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RESEARCH ARTICLE

Evaluation of the biochemical effects and biological efficacy of *Crocus sativus* L. extract in rats with bacterial kidney infection

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Abstract

One major issue affecting the kidneys is bacterial nephritis. The bacterium that causes acute pyelonephritis is *E. coli*. According to studies, caocin and safranal are considered the most effective compounds found in saffron. They are easily penetrated through the bacterial wall because they are water-soluble, thus inhibiting their effectiveness. In addition to estimating certain elements and ions in the rats' blood and examining the histological alterations, The current study attempts to confirm the effectiveness of *Crocus sativus* extract as an antibacterial in infected animals. In this study, rats were infected with *E. coli* with a dose of $1*10^9$ (CFU) and were subsequently given an aqueous extract of the plant at a dosage of 150 mg/kg for 14 days. The results showed that significantly decrease levels of sodium and potassium in the blood serum in the animal group treated with *C. sativus* extract, while the results show a significant increase in the level of haemoglobin and ferritin in the blood. There was also no significant change in the level of iron in group treated with *C. sativus* extraction compared to the infected animals group. Damage to Bowman's capsule was also evident in the histological architecture of the kidneys in the animals with bacterial infection. Renal filtration was enhanced by treatment with *Crocus sativus* aqueous extract, which also reduced tissue damage to the kidneys. Examined the effects of several amounts of *C. sativus* extract on the *E. coli* bacterium.

Keywords: bacterial nephritis; biochemical blood variables; *Crocus sativus* L.

Introduction

Crocus sativus L. is considered to be an important member of the Iris family, which is grown across the Middle East and parts of Europe, as well as in Iran, India and Greece and is planted across the Middle East and portions of Europe. It is considered as a valuable spice that is expensive due to its lengthy cultivation period, which can last up to 3 years starting with the sowing of seeds and ends with the production of flowers (1, 2). Research has proven that *C. sativus* has an impact on mice weight gain and antibacterial activities. *Crocus sativus* L. makes food more palatable with flavor and brightly colored coloring. Many scientific studies have proven the beneficial use of the C. *sativus* on the health of humans, such as fighting cancer cells and reducing blood pressure because it has the perfect proportion of potassium to sodium ions (3). In addition, iron from C. *sativus* preserves the amount of iron reserves (Ferrtin) and prevents anemia (4, 5). It also contains vitamin B6 needed for the formation of red cells and assists in reducing spasms in the body (6). Potassium available in variable quantities in the C. *sativus* plants is necessary for the functions of nerves, fluid balance and blood pressure control. Additionally, it keeps the kidneys healthy by regulating the ions, avoiding the development of bacterial infections and preserving the health of the kidneys (7). The way the extract functions is by preventing germs from sticking to the kidneys and other urinary tract organs, which inhibits their capacity to infect tissues (8). The current study seeks to assess certain ions such as (Na^+, K^+, Fe^{+++}) in the blood of laboratory rats, review the histological effects and examine the protective role derived from the C. *sativus* extract against some of the negative effects induced by the infection of the kidneys by bacteria.

Material and Methods

Ethical Approval

Ethical approval was obtained from the Animal Welfare Committee of the Battalion of Veterinary Medicine, University of Mosul after a complete review UM.VET.2023.041, as the committee recommends internationally recognized ethical values in the field of animal experiments and research.

Bacterial isolate

We utilized the VITEK® 2 Compact system (bioMérieux, Marcy-l'Etoile, France) to analyze the biochemical responses found on microbial identification cards using sophisticated

colorimetry technology in order to identify the bacteria and their sensitivity to antimicrobial agents. Once these cards are inoculated with an unknown organism, they may be read by the system's internal optics. System identification of the organism is achieved by comparing the findings to known responses in the VITEK 2 database. The technology uses various visible spectrum wavelengths to interpret the test reactions using a transmittance optical device. An algorithm is utilized to eliminate misleading readings brought on by tiny bubbles and each reaction is checked for turbidity or colored substrate metabolic products every 15 min while it is incubating.

Preparation of *Crocus sativus* extract

Aqueous extraction

Crocus sativus was purchased from the neighborhood market in Mosul city, Iraq. For the purpose of extracting juice, the *C. sativus* was first thoroughly cleaned with distilled water, dried and then mashed using an electric blender and sieved through layers of muslin paper. Allowed the material to air dry entirely at ambient temperature. In order to make the aqueous extract, 10 ml of sterile distilled water were used to soak 1 g of powdered *C. sativus* For around 24 h, the solution was kept at 25°c. In order to create a fine powder, the filtrate was finally filtered through Whatman filter paper and allowed to dry (9).

Oil extraction

The presence of lipid-soluble vitamins was checked. fatty compounds and volatile oils were extracted by soaking 100 grams of the plant powder in petroleum ether solvent (60-80°C) for 1 day, then the soaked plant powder was placed in the Clevenger extraction device For 3 days until the color of the solvent became transparent, The solvent is heated continuously in a water bath until it reaches the boiling point. The excess solvent is then removed at the end of the extraction process, thus obtaining the materials that were dissolved in the solvent. The solvent is evaporated using a rotary evaporator and placed under low pressure to completely get rid of the solvent. Then the extract is stored in a cool, dry place in an opaque bottle (10, 11).

Diagnosis of fatty compounds using HPL-C technique

The fatty compounds were identified using the HPL-C technique in the laboratories of the Ministry of Science and Technology, Baghdad, Iraq. The type of device was SYKAM, Germany the type of column C18-ODS at a wavelength of 360 nanometers. Standard solutions were prepared at different concentrations and then injected into a column. The fatty and aqous compounds of the plant extract were also prepared at a concentration of 2000 ppm, the retention time of the peaks of the oil compounds of the plant extract with the retention time of the standard solution.

Evaluation of the *in vivo* antibacterial effect of the extract using rat model

In this investigation, 18 male white rats weighing between 250 and 320 g were employed; they were 2 to 3 months old. The rats were housed in the College of Veterinary Medicine, University of Mosul's animal home. They were split into 3 groups, with 6 animals in each group, as indicated by the arrangement below (Fig. 1). Throughout the experiment, distilled water was administered to the first group, which was a negative control. The second group, a positive

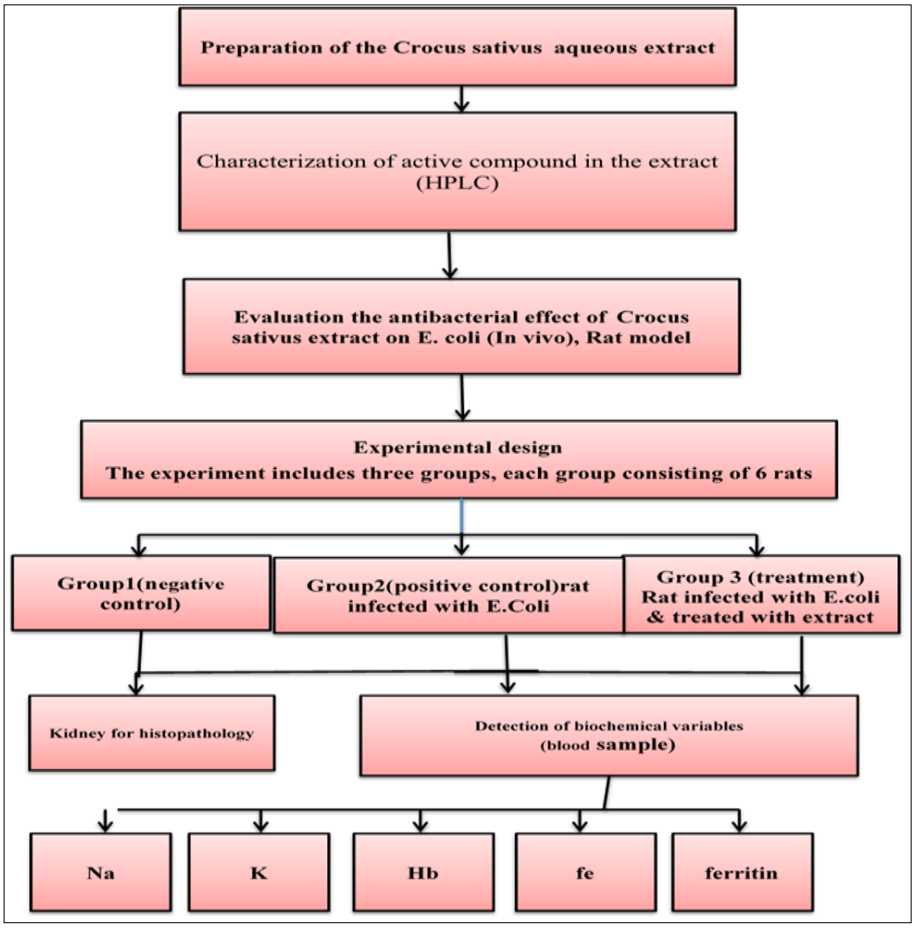

Fig. 1. General outline of the study.

control, had an *E. coli* infection that resulted in Urinary tract infections UTIs at $1*10^9$ (CFU) (12). The third group (treatment) received a Concentration of extraction (150 mg/ kg) therapy for 14 days after contracting an *E. coli* infection. When treatment with the aqueous extract is carried out over a period of (14 days) , it was given anesthesia and then dissected. Kidneys were removed for histological analysis and blood was taken for biochemical testing.

Evaluation of iron, sodium and potassium

An atomic absorption device applied by Analyze American company was utilized to determine the concentration of iron element. Also, a flame hoist device , which measures emission intensity, was used to estimate the elements Na and K Ferritin concentration was estimated using the (ELISA) From a company Biotek American company, model 2018 (13-15).

Estimation of hemoglobin concentration

Measuring the absorbance of the cyanmethemoglobin at 540 nanometers in a photoelectric calorimeter against a standard solution. The concentration was obtained by plotting the absorbance of the sample with standard solutions versus the concentrations of standard solutions (16).

Histopathological study of kidney

At the end of each experimental period, 2 animals from each group were scarified. Changes of the kidneys were observed macroscopically. The tissue was taken and kept in 10 % formaldehyde solution to study the histological changes of the kidneys, Hematoxylin (H) and eosin (E) stains are used to stain tissues to clarify the details of cells and make them visible under the microscope. Hematoxylin is used to stain the nuclei of cells in blue or purple, while eosin stains are used to stain the cytoplasm in pink or red. (17).

Statistical evaluation

The statistical software SPSS 25 was used to record and analyze the biochemical variable data in order to calculate the mean and standard deviation (SD). Results were compared using the Duncan test and the difference between the values was determined, using the T-test. A significant difference was defined as the probability value ($p \leq 0.01$) (18).

Results

The Saffron plant is one of the most important natural plants that contain minerals and elements necessary for the human body. The % of aqueous extract was calculated by weighing the extract resulting from the separation process divided by the weight of the powder before separation by 100 and was approximately between 10-12 %. The color of the result was transparent and consistent. The *Crocus sativus* is one of the most important natural plants that contain minerals and elements necessary for the human body. The results of HPLC technique was shows in Table 1 and Fig. 2 & 3 indicate that the extract contains many fat-soluble vitamins, such as the important vitamins (E, D, K1, K2 and A) In various forms and types such as Vitamin A, Vitamin D2, Vitamin D3, Vitamin E, Vitamin K2, Vitamin E acetate, Vitamin K1 trans, Vitamin K1 is along with water-soluble vitamins (B6, Nicotine amide) according to the standards available to us.

Table 1. Retention time and area for plant extracts *Crocus sativus* L. extract of the plant

Peak name	R.T (min)	Area (mm)
solvent	04:17.5	4101.8
Vitamin A	05:02.0	360.3
Vitamin D_2	06:06.4	1425.4
Vitamin D_3	12:34.4	279.4
Vitamin E	13:20.8	13153.9
Vitamin K_2	13:42.8	958.5
Vitamin E acetate	14:48.5	1588.5
Vitamin K_1 trans	16:52.4	926.4
Vitamin K_1 sis	17:25.9	520.3
Nicotineamide	04:08.1	3417.0
Vitamin B_6	04:58.9	1741.2

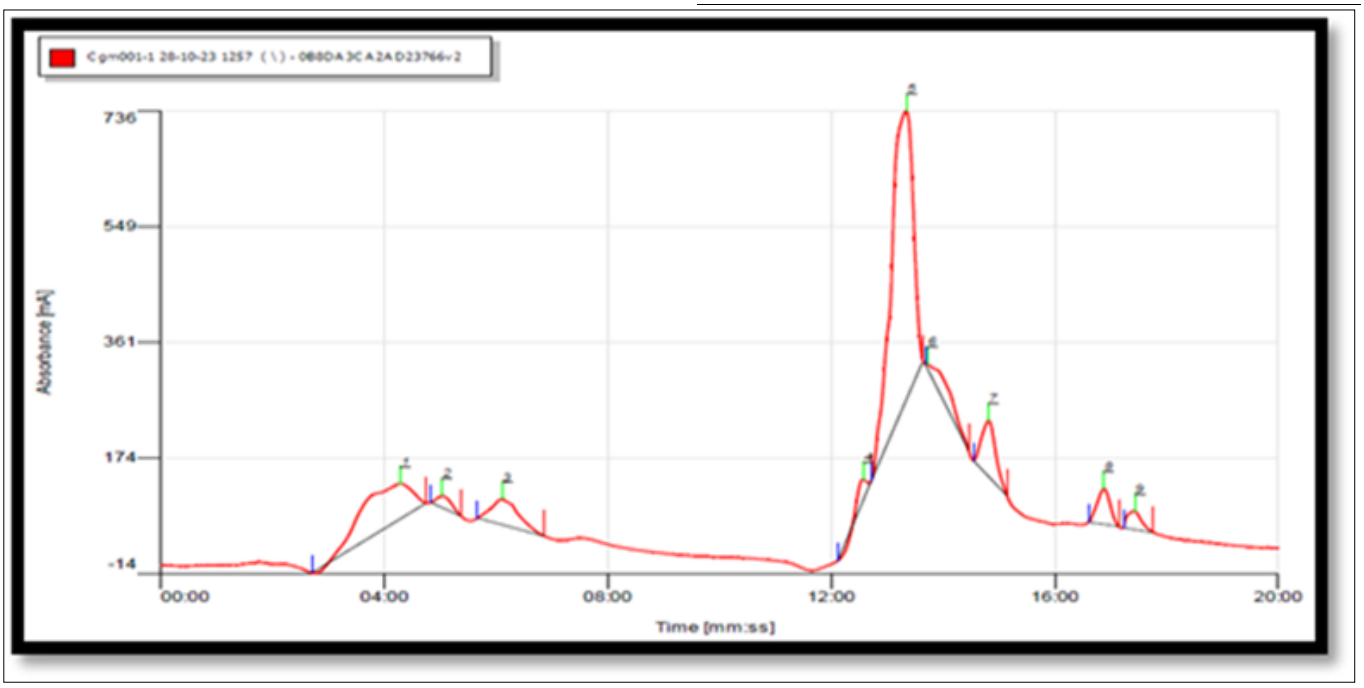

Fig. 2. High Performance liquid chromatography (HPLC) analysis of oily *Crocus sativus* L. extract.

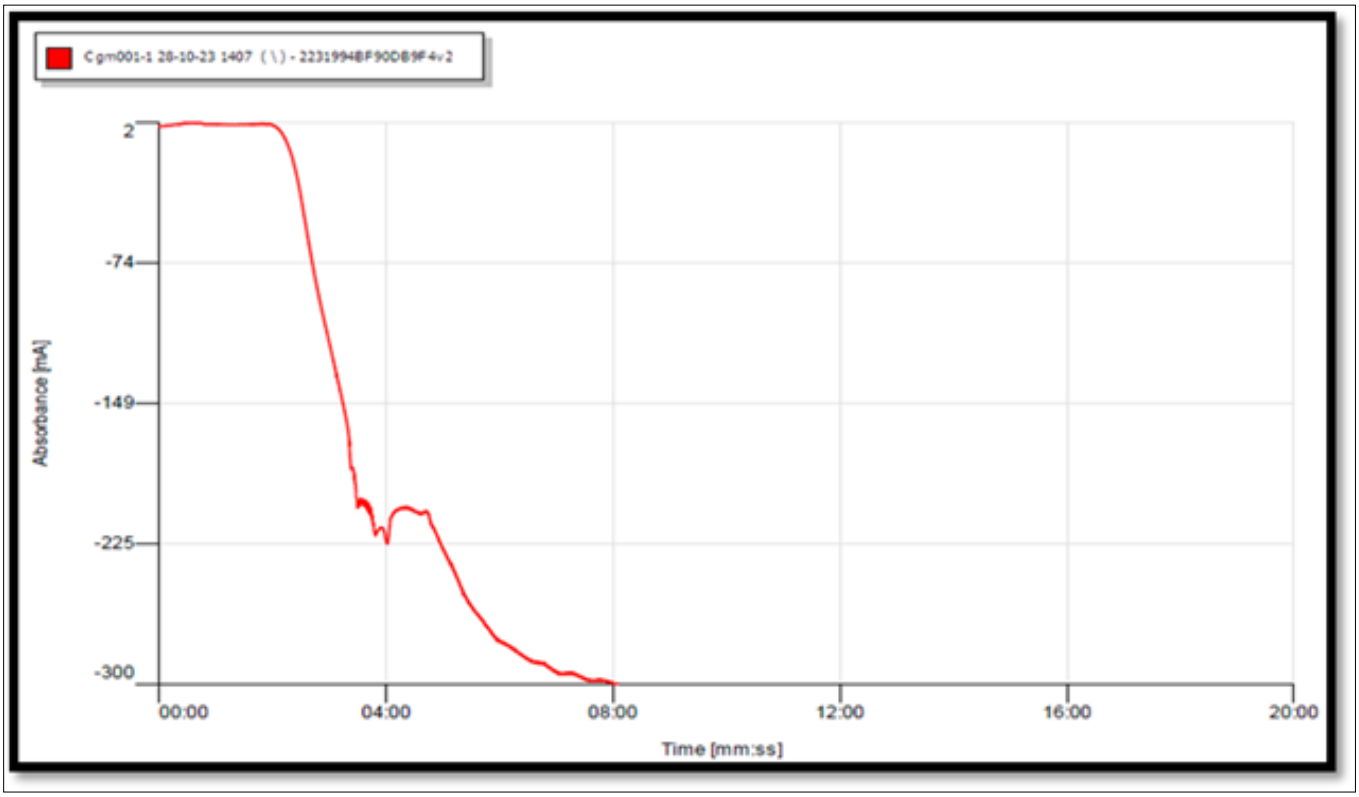

Fig. 3. High performance liquid chromatography (HPLC) analysis of Aqueous extract *Crocus sativus* L. extract.

Table 2 shows a decrease in sodium at probability level (p≤0.01) In the blood serum of animals treated with the aqueous extract compared to animals infected with the bacteria, but an increase in the level of potassium. In the blood serum of animals treated with the aqueous extract compared to animals infected with the bacteria, as the plant contains a higher % of potassium compared to sodium. Also, the results in Table (2) shows a significant increase of ferritin level concentration in animals treated with water extract of the saffron plant compared to animals with bacterial infection of the kidneys, while no significant change occurred in the level of iron in the animals.

The glomerulus and Bowman's spaces comprise the normal number of nephrons in the kidneys of white male rats, according to the relative examination patterns of the kidneys of the control group. In contrast, the kidneys of animals infected with bacteria showed signs of inflammation, degenerative changes in the renal tubules and moderate congestion. When treated with *C. sativus* extract, however, a positive histological change was observed in the blood vessels as compared to the patient groups as seen in. If the tissues of animals treated with bacteria for 7 days showing sloughing in tubular epithelia (black arrow) with dilation in bowman space Fig. 5, also showing distraction in renal tubules (black arrow) with marked dilation in bowman space (red arrow) and severe interstitial hemorrhage Fig. 4(C), But Histopathological section of rat kidney infected with bacteria for 7 days and treated with extract showing mild dilation in bowman space (black arrow) vacuolation in some tubular epithelia (red arrow) with mild inflammatory cells infiltration showing in Fig. 4(D). Histopathological section of rat kidney infected with bacteria for 14 days and treated with extract showing normal glomerular tufts and bowman space (black arrow) also regeneration of tubular epithelia showing in Fig 4 (E, F).

Table 2. The effect of treatments with bacteria and *Crocus sativus* L. extract on biochemical variables in the blood of male laboratory rats.

	Group I	Group II	Group III	p ≤0.01
K µmol /l	3.692±0.04 A	8.413±2.478 B	5.401±2.123 A	0.01
Na µmol/l	133.27±4.165 A	145.60±20.67 B	125.71±11.11 C	0.001
Hb g/dl	13.02±0.58 a	11.95±0.60 b	12.34±0.69 C	0.01
Fe µmol/l	28.66±2.84 A	26.39±3.76 A	25.51±1.57 A	0.01
Ferrtin ng/l	17.71±3.27 A	13.04±2.12 B	15.94±2.51 A	0.001

Different letters indicate a significant difference at a probability level p≤0.01, (mean ±standard deviation).

Group I: Negative control

Group II: Positive control group treated with bacteria

Group III: Aqueous extract of Crocus sativus L After(14)days

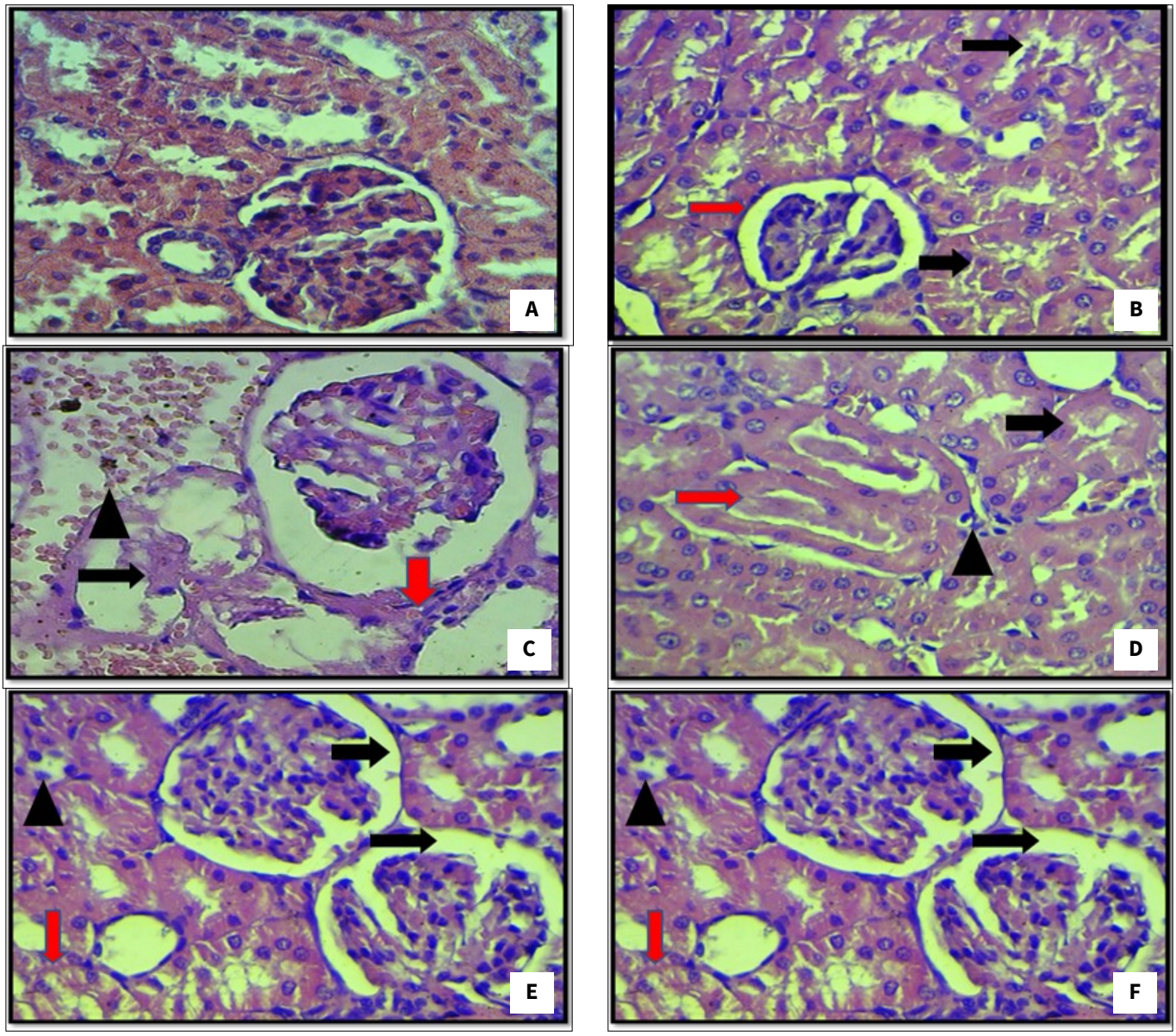

Fig 4. It shows the tissue changes as follows:

A: Normal tissues and normal structure of the kidneys of rats in the control group (H&E, stains,400x). B: Histopathological Kidney tissue section of vaccinated animals with bacteria for 7 days showing sloughing in tubular epithelia (black arrow) with dilation in bowman space (red arrow). (H&E, stains,400x). C: Histopathological section of rat kidney treated with bacteria for 7days showing distraction in renal tubules (black arrow) with marked dilation in bowman space (red arrow) and severe interstitial hemorrhage (head arrow) . (H&E, stains,400x). D: Histopathological section of rat kidney treated with bacteria for 7 days showing cloudy swelling in the renal tubule epithelia (black arrow) also severe sloughing in tubular epithelia (red arrow) with inflammatory cells infiltration (head arrow). (H&E, stains,400x). E: . Histopathological section of rat kidney infected with bacteria for 7days and treated with extract showing mild dilation in bowman space (black arrow). F: Histopathological section of rat kidney infected with bacteria for 14 days and treated with extract showing normal glomerular tufts and bowman space (black arrow) also regeneration of tubular epithelia (red arrow) (H&E, stains,400x).

Discussion

Approximately 70-80% of people on the planet still use medicinal plants-which don't have any negative side effects or contain hazardous chemicals-as their primary source of disease treatment, particularly in underdeveloped nations. The risk of kidney disease was higher in animals treated with saffron plant extract than in animals with bacterial kidney infections. This is because kidney disease is a major public health concern in most countries (19, 20) and kidney disease is linked to heart disease because the high potassium-to-sodium ratio lowers the activity of the angiotensin renin system, which in turn is linked to high blood pressure (21). Compared to animals with kidney infections caused by bacteria, those treated with an aqueous extract of saffron had different concentrations of the bacterial infection in their kidneys. The reason for the decrease in sodium concentration in animals treated with the aqueous extract of *C. sativus* is that it works as a diuretic and regulates the excretion of sodium ions to protect the kidneys from salt accumulation. Sodium is one of the ions that has a specific renal limit, unlike potassium, which is completely absorbed by the kidneys. Therefore, the potassium concentration was higher than sodium in animals treated with the aqueous extract *C. sativus* to maintain osmotic pressure and prevent oxidative damage to the kidneys because it contains quercetin (22).

The main reason for the decrease in hemoglobin in the blood of animals infected with bacterial infection is due

to the oxidative damage caused by bacteria to the kidney tissues, thus reducing the production of red blood cells due to the lack of secretion of the hormone erythropoietin, which is responsible for the production of red blood cells and causing anemia. However, when the animals were treated with the aqueous extract, it led to an increase in the concentration of haemoglobin due to the plant containing many flavonoids that are antioxidants and prevent damage caused by kidney inflammation and increase the concentration of red blood cells (20, 23). There was no significant change in the iron level in the animals treated with bacterial infection compared to the negative control group and the iron concentration remained unchanged after treating the animals with the plant extract, while the decrease in the level of ferritin, which is the iron store in the liver, occurred. It is closely related to the iron concentration and is released from the liver when it is lacking in the blood, as its level decreases in animals infected with bacterial infection due to the high level of histidine when infected with inflammation. However, when the animals were treated with the aqueous extract of saffron, the level of ferritin in the blood increased, as ferritin contains an amount of iron estimated at 23.7 /100 g (24, 25).

Moreover, the aqueous extract has 2 water-soluble vitamins (B6 and nicotinamide) that are significant for anemia and iron deficiency (26, 27). Among the most significant antioxidant components found in the oil and aqueous extract were fat-soluble and water-soluble vitamins, particularly vitamin D3, a deficiency that causes breast cancer (28). Numerous fat-soluble vitamins, including vitamin A and E2,1, which are thought to be among the most significant antioxidants, are present in the oil extract and help shield kidney tissues from oxidative damage and inflammation. Rats with glomerulonephritis treated with retinoids showed decreased albuminuria and decreased cell proliferation. In addition, it controls the migration of epithelial cells and aids in the healing of damaged cells (29 - 31).

Conclusion

The results of the current research showed that the aqueous extract contained vitamins as (B6 and nicotine amide) and the oil extract contained vitamins (E, D, K1, K2 and A). highlighting a higher potassium level and increased ferritin concentration in animals treated with saffron extract. The current results indicated that the *Crocus sativus* L. extract is rich in elements and vitamins through blood biochemical analysis and technical analysis (HPLC). Treating animals with the aqueous extract also led to repairing damage to kidney tissue, with an increase in the level of haemoglobin and a reduction in the level of sodium, along with an increase in the level of ferritin and potassium in the blood during periods of treatment with infected animals having bacterial nephritis.

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Authors' contributions

LAM participated in raising and administering animals and IGT in measuring biochemical variables and writing the research with LAA and assisted in making tissues and determining the dose of bacteria. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: There is no conflict of interest

Ethical issues: None

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