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Oleuropein Protects against the Development of Kidneys Induced by Paracetamol in Albino Male Rats.

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ABSTRACT

Paracetamol treatment is considered one of the treatments used to relieve pain and antipyretic. Therefore, excessive doses and long-term use lead to organ toxicity. Paracetamol treatment is considered one of the treatments used to relieve pain and antipyretic. Therefore, excessive doses and long-term use lead to organ toxicity. The aim of the study was to investigate the protective effect of Oleuropein extracted from olive leaves on the physiological and histological aspects induced by Paracetamol in a rat model. The methods used 25 albino Swiss rats randomly distributed into five groups with the same number. The unit of control is given normal saline. Paracetamol (750 mg/kg) was injected into the group once. In the treatment groups (50 mg/kg, 100 mg/kg, 150 mg/kg). The Administration of Paracetamol's result significantly increased blood urea, creatinine, sodium, and potassium levels, and their blood concentrations decreased with Oleuropein (P 0.05). In addition, Oleuropein extracted from olive leaves relieved some symptoms, including acute vascular congestion caused by a dose of Paracetamol. Compared with paracetamol treatment, there is an infiltration of inflammatory cells and severe nephrotoxicity in the tubules. According to this study, the Oleuropein extracted from olive leaves can be used to prevent kidney damage, and It is not recommended to give Paracetamol, which increases kidney disorders.

1. Introduction

Oleuropein is a phenolic compound found in olive trees (*Olea europaea L.*), although the leaves have higher levels (Anwar *et al.* 2023). It is known for its many health benefits. It acts as a powerful antioxidant that helps protect cells from oxidative stress and damage caused by free radicals. It also has strong anti-inflammatory properties and antimicrobial activity, which can help reduce inflammation in the body caused by various pathogens, including bacteria, viruses, and fungi. Additional properties of Oleuropein include potential anti-cancer, neuroprotective, and anti-diabetic properties (Hassen *et al.* 2015). It effectively and dose-dependently inhibits low-density lipoprotein (LDL) oxidation induced by copper sulfate (Scicchitano *et al.* 2023). According to a study (Ahamad *et al.* 2019). Oleuropein can scavenge nitric oxide and raise the inducible nitric oxide synthase (iNOS) expression level. It was also shown that hypochlorous acid (HOCl) is scavenged by Oleuropein (Hameed *et al.* 2021). During inflammation, neutrophils produce myeloperoxidase HOCl, which can damage proteins, including enzymes. Among the health benefits of Oleuropein are its ability to prevent cardiac arrest, decrease obesity-related disorders, and improve fat metabolism

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(González-Ortega *et al.* 2021). It has antioxidant properties in low-density lipoprotein while reducing plasma levels of total, free, and esterified cholesterol. The protective effect of Oleuropein has been studied in several areas against some toxins, but it has not yet been studied in nephrotoxicity (Topuz & Bayram 2022). Paracetamol toxicity in animals and humans can cause hepatic necrosis and reduce the depletion of glutathione pools in the mitochondria and cytosol. Paracetamol is a potent inducer of cytochrome P450 (Bertolini *et al.* 2006). Paracetamol is an analgesic metabolized by the cytochrome P450 system, resulting in N-acetyl-P-benzoquinone imine (NAPQI) (Sheen *et al.* 2002). The kidney is a secondary target organ for paracetamol toxicity, although nephrotoxicity may be present (Grgic 2022). There is no hepatic toxicity after Paracetamol overdose (Przybyła *et al.* 2021). Kidney disease treatment research completely ignores Paracetamol toxicity in herbal formulations (Nunes 2020).

Covalent binding of active metabolites to large cellular molecules impairs homeostasis in renal cells and the redox state of mitochondria and peroxisomes, releasing reactive oxygen species (Zhu *et al.* 2017). In studies comparing the toxicity of Paracetamol on kidney injuries versus its limited effects on liver injuries, Many studies have focused on exploring natural alternatives to reduce paracetamol toxicity, such as alpha-lipoic acid, quercetin pretreatment against paracetamol-induced GSH levels in renal cells (Barros *et al.* 2017; Chen *et al.* 2020). Acrylic acid is a no inhibitor, and *Phoenix dactylifera* is an antioxidant (Hmidani *et al.* 2020; Bouhlali *et al.* 2021).

Environmental toxins added to medications of clinical interest, such as Paracetamol, could cause toxicity in many organs through metabolic activation (Alchin *et al.* 2022). Fights against free radicals, such as superoxide addition to reactive oxygen types, are highly active. Electoral renal gathering of nonsteroidal anti-inflammatory phototoxins, such as Paracetamol, in animals and humans may lead to a cascade of biochemical reactions (Moshaie-Nezhad *et al.* 2019). Eventually, leads to acute or chronic kidney disease (Ishitsuka *et al.* 2020). Additionally, Paracetamol has been reported to enhance hepatic and renal cell apoptosis (Nithiyanandam & Evan Prince 2023). Thus, the current study investigated the protective effects against paracetamol-induced toxicity.

2. Materials and Methods

2.1. Ethical Approvals

Our experimental procedures were approved by the Scientific Research Ethics Committee at Anbar University No. 116/in 16/10/2022 ethicalapproval@uoanbar.edu.iq.

2.2. Isolation and Purification of Oleuropein

Extract Oleuropein from the dried leaves of olea leaf by mixing 2-propanol: water (9:1). Mix 10 g of leaves with 200 ml of solvent and extract at room temperature for about 1.5 hours. The solution was mixed to obtain a quantity of dry matter, and the solvent was removed under reduced pressure. The crude extract was then partitioned between a methanol: water (3:1) mixture. And a mixture of toluene: petroleum ether (2:1) (100 ml for each phase). Under reduced pressure the aqueous methanol was dehydrated, resulting in 1.8 grams of the sub-extraction. The material was fractionated between water and 2-butanone (100 ml for each phase) to remove sugar and other water-soluble compounds. The organic phase was dried, resulting in a sub-extract. 43% of the initial crude extract and 10% of the plant material were recovered, representing the resulting substance. The Folin-Ciocalteau reagent was used to confirm that the obtained content was phenol, and 0.6 ml of the resulting solution was transferred to a glass tube, then 0.5 ml of Folin-Ciocalteau reagent was added. After 4 minutes, approximately 2 ml of sodium carbonate solution (Na_2CO_3) with a concentration of 200 mg/ml was added, mixed well, and placed on a vortex apparatus. It was then left in a dark place for an hour. Using a concentration of 5 mg/ml, the alirobin and phenol were quantified using high-performance liquid chromatography (HPLC) technique with the following parameters: Mobile phase: Methanol: 1% Formic acid (70:30), Column: C18 (250 × 4.6 id) mm, 5 micrometers site, Flow rate: 1 ml/min, Injected volume: 20 microliters, Wavelength: 254 nm, Instrument = Shimatzu/Japan (Sucharitha *et al.* 2019; Mohammed *et al.* 2020).

2.3. Chemical Materials

Paracetamol is obtained from Samarra Pharmaceutical Factory. Iraqi solvents and other chemicals were obtained from local traders. Paracetamol suspension (1%) was prepared in a normal saline solution. Paracetamol was taken, and

the animals were given 750 mg/kg of this suspension orally through a tube next to the stomach (1 ml).

2.4. Biological Experiment

This study used 25 adult male rats with a body weight of about (235±9 g). Obtained from the Biotechnology Centre/Baghdad. The mice in our study were placed in plastic cages lined with sawdust. The cages were kept clean, and the bedding was changed three times a week. In total, five cages with five mice each were used. In total, five cages with five mice each were used. The appropriate conditions were created for the studied animals regarding ventilation, temperature, and proper lighting. They were provided with constant access to water and fed a standard diet. The experimental rats were divided into five groups: a negative control group that received normal saline. In three groups, this positive control group received a single dose of Paracetamol (750 mg/kg) orally. Combinations of Oleuropein at concentrations of 50 mg/kg, 100 mg/kg, and 150 mg/kg, respectively, were administered for 15 days.

2.5. Biochemical Tests

Measurement of markers of nephrotoxicity: Mice were subjected to an overnight fast before necropsy. Blood samples were collected from the posterior orbital sinuses of the eye before and during dissection and from the portal vein. Blood samples were preserved in tubes of heparinization and placed in a centrifuge at 3,000 rpm for 15 minutes at four °C using a centrifuge. Plasma and serum were collected to measure renal K+ levels and indices of toxicity. Urea, uric acid, creatinine, and sodium levels were estimated using specific kits (Mahl 2000).

2.6. Histopathological Observation

Kidneys were cut to an appropriate size, and 10% of the volume was collected. Fix with normal saline. When these tissue samples are properly fixed Paraffin is embedded and processed as a standard program. Section thickness is 3-5 µ for microscopy purposes, stain with Mayer's hematoxylin and eosin Survey (Jemai *et al.* 2010).

2.7. Statistical Analysis

Numerical data expressed as mean ± standard error Statistical analysis using CRD and ANOVA Graph Pad Prism software. A probability of less than 5% ($P<0.05$) is considered important.

3. Results

3.1. Physiological Effects of Oleuropein

Oleuropein was shown to have a protective role on nephrotoxicity caused by Paracetamol in rats, as shown in (Figure 1). Meanwhile, administration of individual dose of Paracetamol at 750 mg/kg increased the urea, creatinine levels, sodium, and potassium compared to the expected levels in the control group (normal saline). This condition may be attributed to impaired renal function (source). In addition, a significant increase in both sodium and potassium levels was observed (Figure 1). On the other hand, treatment with Oleuropein at a concentration of 150 mg/kg provided better protection against the studied agents. A 10-day pretreatment showed a protective effect against toxicity by reducing the rise in serum urea and creatinine levels compared to the protective effect alone. Serum concentrations of creatinine, uric acid, and urea were significantly increased ($P<0.05$) in the paracetamol-treated group compared to normal animals, indicating severe nephrotoxicity (Figure 1). Treatment with Oleuropein caused a significant decrease in the concentrations of creatinine 0.3 mg/ml and urea in the blood compared to Paracetamol. Overdose of toxic Paracetamol usually manifests as increased metabolism Derangements, including serum electrolytes, urea, and creatinine.

3.2. Histopathological Study

The biochemical normal kidney in (Figure 1) shows proper lobular organization of the glomerulus and surrounding Bowman's capsule, lined with squamous (squamous) epithelial cells (arrows) that show consistent distance between the glomerulus and the capsule wall. (Bowman, Space). The proximal tubular (PT) lining has a typical cuboidal epithelium with brush borders. The distal convoluted tubule shows a relatively uniform distinction lumen, the kidneys of paracetamol-treated mice show severe tubular damage with partial rupture of Bowman's capsule, tubular necrosis of the proximal and distal convoluted tubules, and tubular luminal debris containing cellular casts (Figure 2). In addition to proximal and distal tubular desquamation, tubular dilatation and necrosis with infiltration of inflammatory cells are also present. In addition, there is severe tubular dilatation with erosion and swelling of the tubules, along with amyloid deposition in the glomerulus, consistent with the thickening of Bowman's capsule. The histological sections of the kidneys treated with Oleuropein showed

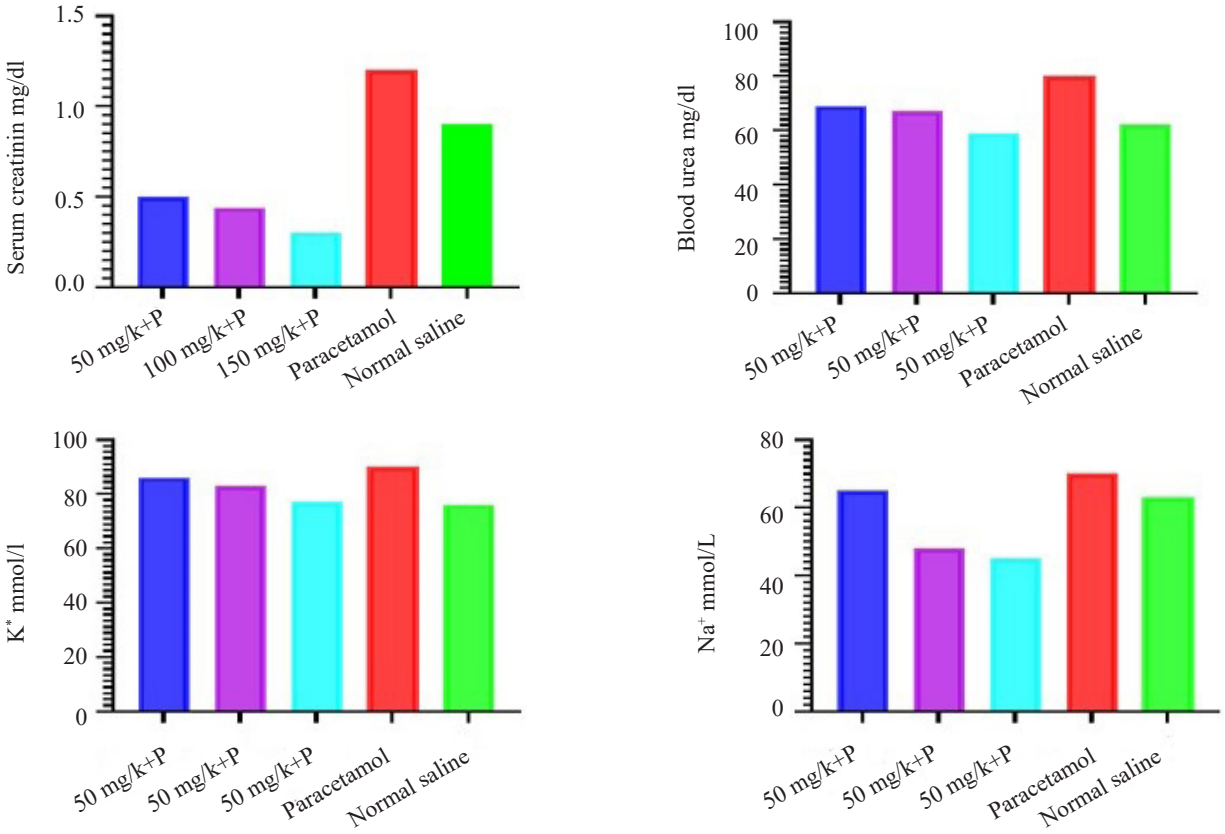

Figure 1. Effect of Oleuropein pretreatment on some physiological parameters in mice with paracetamol-induced nephrotoxicity. Control (normal saline solution), Paracetamol; A: Creatinine levels, B: Urea levels, C: Na+ Concentration, B: K Concentration. All values are presented as mean ± SEM. (n = 8. P <0.05)

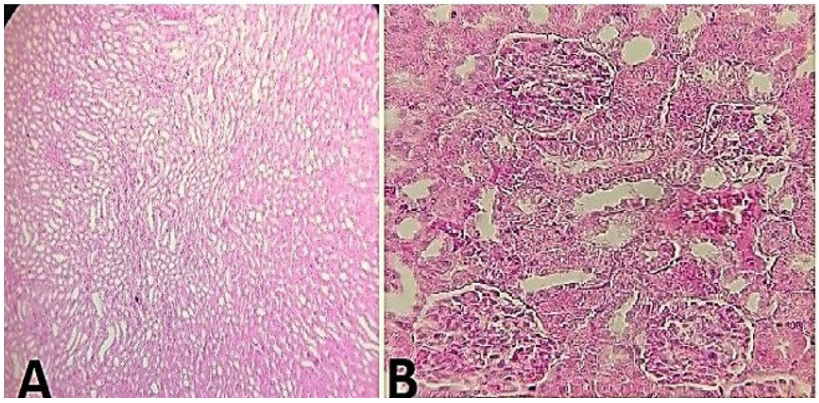

Figure 2. The histological section of a mouse kidney from the control group was normal slane, showing the normal structure of the tubules and glomeruli of the kidney (A = 10x, B = 40x).

a histological appearance similar to the control group given orally at 50, 100, and 150 mg/kg doses. There was a tendency for kidney tissue to adopt a histological pattern with minimal inflammatory infiltration, renal vein dilation, and interstitial hemorrhage (Figure 2). The present results are consistent with those observed in (Figure 1), where elevated serum urea and creatinine levels were observed after administration of Paracetamol 750 mg/kg body weight in rats. In addition, since there was a strong association between nephrotoxicity and autonomic stress in oxen, urea and creatinine levels were found. Histological findings in glomeruli confirmed these biochemical changes and interstitial necrosis in the untreated control

group. However, daily paracetamol treatment for 15 days provided dose-dependent renal protection in renal-impaired paracetamol rats at a dose of 750 mg/kg to provide maximum protection for (Figure 3) showing the histological appearance close to normal with Bowman's capsule dilated and (Figure 4) showing-acute blood congestion in the blood vessels. Inflammatory cell infiltration. -And severe watery degeneration in the tubules. (Table 1) shows that the measurements of the internal parameters of the kidneys in the groups that were dosed with Oleuropein were not affected.

4. Discussion

This study used Paracetamol, which can cause toxicity to many organs and occurs through the metabolism of highly reactive free radicals, including superoxide and reactive oxygen species (Canayakin *et al.* 2016). Olive leaf extract, Oleuropein, was used as a therapeutic substance, as the main active compound in olive leaf extract is Oleuropein, which is a polyphenol with antioxidant, anti-inflammatory, anti-atherosclerosis, anti-cancer, antimicrobial, and anti-viral properties (Omar 2010; Ahamad *et al.* 2019). Oleuropein has

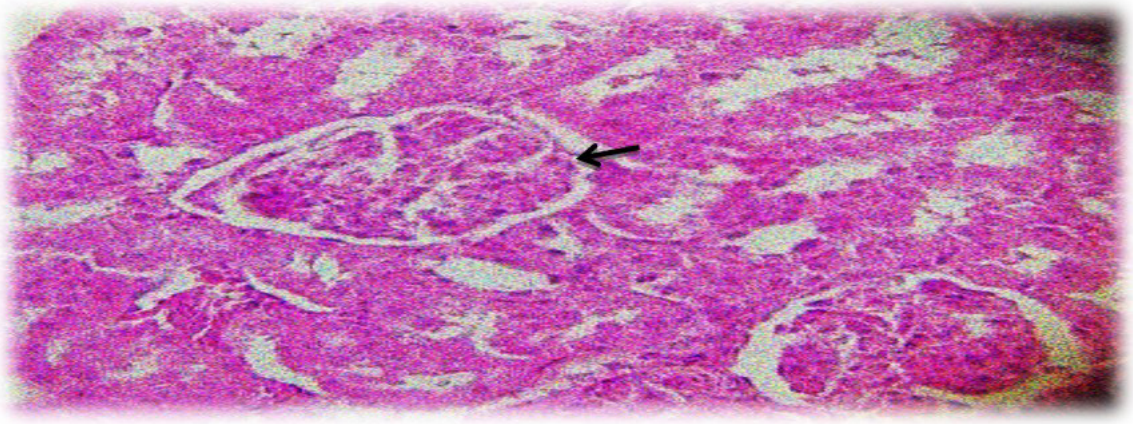

Figure 3. Histological section of a mouse kidney from the group treated with aspirin at a concentration of 150 mg/kg and paracetamol 750 mg/k, showing the histological appearance close to normal with Bowman's capsule dilated (40x)

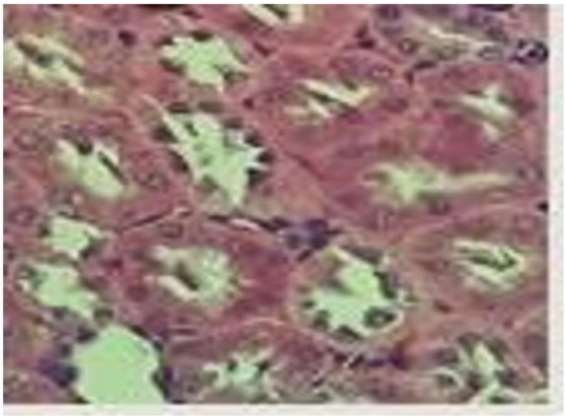

Figure 4. The histological section of a mouse kidney from the group treated with Percitol at a concentration of 750 mg/kg showed acute blood congestion in the blood vessels. Inflammatory cell infiltration. - And severe watery degeneration in the tubules. (40x)

Table 1. Shows the values of kidney averages with the effect of different treatments

Features	Oleuropein 50 mg/k	Oleuropein 100 mg/k	Oleuropein 150 mg/k	Normal saline	Paracetamol
The proximal convoluted tubule	42.712±0.808	36.841±0.687	30.973±0.744	30.877±0.779	55.932±0.931
Distal convoluted tubules	39.748±1.122	33.549±1.015	27.959±0.284	27.862±0.370	60.668±0.574

Shows that the measurements of the kidneys' internal parameters in the groups dosed with Oleuropein were unaffected

been shown to have potent antimicrobial activity against Gram-negative and Gram-positive bacteria and Mycoplasma (Bisignano *et al.* 1999; Furneri *et al.* 2002). In this study, paracetamol administration caused a significant increase in urea and creatinine levels in the blood, leading to kidney damage. These results were consistent with the results of previous studies (Anthony *et al.* 2012; Saxena *et al.* 2012; Mandal *et al.* 2015; Sini *et al.* 2017). What reported this was There is a significant increase in urea and creatinine when taking excessive doses of Paracetamol. In our study, the use of oleuropein extract caused a significant decrease in the level of creatinine and urea in the blood, and this is consistent with the results of previous studies (Zari & Al-Attar 2011; Taha *et al.* 2020), which indicated that olive leaf extract reduces kidney toxicity caused by Carbendazim in mice. Treatment with olive leaf extract also led to a decrease in the level of sodium and potassium in blood serum, and this is consistent with a study (Azab *et al.* 2017), which indicated that olive leaves, rosemary, and sesame significantly protect the kidneys against nephrotoxic agents and diseases resulting from dysfunction-kidneys in humans and experimental animals. Selective renal accumulation of nonsteroidal anti-inflammatory phototoxins, including Paracetamol in animals and humans, is thought to trigger a cascade of biochemical reactions culminating in acute or chronic nephropathy (Tejo 2021). However, blood urea concentration is often considered a more reliable indicator of kidney function than serum creatinine (Soliman *et al.* 2020). Blood urea nitrogen is found in liver protein derived from diet or tissue sources and is normally excreted in the urine. On the other hand, creatinine is mainly derived from endogenous sources via tissue creatinine degradation (Ahmed *et al.* 2021). In this study, administering a nephrotoxic dose of Paracetamol to rats significantly increased urea, creatinine, and uric acid levels in the paracetamol group. Within 1 Within of exposure, it is compared to a normal control group.

Some olive extracts significantly protect body tissues, as has been proven (Hassan *et al.* 2022). The ability to reduce cellular changes and apoptosis resulting from the effects of chemicals and drugs, such as giving phenols to experimental animals, has good protection against the kidneys (Asghari *et al.* 2022; Deniz & Necati 2023). Due to the aromatic hydrocarbons, infection of treated animals with the treated extract resulted in marked recovery of the histopathological structure of the kidneys with very mild cellular damage, which is rich in antioxidants, anti-inflammatory and anti-apoptotic properties and free of radical scavenging activities (Rezaieg & Musleh 2019; Micheli *et al.* 2023; Pirković *et al.* 2023; Huldani *et al*. 2024).

The measurements of the internal parameters of the kidneys were not affected in the groups dosed with the drug oleuropein, as they showed a concentration of 150 mg/kg, and the result was very close to the control group. The reason may be due to the role of phenols, which showed greater protection against the damage that the carcinogen may cause to the internal properties of the kidneys if They were taken only without the plant extract. The reason may be attributed to the fact that plant phenols, including exercise, have a protective effect in restoring the normal shape of kidney tissue due to their anti-inflammatory activity (Fayez *et al.* 2023; Hsu *et al*. 2024). These results are consistent with (Ghrayeb *et al.* 2023), who observed an increase in blood urea and creatinine in rats after ingesting 1 g/kg body content of Paracetamol. This increase in urea and creatinine levels is due to a high correlation between nephrotoxicity and autonomic stress (Bucciantini *et al.* 2021). Daily treatment with Oleuropein confers renal protection in paracetamol rats with renal impairment in a dose-dependent manner.

The marked improvement in alleviating the toxic effects of Paracetamol, such as renal congestion, is what confirms the renal protection of the extract, inflammatory cells, tubular and peritubular necrosis, and the presence of intraluminal casts suggestive of massive necrosis. It is possible that the protective effect of the extract is mediated by antioxidants and/or free radical scavenging activities (Taticchi *et al.* 2019), and some research has shown the importance of olive extracts as anti-stress and tissue protection. Studies on medicinal plants with nephroprotective properties have shown that they mediate their protection through links to antioxidant free radical scavenging activity and/or due to the high concentration of flavonoids and alkaloids (Beauchamp 2019; Khayyat 2021; Fadil *et al.* 2023). In conclusion, excessive doses and long-term use of paracetamols lead to organ toxicity. The study's results indicate that using olive leaf extract for Oleuropein protects the kidneys from damage resulting from toxicity resulting from excessive doses of paracetamols.

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