±0.03
Ethanolic *Mixture*	0.74±0.01	32.00±1.00	4.05±0.02
L.S.D 0.05	0.12	0.87	0.23

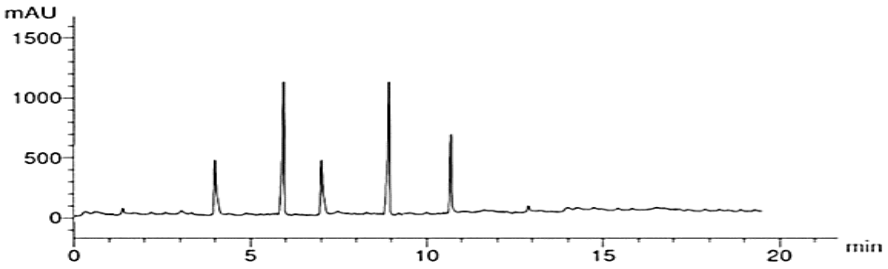

Figure 1: Identification of ethanolic extract of *C. proximus* by HPLC.

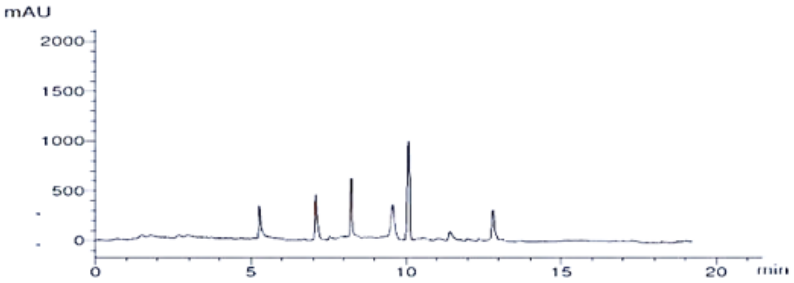

Figure 2: Identification of ethanolic extract of *P.sativum* by HPLC

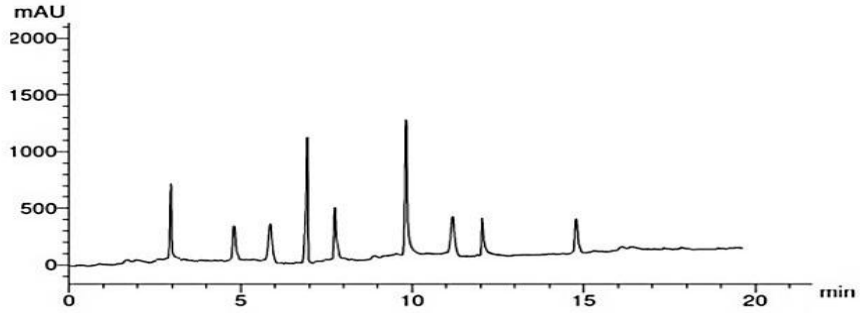

Figure 3: Identification of ethanolic extract of mixture of two plants by HPLC.

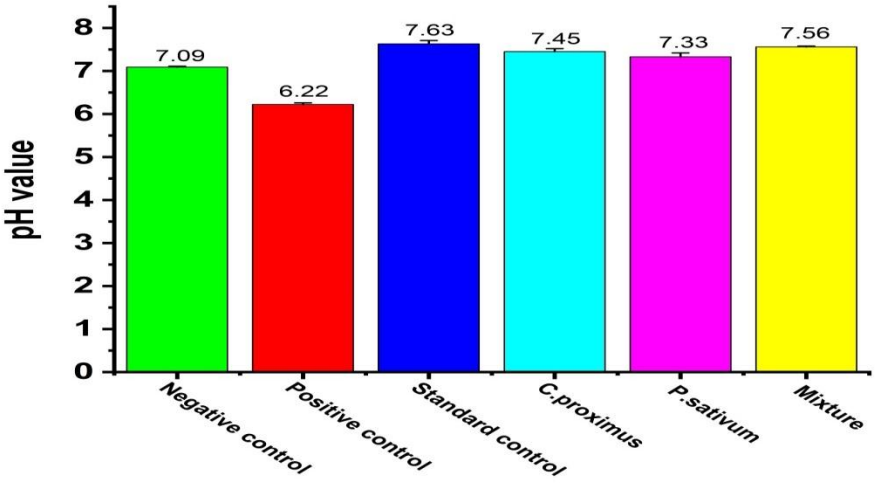

Figure 4: Estimation of Urine pH.

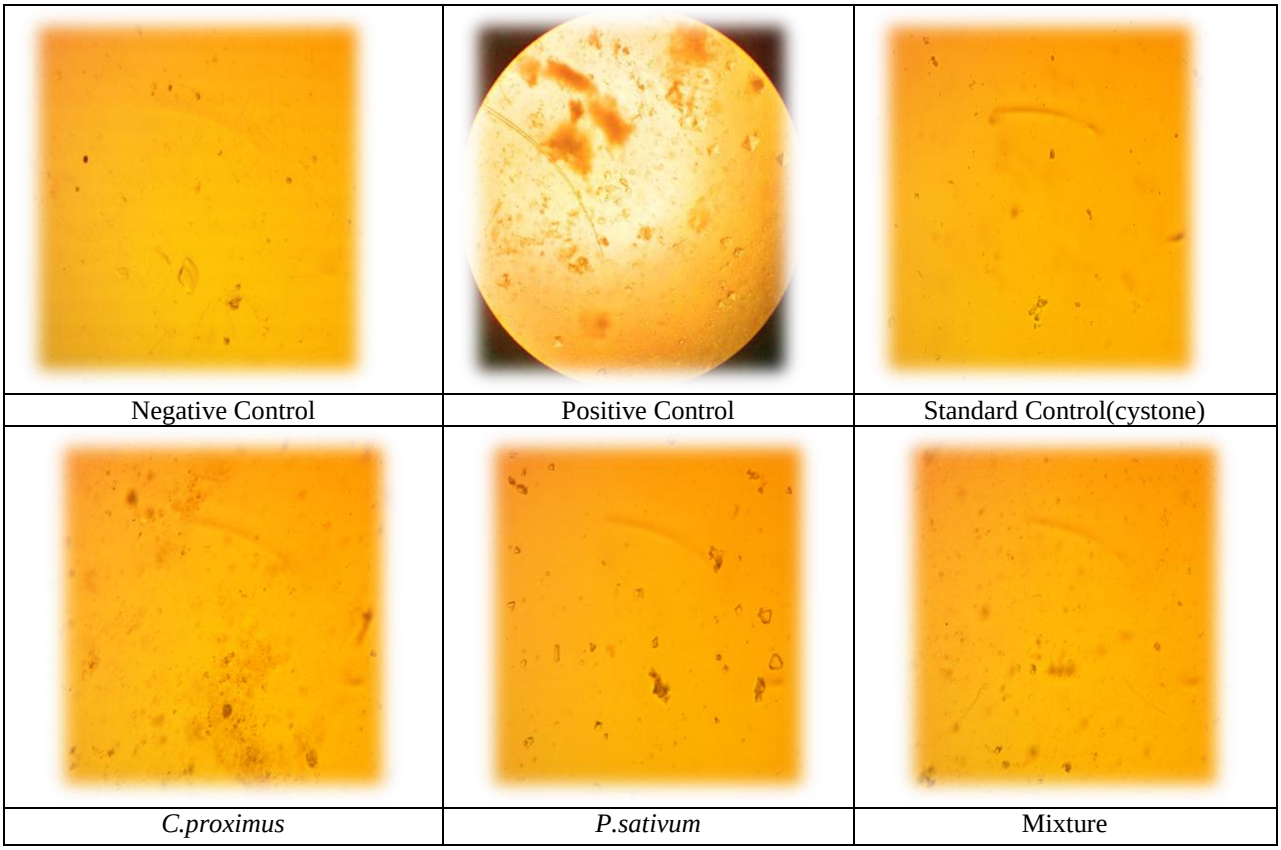

Figure 5: Microscopic examination of urine samples (x40).

Figure 6: Formation of stone.

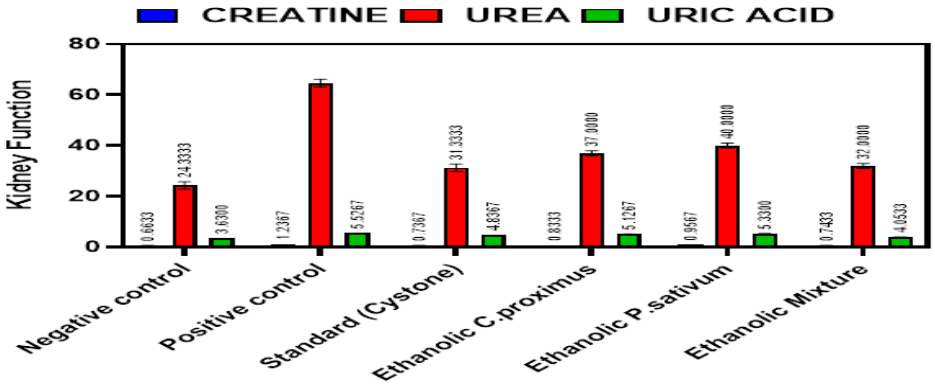

Figure 7: kidney function test:

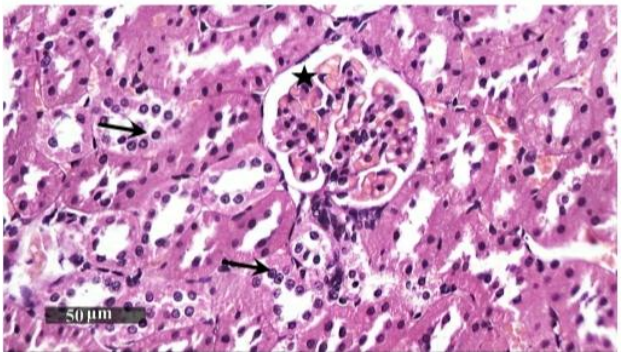

Figure 8: Microscopic examination of kidney samples from normal control samples.

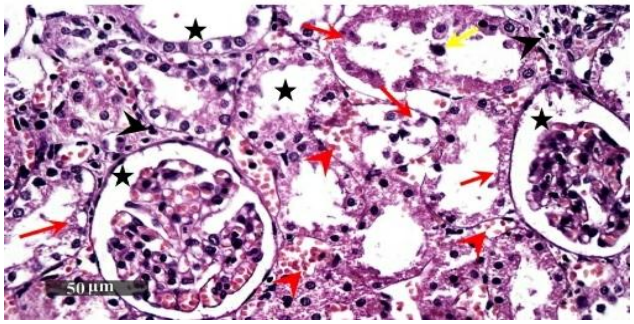

Figure 9: Microscopic examination of kidney samples from positive control samples.

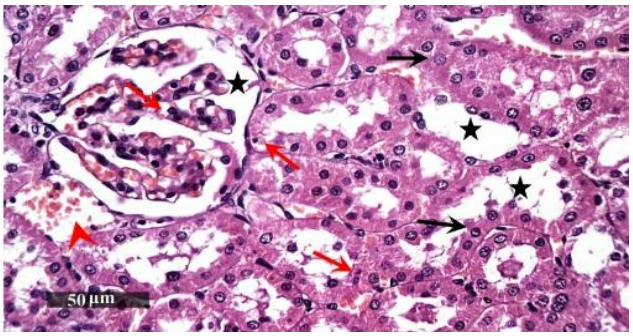

Figure 10: Microscopic examination of kidney samples from standard control samples (Cystone).

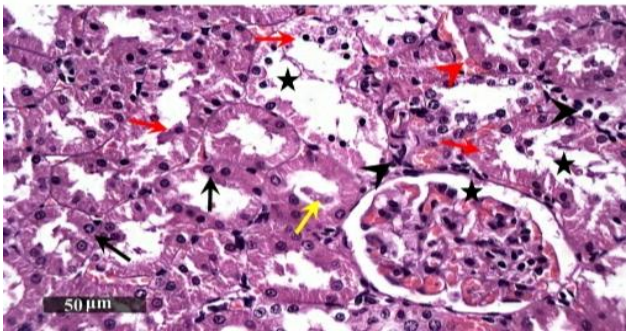

Figure 11: ***Microscopic*** examination of kidney samples from *C. proximus* extracts samples**.**

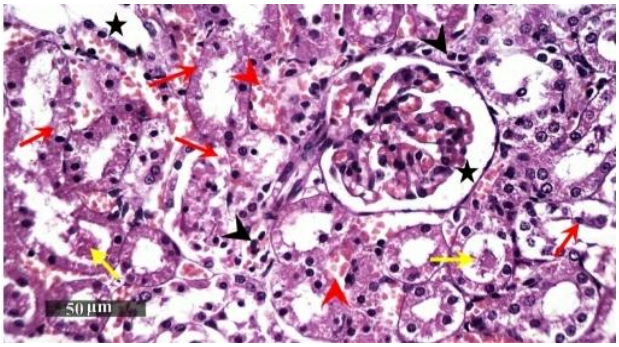

Figure 12: Microscopic examination of kidney samples from ***P. sativum*** extracts samples.

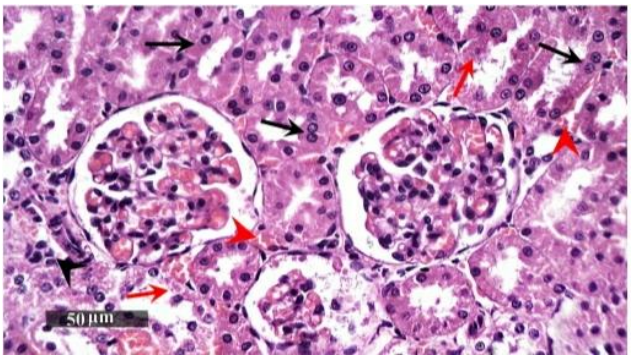

Figure 13: Microscopic examination of kidney samples from mixture Extract samples.

تأثير المستخلص الإيثانولي لنباتات الحلفابار والبقدونس ضد حصوات الجهاز البولي

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الملخص العربى

تهدف هذه الدراسة لتقييم التأثير المضاد لحصوات الكلي لنباتات الحلفابار والقدونس وخليط من نفس النباتين بجرعة (500 مجم / كجم من وزن الجسم / يوم) على حصوات الكلى الناتجة عن الإيثيلين جلايكول في ذكور الفئران البيضاء وتمت مقارنتها مع عقار السيستون كدواء مرجعي بجرعة (750 مجم / كجم من وزن الجسم / يوم). تم إحداث تراكم للأملاح المسببة للحصوات في البول بواسطة الإيثيلين جلايكول (0.75٪ حجم / حجم) وكلوريد الأمونيوم (1٪ وزن / حجم) في مياه الشرب. تم تقسيم الحيوانات إلى ست مجموعات. وهي مجموعة الفئران الطبيعية ، مجموعة الفئران المصابة بحصوات الكلي ، مجموعة الفئران المعالجة بعقار السيستون ، مجموعة الفئران المعاملة بنبات الحلفا بار ، مجموعة الفئران المعاملة بنبات البقدونس ومجموعة الفئران المعاملة بخليط من النباتين بنسبة (1: 1). أظهرت النتائج أن المجموعات المعاملة بالمستخلصات النباتية أعادت المؤشرات البيوكيميائية المرتفعة في سيرم الدم كوظائف الكلي (الكرياتينين واليوريا وحمض البوليك) الي مستوياتها الطبيعية. بالإضافة إلى ذلك ، أعادت مجموعات المستخلصات النباتية درجة حموضة البول إلى الفحص الطبيعي والمجهري لبلورات أكسالات الكالسيوم مع تحسين وظائف الكلى. أكدت الفحوصات الميكروسكوبية والمرضية النتائج المذكورة أعلاه وكشفت أن المستخلصات النباتية لها دور في الوقاية المحتملة على تثبيط حصوات الكلى وانخفاض محتوى الكالسيوم في البول. في الختام: تشير نتائج هذه الدراسة إلى وجود تأثيرات مضادة لحصوات الكلي في هذه النباتات ضد حصوات أكسالات الكالسيوم بواسطة مزيج من مضادات الأكسدة ومدرات البول وتأثيرات قلوية البول ونقص الكالسيوم. أكدت النتائج أن خليط النباتين كان أفضل من حيث الفعالية ، يليه نبات الحلفابار ثم نبات البقدونس.

الكلمات الاسترشادية: حصوات الكلي، وظائف الكلي، الفيتوكيميكال، البقدونس، والحلفا بر.