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Detection of the Ameliorative and Antioxidant Effects of Pumpkin Seed Oil and Olive Oil on DBP-Induced Nephrotoxicity in Male Albino Rats by Investigating their Protective Properties on Physiological and Histological Measures

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Abstract

THE present research assessed the protective properties of pumpkin (Cucurbita pepo L) and olive (Olea europaea L) oil on di-n-butyl phthalate (DBP)-induced renal damage in rats. This study employed 30 albino male rats, six per group. Group one "control" (C); group two (DBP) was treated with DBP (500 mg/kg/day); group three (DP) was treated with DBP and supplemented with pumpkin oil (288 mg/kg bwt/daily); group four (DO) was treated with DBP and supplemented with olive oil (1.5 ml/kg bwt/daily); and group five (DPO) was treated with DBP and supplemented with both pumpkin and olive oils. The experiment lasted for two months. Serum levels of creatinine, urea and uric acid, oxidative stress markers and histological changes, were investigated. TAC, SOD, and ALP levels decreased significantly, but MDA, creatinine, urea and uric acid levels increased. Histopathological lesions in kidney tissue of the DBP-exposed group were found. In conclusion, supplementing rats with pumpkin or olive oil under oxidative stress resulted in better oxidative state of the kidney, kidney function tests, and renal tissue histology.

Keywords: Nephrotoxicity, pumpkin oil, olive oil, dibutyl phthalate.

Introduction

Nephrotoxicity is the more common renal illnesses that happens when the animals and human are exposed to toxins or drugs at certain quantities [1]. Dibutyl phthalate (DBP), the most often used plasticizer, increases the flexibility and elasticity of plastic polymers, resulting in greater quality [2]. Dibutyl phthalate (DBP) can harm the kidney, causing acute renal failure and chronic interstitial nephritis. It causes tubular damage by necrotizing the epithelial coating of renal tubules and disrupting the function of key cellular components responsible for water and electrolyte transport [3]. In the kidney, dibutyl phthalate (DBP) generates renal oxidative stress [4]. According to [5], ROS can activate nuclear factor kappa β, which is responsible for inducing inflammation. Kidney tissue is extremely vulnerable to damage caused by oxidative stress [6] Medicinal herbs are frequently utilized since they are effective and have few adverse effects [7]. Several medicinal plants are recommended for the treatment of renal injury [8]. Pumpkin has been widely utilized as a functional food and medication [9]. Pumpkin (Cucurbita pepo L.) includes macro- and micro-constituents [10]. It includes unsaturated fatty acids (oleic and linoleic acids), antioxidants, vitamins (carotenoids and tocopherol), trace elements (zinc and selenium), proteins and phytosterols [11,12]. Pumpkin seed oil (PSO) reduces liver damage and oxidative stress generated by sodium nitrate in rats [13]. Furthermore, it mitigates the oxidative stress caused by tramadol analgesic treatment [14]. PSO anti-inflammatory action was ascribed to the activation of antioxidant mechanisms and decreasing of lipid peroxidation [15]. The possible activity of PSO in wound healing of rats, as well as the presence of well-organized collagen fibers and the absence of an inflammatory cellular response [16].

Olive oil (Olea europaea L) contains many antioxidants, the majority of which are phenolic compounds categorized into a hydrophilic group, a lipophilic group, and oleuropein [17]. Various studies have reported that olive extracts have a variety of pharmacological actions, including lowering LDL blood cholesterol levels, being antioxidant, antimicrobial, anti-inflammatory, anti-atherosclerotic, hypotensive, cardioprotective, anti-cancer, anti-thrombotic and hypoglycemic [18]. Olive oil decreases resistance of insulin caused by a diet high fat in rats and alleviates liver inflammation

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[19]. Thus, olive oil is used to alleviate the adverse effects of various diseases and toxicities [20, 21, 22, 23]. Olive oil, for example, improves the pathologic alterations in tissues caused by experimental autoimmune encephalomyelitis in organs such as kidney, liver, heart, and intestines [24]. Furthermore, olive oil decreases oxidative stress, platelet aggregation, and damage of cell caused by hypoxia in rat brain tissues [25].

The present research intended to detect the ameliorative and an antioxidant effects of pumpkin seed oil and olive oil on DBP-induced nephrotoxicity in male albino rats by investigating their protective properties on physiological and histological measures.

Material and Methods

Thirty adult male albino rats (Sprague-Dawley strain, weighing 150g) were obtainedt from the Giza Ophthalmic Institute's animal facility. The rats were divided into five equal groups at random. The first group served as the negative control (C); the second group (DBP) got DBP therapy for two months at a dosage of 500 mg/kg bwt.; and the third group (DP) received DBP treatment for two months at a dosage of 500 mg/kg bwt. as well as 288 mg/kg bwt./daily) of pumpkin oil [26], group four (DO) were treated with DBP at a dose of 500 mg/kg bwt. and supplemented with olive oil (1.5 ml/kg bwt/daily), and group five (DPO) were treated with DBP at a dose of 500 mg/kg. bwt. and supplemented with both pumpkin and olive oils, The experiment lasted for two months. Feed and water were given unlimited access for the duration of the 60-day experiment.

Chemicals and reagents

Dibutyl phthalate (DBP) was purchased from Sigma-Aldrich, Germany. Pumpkin seed oil and olive oil were obtained from Egypt's Arab Company for Pharmaceutical and Medicinal Plants, or "MEPACO,". Randox Laboratories Ltd., Diamond Road Crumlin, Co. Antrim, United Kingdom, BT294QY, supplies all biochemical assay kits.

Collection and analyzing samples

After two months of testing, all animals were given a 12-hour fast before having their blood collected. After allowing samples to clot for 20 minutes at room temperature, they were centrifuged at 3000 rpm for 10 minutes to separate serum for creatinine, urea, and uric acid tests. Following blood collection, the animals were put to sleep using an anesthetic regimen that comprised cervical dislocation and intraperitoneal injections of xylazine (30 mg/kg) and ketamine (300 mg/kg). The kidneys of the killed animals were taken and examined. A kidney was weighed after being carefully cleaned with ice-cold normal saline (10% w/v). The kidney tissues were homogenized in saline buffered with phosphate (pH 7, 50 mmol/l). To assess lipid peroxidation expressed in malondialdehyde (MDA) level [27], total antioxidant capacity (TAC) [28]), superoxide dismutase activity (SOD) [29], and alkaline phosphatase (ALP) activity [30], homogenates were centrifuged at 10,000 rpm (4°C) for 10 minutes.

Immunohistochemistry and histopathology

Histopathological analysis

Rats in each group had their kidneys quickly removed, and they were preserved for 24 hours in 10% neutral buffered formalin (NBF). Higher and higher alcohol dilutions were used to dry the materials. A manual microtome was used to cut the specimens into 5-µm paraffin slices after they had been cleaned in xylene and embedded in parablast. Slices of dewaxed tissue were stained with hematoxylin and eosin (H&E) for histological analysis [31].

Immunohistochemistry

It was done on deparaffinized kidney slices from each group, which were then put on positively charged glass slides. Tissue slices were rehydrated with decreasing levels of alcohol. The antigen retrieval procedure was carried out in a microwave oven at 500 W for 10 minutes using sodium citrate buffer (PH6.0). The activity of endogenous peroxidase was inhibited by administering hydrogen peroxide. Thermo Scientific, Cheshire, UK, used UV Block reagent to prevent non-specific binding.

Proliferating Cell Nuclear Antigen (PCNA)

Slides containing primary antibodies against PCNA (Thermo Scientific) were treated overnight at 4ºC to detect mitosis following antigen extraction and non-specific binding inhibition. The streptavidin-biotin-peroxidase method was used after biotinylated secondary antibodies. Diaminobenzidine was used to observe the immunological response. Every section was counterstained with hematoxylin. Following each treatment, PBS was used to clean the slices. By replacing the primary or secondary antibodies with PBS, negative controls were added [32].

Cyclooxygenase enzyme 2 (COX-2)

Tissue slices were incubated overnight at 4ºC in a humidified environment with rabbit anti-COX2 polyclonal antiserum (Cayman Chemical, Ann Harbor, MI) at a 1:50 dilution after antigen extraction and non-specific binding blocking. Before applying a biotinylated goat anti-rabbit antibody (Thermo Scientific, USA) for 10 minutes, the slices were washed again with PBS. PBS was used to clean the pieces once again. Finally, Streptavidin Peroxidase (Thermo Scientific, USA) was used on the slices. Slides were treated with 3, 30 diaminobenzidine tetrahydrochloride (DAB, Sigma) for 10 minutes to examine the response. Hematoxylin was used to counterstain the slides, which were subsequently

dried. Primary antibodies were removed, and PBS served as a negative control.

PCNA and COX2 stained sections were analyzed and photographed at Cairo University's Faculty of Dentistry with a Leica Qwin 500 analyzer computer system (Leica Microsystems, Switzerland). The image analyzer program's measurement units (pixels) were automatically translated to micrometer units via calibration.
PCNA and COX2 immunostaining were quantified as area percentages in five fields within each group using light microscopy projected onto a screen at x400 magnification. Regardless of staining intensity, regions with PCNA and COX2 positive brown immunostaining were chosen for further investigation.

Statistical analysis

The SPSS version 18.0 was used to examine all quantitative data. The data was shown as mean ± SE. After comparing the means of various groups using a one-way analysis of variance, the test of Duncan was conducted. A p-value of less than 0.05 was considered statistically significant.

Results

Impact of supplementing pumpkin and olive oils on SOD, TAC, MDA, and ALP in rats exposed to DBP nephrotoxicity.

The average antioxidant characteristics of renal tissue were shown by Figure 1. DBP exhibited considerably greater levels of MDA and significantly decreased of SOD, TAC, and ALP levels than the control group ($P < 0.05$). Comparing the DBP group to those treated with DBP and supplemented with pumpkin oil and olive oil, revealed a significant improvement in each of these groups. Additionally, when compared to all other groups, in comparison to the negative control group, the DOP group had significantly higher levels of SOD, TAC, and ALP. *Supplementing with pumpkin and olive oils affected blood urea, creatinine, and uric acid levels in rats subjected to DBP nephrotoxicity (Mean±SD).*

Fig. 2 showed the average serum urea, creatinine, and uric acid levels. Serum urea, creatinine, and uric acid levels increase in DBP-treated groups, but reduced in pumpkin oil and olive oil-supplemented groups compared to the control group ($P<0.05$).

Results of immunohistochemistry and histopathology Examination with a light microscope.

When viewed under a light microscope, the renal cortex of the control group revealed typical renal corpuscles as well as proximal and distal convoluted tubular structures. Bowman's capsule was made up of two layers: parietal and visceral was separated by the urinary gap, encircled each renal corpuscle's glomerular capillary tuft. The proximal convoluted tubules (PCs) were surrounded by truncated pyramidal cells with distinct brush boundaries. The cells appeared to have acidophilic cytoplasm and large, spherical nuclei. The low cubic cells surrounded the distal convoluted tubules (DC). The nuclei cells were rounder, and their cytoplasm was less acidophilic (Fig 3.a) A Henel's loop, collecting tubules coated with simple cuboidal cells, and blood capillaries in the interstitial tissue comprised the renal medulla. (Fig.3.b). Comparison with control group, DBP-exposed group showed a number of histological changes in the renal tissue. The cortex had glomerular degeneration and a significant urine gap, whereas the other glomeruli exhibited modest expansion. Furthermore, the interstitial tissue looked to be full of blood. The epithelial lining of (PC) showed lack of brush boundaries and enlarged karyolitic nuclei. Others exhibited desquamated epithelium. Also, the identical changes are noted in the (DC). Some distal convoluted tubules revealed flattened lining epithelial cells, while others lost their nuclei and cytoplasm inside the lumen. Furthermore, hyalinization was seen in several tubules (Fig. 3.c).

Hyalinization was seen in several regions of the renal medulla, as were proteinaceous casts inside the tubules. In addition to vascular congestion and perivascular oedema in the tubules. Additionally, there was lymphocytic infiltration. Flattened collecting tubule epithelial cells. Some tubules exhibited enlarged karyolitic nuclei (Fig.3.d). The structure of the kidney tissue improved in the pumpkin oil and olive oil co-treated groups, with minor histological changes found in the kidney cortex and medulla (Fig.3.e-3.f). However, the kidney cortex and medulla of the co-treated group with pumpkin and olive oil showed nearly normal results (Fig. 3.g & 3.h).

Immunohistochemistry In Figure 4, the renal tissue of the DBP-exposed group exhibited a higher level of brown immunoreactivity for both the nuclear apoptotic factor PCNA and the cytoplasmic apoptotic factor COX2 than the control group. Conversely, both PCNA and COX2 levels in the renal tissue of the co-treated groups improved (Figs. 5 and 6).

Discussion

The kidneys are responsible for a wide range of biological functions. They primarily function to maintain the homeostatic balance of physiological fluids by filtering and secreting minerals and metabolites from the blood, as well as removing nitrogenous wastes [33].

The current investigation discovered that the DBP group had decreased antioxidant levels (SOD and TAC) and renal enzyme indicators (ALP), as well as a significant rise in serum creatinine, urea, uric acid, and lipid peroxidation (MDA). DBP promotes the generation of reactive oxygen species (ROS). Cells' first line of defense against oxidative damage is the presence of free radical scavenging enzymes like

superoxide dismutase and catalase [34]. ROS (such as OH-) may easily damage the human plasma membrane, which is rich in polyunsaturated fatty acids. Malondialdehyde (MDA), the byproduct of lipid peroxidation, is used to measure the process, also known as the lipid peroxidation reaction [35]. The findings revealed that the group treated with DBP exhibited a substantial drop in ALP. Alkaline phosphatase levels have been proposed as indications of renal function. The findings are consistent with those of [36], who reported that rats subjected to oxidative stress exhibited lower activity of lactate dehydrogenase, gamma glutamyl transferase, and alkaline phosphatase. Germ cell loss was related with decreased activity [37].

The reduction in tissue ALP activity might be attributed to DBP-induced nephrotoxicity, which damages the brush border membrane of renal tubular cells. ALP may be a marker for renal participation in disease processes, as well as a sign of and renal participation in disease processes [38]. Furthermore, urea concentrations increase exclusively following DBP-induced parenchymal tissue damage [3]. Endogenous creatinine production occurs through tissue creatinine breakdown, and its removal allows for a relatively exact evaluation of GFR. After passing through the glomeruli and into tubular urine, DBP binds to phospholipids in the brush border membrane of proximal tubular cells, and the toxin enters cells via adsorptive/receptor-mediated endocytosis after binding to acidic lipids. DBP-treated mice show proximal tubular cell death [39]. Elevated serum urea, uric acid, and creatinine levels may be induced by DBP boosting Ca+2 entry in mesangial cells, resulting in a slower glomerular filtration rate [2].

Our microscopic study of the DBP-exposed group revealed renal tissue with several histological changes. This was addressed by [34] who stated that the kidney tissue is very susceptible to toxicants due to its involvement in the filtering of a large volume of blood, which concentrates toxicants in the renal tubules.

The renal cortex showed glomerular deterioration, a large urinary gap, and mild glomerular enlargement, which is similar with the findings of [40].

In the DBP-exposed group, COX2 demonstrated strong immunological reactivity in the epithelial lining of the renal tubule.

According to [41], tubular necrosis is produced by an increase in intracellular free oxygen radicals, which results in irreversible cellular death via lysosomal enzyme activation. Additionally, tubular vacuolization was seen in the DBP-exposed group. [41, 42] found that plasma membrane damage produces reversible cellular swelling and tubular vacuolization, resulting in loss of osmotic balance and elevated intracellular calcium. Furthermore, PCNA demonstrated significant positivity in the nuclei of the afflicted tubules. Some renal tubules revealed enlarged and karyolitic nuclei, as reported by [43].

Our histological results are congruent with those of [43], who found necrosis and loss of brush boundaries in the epithelial lining of (PC) in the DBP-exposed group. In addition, hyalinization occurs in certain tubules. Cortical regions in the DBP group exhibited widespread tube necrosis, desquamation and dilatation, brush boundary loss, and the occasional presence of protein casts. Tubular morphology appeared to be normal in the medulla. Mohamadi [44] found vascular congestion, perivascular oedema in between tubules, and lymphocytic infiltrations, which are compatible with our results.

Medicinal plants and herbal drugs are extensively used to treat a range of ailments due to their effectiveness, few side effects, and inexpensive cost. According to [45], antioxidants are vital to the body's ROS defense mechanism. The antioxidants are divided into two categories: enzymatic antioxidants and non-enzymatic antioxidants, which include phenolic compounds (olive), vitamins E, and C [46]. In this study, co-treated groups who received DBP and were supplemented with pumpkin and/or olive oil had a significant drop in lipid peroxide, an increase in antioxidant indices, and a decrease in urea, creatinine, and uric acid. The results were comparable to those of [47]. who discovered that pumpkin polysaccharide may lower MDA levels in tumor mice's blood while increasing SOD and GSH-Px activity. Furthermore, [48] found that adult male albino rats given pumpkin seed oil consistently had significantly higher blood glutathione levels. Pumpkin seeds are rich in fatty and linoleic acids [49]. They may improve health and prevent chronic illnesses [50].

Most of the antioxidant molecules in olive oil are made up of vitamin E and phenolic chemicals found naturally in fruits, vegetables, and grains., these chemicals have a wide range of physiological properties and can alleviate oxidative stress [17].

The current findings are comparable with those of [51], who found that olive products boosted antioxidant enzymes in rabbits under oxidative stress.

The increase in ALP activities might be due to the fact that antioxidants have a role in preserving tissue physiological integrity by guarding against enzyme leakage [52] rise in DBP-induced blood creatinine and urea levels and uric acid, which might be attributed to the protective action of pumpkin and olive oils against DBP-induced nephrotoxicity, as seen by the lower serum urea and creatinine concentrations. These findings are consistent with those published by [33], who found that olive leaf

reduces serum urea and creatinine. At the microscopic level, our findings revealed no significant difference between the pumpkin oil and olive oil co-treated groups, which were identical to the standard cytoarchitecture. In contrast, the pumpkin oil and olive oil co-treated groups showed healing in renal tissue with mild affection. These affections were more common in the olive oil co-treated group than the pumpkin oil co-treated group.

The protective effect of olive oil on carbendazim-exposed rats and discovered a substantial rise in histopathological abnormalities [53]. According to [18] this improvement is due to olive's ability to prevent deterioration in the antioxidant defense system and direct free radical scavenging. Furthermore, [22] demonstrated that olive oil had a beneficial effect on renal tissue histological changes. Pumpkin has anti-apoptotic effects and can decrease BAX gene expression while raising BCL-2, which explains the improvement in renal tissue after co-treatment [54].

Conclusion

Rats fed pumpkin and olive oils have improved kidney function (antioxidant and enzyme levels) and reduced urea, uric acid, creatinine, and oxidative stress. Additionally, there is better protection against DBP-induced nephrotoxicity when pumpkin oil and olive oil are combined.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The research was done at the Physiology Department of Cairo University's Faculty of Veterinary Medicine. All experimental procedures were approved by the Animal Welfare and Use Committee (IACUC) of Cairo University's Faculty of Veterinary Medicine under protocol number (VetCU1022019075).

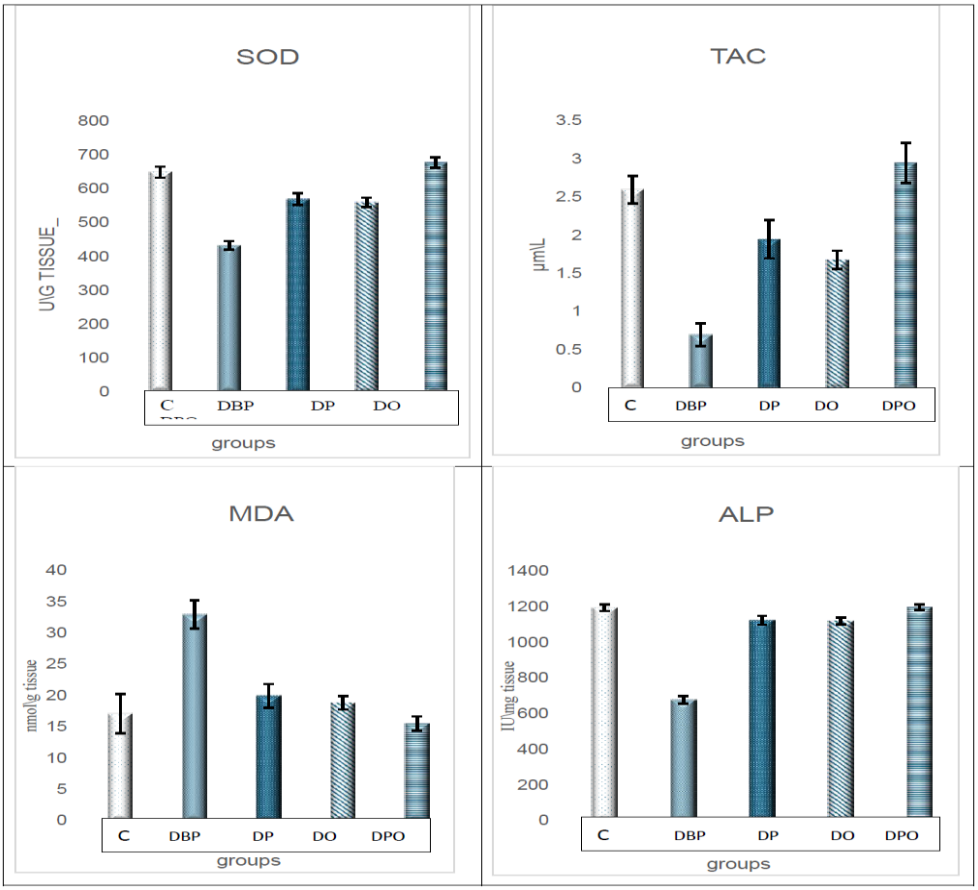

Fig. 1. Impact of pumpkin oil and olive oil supplementation on antioxidant parameters (SOD and TAC), MDA and ALP of rat subjected to DBP nephrotoxicity (means ± SE).

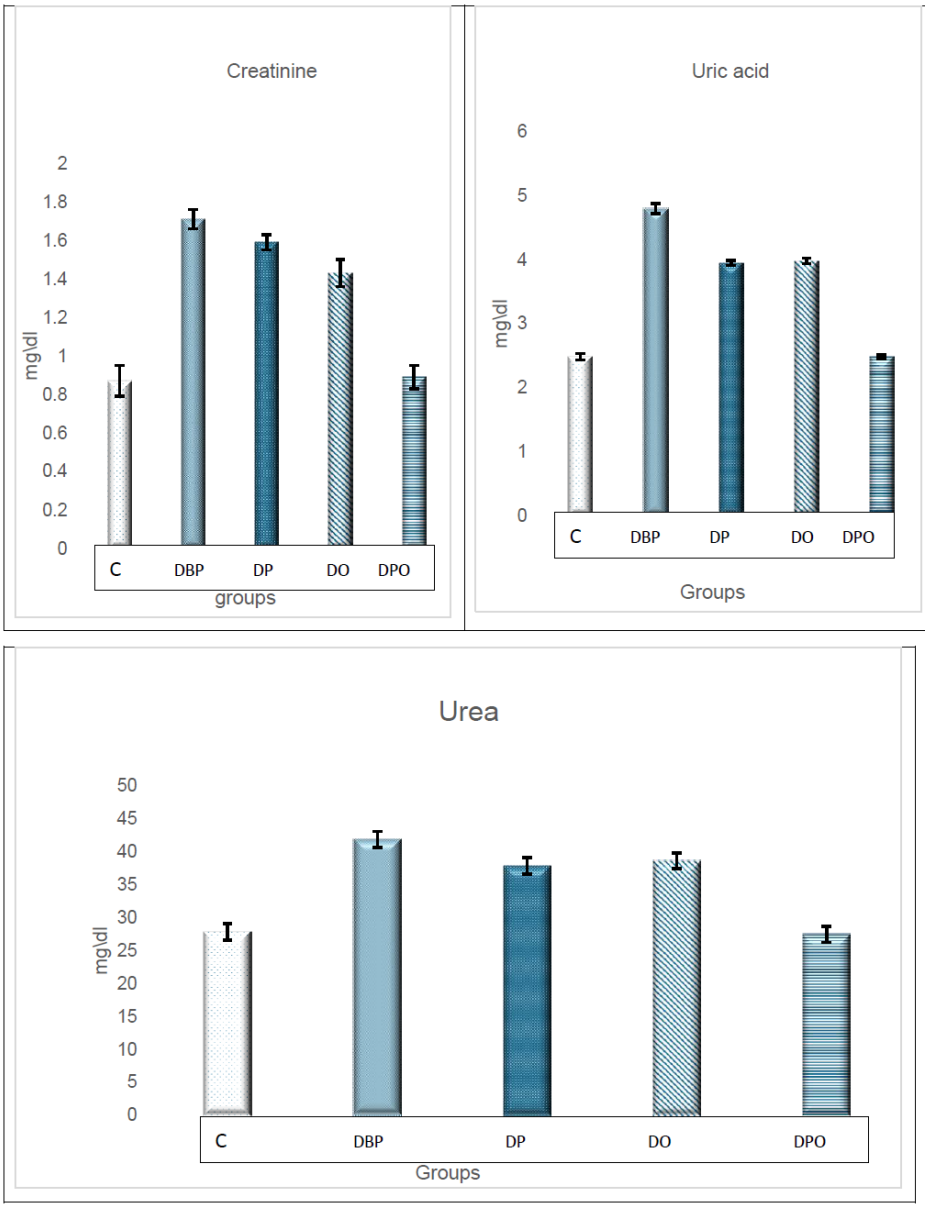

Fig. 2. Effect of pumpkin oil and olive oil supplementation on kidney parameters of rat subjected to DBP nephrotoxicity (means ± SE).

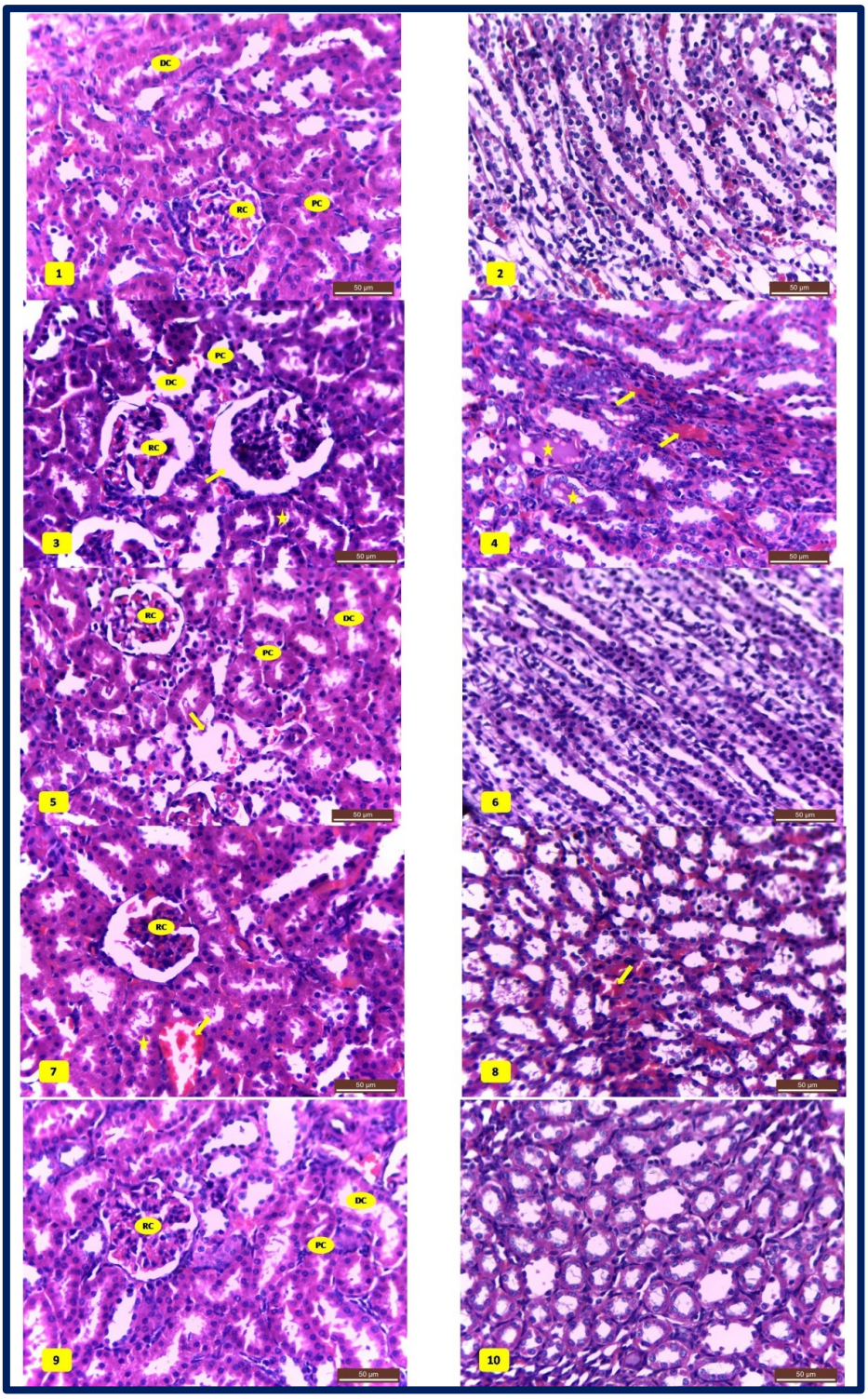

Fig. 3. (1&2) C group normal renal tissue. (3) DBP group showed degenerated and enlarged renal corpuscle (RC) with wide urinary space (arrow), the proximal convoluted tubules (PC) showed loss brush borders, distal convoluted tubules (DC) appeared with flattened cells, others with desquamated cells in their lumen (star), (4) medulla showed hyalinization in some areas and the intra-tubular proteinaceous casts (star) and congestion (arrow). (5&6) DBP group some tubules still affected (arrow). (7&8) DO group some areas still congested (arrow). (9&10) DPO group the renal tissue is nearly normal. H&E stain 400x.

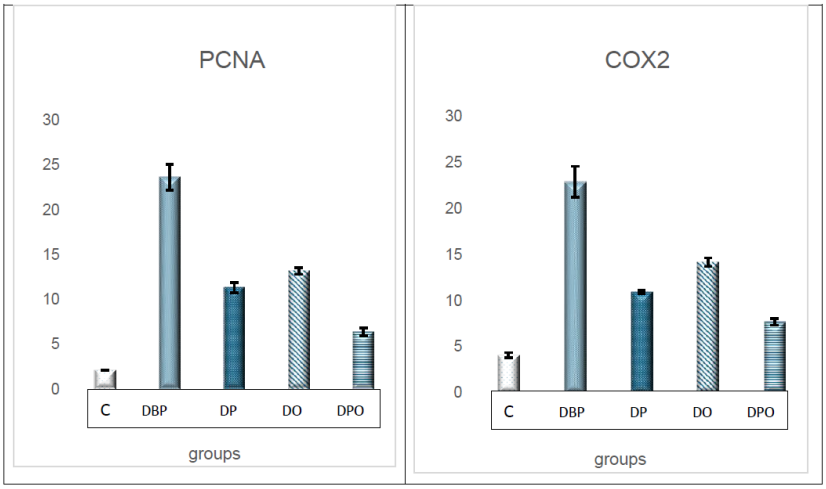

Fig. 4. Impact of pumpkin oil and olive oil supplementation on PCNA and COX2 immunohistochemistry of rat subjected to DBP nephrotoxicity (means ± SE).

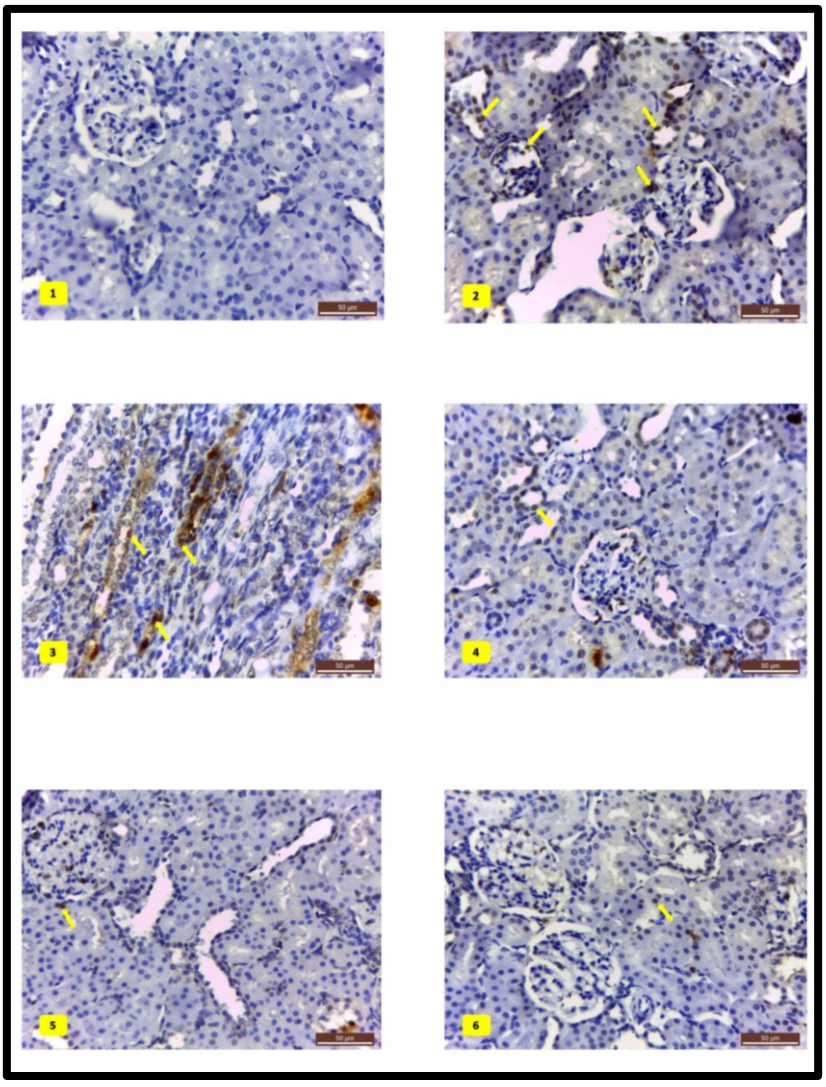

Fig. 5. Brown positive immune-reactivity in nuclei of renal tissue (arrow). (1) C group. (2&3) DBP group. (4) DP group. (5) DO group. (6) DOP group. PCNA, 400x.

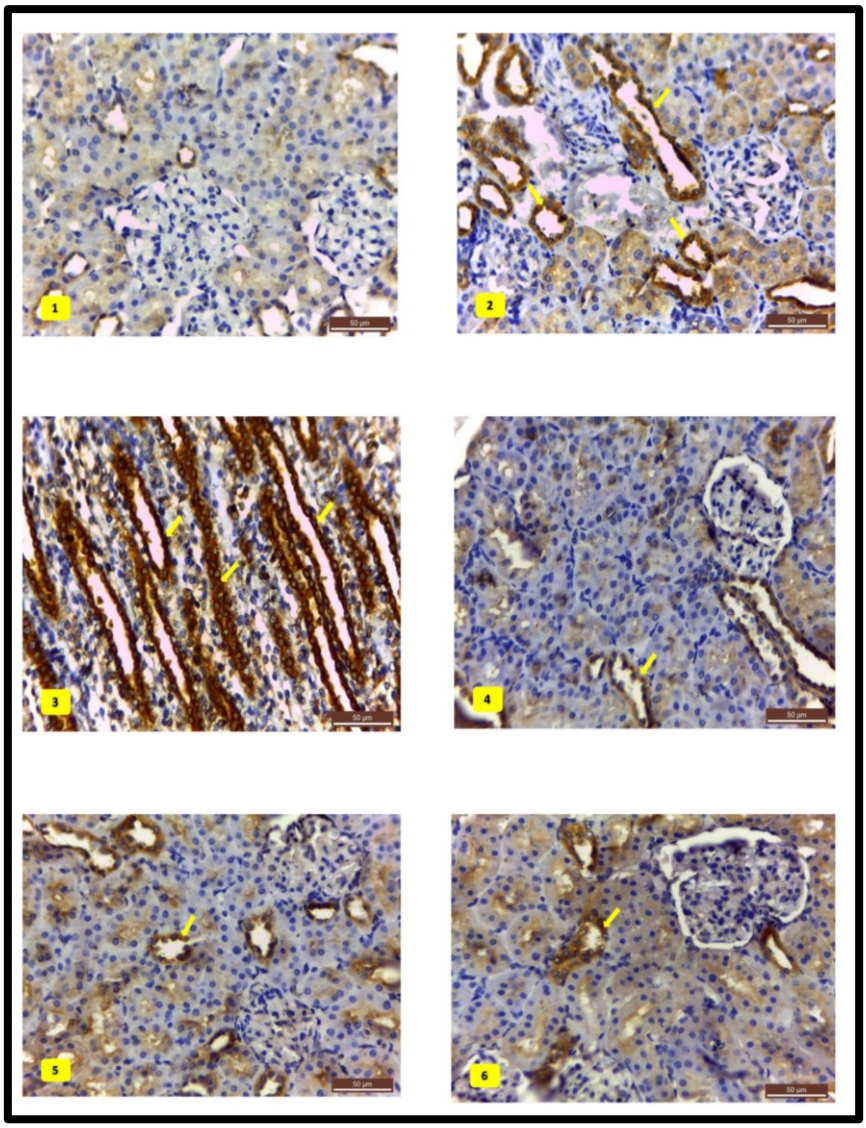

Fig. 6. (1to6) brown positive immune-reactivity in cytoplasm of renal tissue (arrow). (1) C group. (2&3) DBP group. (4) DP group. (5) DO group. (6) DOP group. COX2, 400x

References

1. Gamaan, M.A., Zaky, H.S. and Ahmed, H.I. Gentamicin-induced nephrotoxicity: A mechanistic approach. Azhar *International Journal of Pharmaceutical and Medical Sciences*, **3**(2),11-19.(2023). doi: 10.21608/AIJPMS.2023.161755.1167 . https://aijpms.journals.ekb.eg/
2. Chang, W.H., Herianto, S., Lee, C.C., Hung, H. and Chen, H.L. The effects of phthalate ester exposure on human health: A review. *Sci Total Environ*, **786**, 147371(2021).
3. Cheng, L., Li, J., Cheng, J. and Wu, Z. Dibutyl phthalate-induced activation of ROS and ERK1/2 causes hepatic and renal damage in Kunming mice. *Human & Experimental Toxicology*, **38**(8), 938-950 (2019). doi:10.1177/0960327119843583
4. Wang, X., Yan, X., Yang, Y., Yang, W., Zhang, Y., Wang, J., Ye, D., Wu, Y., Ma, P. and Yan, B. Dibutyl phthalate-mediated oxidative stress induces splenic injury in mice and the attenuating effects of vitamin E and curcumin. *Food Chem Toxicol.,* **136**,110955(2020). doi: 10.1016/j.fct.2019.110955. Epub 2019 Nov 8. PMID: 31712109.
5. Lopez-Novoa, J.M., Quiros, Y., Vicente, L., Morales, A.I. and Lopez-Hernandez, F.J. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney Int*., **79**(1), 33-45 (2011). doi: 10.1038/ki.2010.337. Epub 2010 Sep 22. PMID: 20861826.
6. Ozbek, E. Induction of oxidative stress in kidney. *Int. J. Nephrol.*, 1–9(2012).
7. Tohidi, M., Khayami, M., Nejati, V. and Meftahizade, H.. Evaluation of antibacterial activity and wound healing of Pistacia atlantica and Pistacia khinjuk. *J. Med. Plant Res*., 5, 4310–4314 (2011).

8. Palani, S., Raja, S., Kumar, R.P., Jayakumar, S. and Kumar, B.S. Therapeutic efficacy of Pimpinella tirupatiensis (Apiaceae) onacetaminophen induced nephrotoxicity and oxidative stress in male albino rats. *Int. J. PharmTech. Res*., **1**, 925–932 (2009).

9. Zaremba, A., Hs, M., Jedrusek-Golinska, A., Przeor, M. and Szymandera-Buszka, K. The Antioxidant Properties of Selected Varieties of Pumpkin Fortified with Iodine in the Form of Potassium Iodide and Potassium Iodate. *Foods*, **12**, 2792 (2023). https://doi.org/ 10.3390/foods12142792.

10. Bakeer, M.R Focus on the effect of dietary pumpkin (Cucurbita moschata) seed oil supplementation on productive performance of growing rabbits. *Journal of Applied Veterinary Sciences*, **6** (2), 22 – 26 (2021). https://javs.journals.ekb.eg.

11. Makni, M., Fetoui, H., Gargouri, N.K., Garoui, M., Jaber, H., Makni, J., Boudawara, T. and Zeghal, N. Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in u-3 and u-6 fatty acids in hypercholesterolemic rats. *Food ChemToxicol.,* **46**, 3714e20 (2008).

12. Elhamalawy, O. Protective effect of pumpkin seed oil against lead acetate toxicity in male mice. *Al-Azhar Journal of Pharmaceutical Sciences*, **58**(2), 115-129(2018).

13. Rouag, M., Berrouague, S., Djaber, N., Khaldi, T., Boumendjel, M., Taibi, F., Abdennour, C., Boumendjel, A. and Messarah, M. Pumpkin seed oil alleviates oxidative stress and liver damage induced by sodium nitrate in adult rats: biochemical and histological approach. *African Health Sciences*, **20**(1), 413-425(2020).

14. Ekpono, E.U., Eze, E.D., Adam, A.M., Ibiam, U.A., Obasi, O.U., Ifie, J.E., Ekpono, E.U., Alum, E.U., Noreen, S., Awuchi, C.G. and Aja, P.M. Ameliorative Potential of Pumpkin Seed Oil (Cucurbita pepo L.) Against Tramadol-Induced Oxidative Stress. *Dose-Response*, **22**(1), 15593258241226913 (2024).

15. Arslanbaş, E., Kara, H., Karayiğit, M.Ö., Doğan, H.O. and Yildiz, Ş.N. Anti-inflammatory activity of Turkey source pumpkin seed oil in rat oedema model. *Acta Poloniae Pharmaceutica-Drug Research*, **77**(2), 305-312 (2020).

16. Bardaa, S., Ben Halima, N., Aloui, F., Ben Mansour, R., Jabeur, H., Bouaziz, M. and Sahnoun, Z. Oil from pumpkin (Cucurbita pepo L.) seeds: evaluation of its functional properties on wound healing in rats. *Lipids in health and disease*, **15**, 1-12 (2016).

17. Guclu, G., Kelebek ,H. and Selli ,S. Antioxidant activity in olive oils, *Olives and Olive Oil in Health and Disease Prevention (Second Edition)* , Chapter 26 - Pages 313-325(2021).

18. Bakeer, M., Abdelrahman, H. and Khalil, K. Effects of pomegranate peel and olive pomace supplementation on reproduction and oxidative status of rabbit doe. *Journal of Animal Physiology and Animal Nutrition*, **106**, 655–663 (2022). https://doi.org/10.1111/jpn.13617.

19. Lama, A., Pirozzi, C., Mollica, M.P., Trinchese, G., Di Guida, F., Cavaliere, G., Calignano, A., Mattace Raso, G., Berni Canani, R. and Meli, R. Polyphenol-rich virgin olive oil reduces insulin resistance and liver inflammation and improves mitochondrial dysfunction in high- fat diet fed rats. *Molecular Nutrition & Food Research*, **61**(3),1600418 (2017).

20. Kumral, A., Giris_, M., Soluk-Tekkes_in, M., Olgaç, V., Dog˘ru-Abbasog˘lu, S., Türkog˘lu,Ü. and Uysal, M. Effect of olive leaf extract treatment on doxorubicininduced cardiac, hepatic and renal toxicity in rats. *Pathophysiology,* **22**, 117–123 (2015).

21. Al-Attar, A.M., Alrobai, A.A. and Almalki, D.A. Effect of Olea oleaster and Juniperus procera leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. *Saudi J. Biol. Sci.,* **23**, 363–371(2016).

22. Al-Attar, A.M., Alrobai, A.A. and Almalki, D.A. Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice. *Saudi J. Biol. Sci*., **24**, 13–22 (2017).

23. Lombardo, L., Grasso, F., Lanciano, F., Loria, S., & Monetti, E. Broad-spectrum health protection of extra virgin olive oil compounds. *Studies in Natural Products Chemistry*, **57**, 41-77 (2018).

24. Conde, C., Escribano, B.M., Luque, E., Feijóo, M., Caballero-Villarraso, J., Valdelvira, M.E., Ochoa-Sepúlveda, J.J., Lillo, R., Paz, E., Santamaría, A. and Agüera, E. Extra-virgin olive oil modifies the changes induced in non-nervous organs and tissues by experimental autoimmune encephalomyelitis models. *Nutrients,* **11**(10), 2448 (2019).

25. González-Correa, J.A., Muñoz-Marín, J., Arrebola, M.M., Guerrero, A., Narbona, F., López-Villodres, J.A. and De La Cruz, J.P. Dietary virgin olive oil reduces oxidative stress and cellular damage in rat brain slices subjected to hypoxia–reoxygenation. *Lipids,* **42**, 921-929 (2007).

26. Hong, H., Kim, C. and Maeng, S. Effects of pumpkin seed oil and saw palmetto oil in Korean men with symptomatic benign prostatic hyperplasia. *Nutr. Res. Pract*., **3**, 323–327(2009).

27. Yoshioka, T., Kawada, K., Shimada, T. and Mori, M . Lipid peroxidation in maternal and cord blood and protective mechanisms against activated oxygen toxicity in blood. *Am. J. Obstet. Gynecol.,* **135**, 372-376(1979).

28. Koracevic, D. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol,*. **54**, 356-361 (2001).

29. Jewett, S.L. and Rocklin, A.M. Variation of one unit activity with oxidation rate of organic substrate in indirect superoxide dismutase assays. *Analyt Biochem.,* **212**, 555-559 (1993). doi:10.1006/abio.1993.1368.

30. Tietz NW. Fundamentals of clinical chemistry. W.B. Saunders, *Philadelphia* (1970).

31. Bancroft, J.D. and Gamble, M. Theory and Practice of Histological Techniques, 6th ed.Churchill Livingstone/Elsevier, *Philadelphia,* PA (2008).

32. Ramos-Vara, J.A. Technical aspects of immunohistochemistry. *Veterinary Pathology*, **42**(4), 405-426 (2005).

33. Abd El-Rahman, H.S.M. The Effect of Olive Leaf Extract and α-Tocopherol on Nephroprotective Activity in Rats. *J. Nutr. Food Sci.,* **6**, 479 (2016). doi:10.4172/2155-9600.1000479.

34. Kang, H., Kim, S. and Lee, G. Urinary metabolites of dibutyl phthalate and benzophenone-3 are potential chemical risk factors of chronic kidney function markers among healthy women. *Environ Int.,* **124**, 354–360 (2019).

35. Agarwal, A., Saleh, R.A. and Bedaiwy, M.A. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil. Steril.,* **79** , 829-843 [2003).

36. Sawiress, F.A.R., Ziada, M.S., Bebawy, W.S.F. and Amer, H.A. Effect of ginseng extract supplementation on testicular functions in diabetic rats, .*Endocrine Regulations*, **45**, 139–148(2011). doi:10.4149/endo_2011_03_139.

37. Kumar, M., Sharma, M.K., Saxena, P.S. and Kumar, A. Radioprotective effect of Panax ginseng on the phosphatases and lipid peroxidation level in testis of Swiss Albino mice, .*Biol. Pharmaceut. Bull*, **26**, 308-312 (2003).

38. Leibovitch, I., Ben-Chaim, J., Ramon, J. and Goldwasser, B. Increased serum alkaline phosphatase activity: a possible indicator of renal damage .*J. Clin. Lab. Anal.,* **5**(6),406-419 (1991).

39. Suzuki, C.K., Kutejová, E. and Suda, K. Deregulated T Cell Activation and Autoimmunity in Mice Lacking Interleukin-2 Receptor Beta. *Science*, **268**, 1472-1476 (1995).

40. Stojiljkovic, N., Dragan, M., Slavimir, V., Milan, S. and Ivan, J. ,Glomerular basement membrane alterations induced by gentamicin administration in rats, *Experimental and Toxicologic Pathology*, **60**(1), 69-75 (2008). ISSN 0940-2993, https://doi.org/10.1016/j.etp.2008.02.007.

41. Kumar, V., Kotran, R. S. and Robbins, S. L. "Pathologic Basis of Disease," 6th ed., Chap. 1, ed by Kumar V., Cotran R. S., W.B. Saunders Company, Philadelphia, , pp. 15—18 (1999).

42. Cos¸kun, S., Özge, U., Nil Üstündag,˘ C¸, Omunog,˘ L.U., Sanem Gokc¸,E.N., Selma, B. and Müjgan, C. Gentamicin-Induced Nephrotoxicity in Rats Ameliorated and Healing Effects of Resveratrol. *Biol. Pharm. Bull.,* **30**(1) 79—83 (2007).

43. Irfan, M., Qadir, T .M. and Khalid P. Lone, Bushra Munir and Waqas, Sami. Gentamycin Induced Nephrotoxicity In Albino Mice. *Biomedica,* **26**,161-165 (2010).

44. Mohamadi, Y., Najafi, H. and Madani, S.H. Protective effect of crocin on gentamicin-induced nephrotoxicity in rats, .*Iran J Basic Med Sci.;* **19**, 337-343 (2016).

45. Boxin, O.U., H. Dejian, H., Maureen, A.F. Judith and Elizabeth, K.D. Analysis of antioxidant activities of common vegetables employing oxygen aadical. Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assays: A comparative study. *J. Agric. Food Chem*., **5**(31), 223-228 (2002).

46. Record, I.R., Dreosti, I.E., McInerney J.K. Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture, .*Br. J. Nutr.*, **85** , 459–464 (2001).

47. Xu, G.H. A Study of the Possible Antitumour Effect and Immunompetence of Pumpkin Polysaccharide. *J. Wuhan Prof. Med. Coll*., **28**, 1-4 (2000).

48. Ahmed, H.H., Abdel-Rahman, M., Ali, R.S. and Abdel Moniem, A.E. Protective effect of Ginkgo biloba extract and pumpkin seed oil against neurotoxicity of rotenone in adult male rats. *J. Appl. Sci. Res*., **5**, 622e35 (2009).

49. Bakeer, M.R., Saleh, S.Y., Gazia, N., Abdelrahman, H.A., Elolimy, M. and Abdelatty, A.M. Effect of dietary pumpkin (Cucurbita moschata) seed oil supplementation on reproductive performance and serum antioxidant capacity in male and nulliparous female V-Line rabbits. *Italian Journal of Animal Science*, **20**(1),419-425 (2021). https://doi.org/10.1080/1828051X.2021.1889406.

50. Kumar A, Roberts, D., Wood, K.E., Light, B., Parrillo, J.E., Sharma, S., Suppes, R., Feinstein, D., Zanotti, S., Taiberg, L., Gurka, D., Kumar, A. and Cheang, M. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human. *Septic Shoc,* **34**(6), 1589-1596 (2006).

51. Al-Azzawie, H.F. and Alhamdani M.-S.S. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Journal of Nutrition*, **134**, 785-791(2006).

52. Cerolini, S., Zaniboni, L., Maldjian, A. and Gliozzi, T. Effect of docosahexaenoic acid and α-tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation, *Theriogenology,* **66**, 877–886 (2006).

53. Zari, T.A. and Al-Attar, A.M. Therapeutic effects of olive leaves extract on rats treated with a sublethal concentration of carbendazim. *Eur. Rev. Med. Pharmacol. Sci.,* **15**, 413–426(2011).

54. Kandeil, M.A.M., Hassanin, K.M.A., Mohammed, E.T., Safwat, G.M. and Mohamed, D.S. pumpkin and Vitamin E as Potent Modulators of Apoptosis in Gentamicin-induced Rat Nephrotoxicity. *Asian Journal of Biochemistry,* **13**, 1 – 8 (2018).

اكتشاف تأثيرات مضادة الأكسدة لزيت بذور اليقطين وزيت الزيتون على السمية الكلوية الناجمة عن ثنائي بوتيل هيدروكسي بروبيل في ذكور الفئران البيضاء من خلال التحقيق في خصائصها الوقائية على التدابير الفسيولوجية والنسيجية.

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الملخص

قام البحث الحالي بتقييم الخصائص الوقائية لزيت اليقطين (Cucurbita pepo L) وزيت الزيتون (Olea europaea L) على تلف الكلى الناجم عن ثنائي-n-بوتيل فثالات (DBP) في الفئران. استخدمت هذه الدراسة 30 فأرًا ذكرًا مهقًا، ستة لكل مجموعة. المجموعة الأولى "مجموعة التحكم" (C)؛ المجموعة الثانية (DBP) عولجت بـ DBP (500 مجم / كجم / يوم)؛ المجموعة الثالثة (DP) عولجت بـ DBP ومكملات زيت اليقطين (288 مجم / كجم وزن الجسم / يوم)؛ المجموعة الرابعة (DO) عولجت بـ DBP ومكملات زيت الزيتون (1.5 مل / كجم وزن الجسم / يوم)؛ والمجموعة الخامسة (DPO) عولجت بـ DBP ومكملات زيت اليقطين وزيت الزيتون. استمرت التجربة لمدة شهرين. تم التحقيق في مستويات الكرياتينين واليوريا وحمض البوليك في المصل وعلامات الإجهاد التأكسدي والتغيرات النسيجية. انخفضت مستويات TAC وSOD وALP بشكل ملحوظ، ولكن مستويات MDA والكرياتينين واليوريا وحمض البوليك زادت. تم العثور على آفات نسيجية مرضية في أنسجة الكلى في المجموعة المعرضة لـ DBP. في الختام، أدى تناول مكملات اليقطين أو زيت الزيتون للفئران تحت الإجهاد التأكسدي إلى حالة أكسدة أفضل للكلى واختبارات وظائف الكلى ونسيج أنسجة الكلى.

الكلمات الدالة: سمية الكلى، زيت اليقطين، زيت الزيتون، ثنائي-n-بوتيل فثالات.