Arabica coffee and olive oils mitigate malathion-induced nephrotoxicity in rat: *In silico*, immunohistochemical and biochemical evaluation

Khalid M. Al-Asmari a,⁎, Hisham N. Altayb b, Atef M. Al-Attar a,c,⁎, Safa H. Qahl d, Saed A. Al-Thobaiti e, Isam M. Abu Zeid a,c

a Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia
b Department of Biochemistry, Faculty of Science, King Abdulaziz University, Building A 90, Jeddah 21589, Saudi Arabia
c Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz University, P.O. Box 80200, Jeddah 21589, Saudi Arabia
d Department of Biology, College of Sciences, University of Jeddah, Jeddah, Saudi Arabia
e Department of Biology, University College Turabah, Taif University, Saudi Arabia

⁎ Corresponding authors at: Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.

E-mail addresses: hemmahom@kau.edu.sa (H.N. Altayb), khalid19791984@gmail.com, aterf.e.2000@yahoo.com (A.M. Al-Attar), shqahal@uoj.edu.sa (S.H. Qahl), saed@tu.edu.sa (S.A. Al-Thobaiti), abuzeidmm@yahoo.com (I.M. Abu Zeid).

Abstract

Malathion (MAL) is an organophosphate insecticide that disrupts the body’s antioxidant system; it is one of the earliest organophosphate insecticides extensively used as dust, emulsion, and vapor control a wide variety of insect pests under different conditions. This experimentation aims to evaluate the influence of Arabica coffee oil and olive oil on MAL-induced nephrotoxicity in male rat. 6 sets bearing the same number of animals were applied to this experiment. Each set comprised 10 rats. The first set of rats was used as the control group; rats in the second set were exposed to MAL measured at 100 mg/kg body weight for 7 weeks. Animals in the third and fourth set were treated with 400 mg/kg body weight of Arabica coffee oil and olive oil, and 100 mg/kg body weight of MAL. The fifth, together with the sixth set, were fed with a similar proportion of Arabica coffee oil and olive oil as administered to the third set of rats. After the experimental duration, rats of group 2 showed severe biochemical alterations, including significant increases of creatinine, uric acids, and urea nitrogen (BUN), resulting in marked decreases in serum albumin values and total protein (TP). Severe histopathological and immunohistochemical modifications of kidney tissues were observed in exposed MAL-intoxicated rats. Administration of these oils reduced the detected biochemical, histopathological modifications caused by MAL intoxication. Two active ingredients in Arabica coffee oil (oleic acid) and olive oil (hydroxytyrosol) showed good cyclooxygenase-2 (COX 2) interaction. Moreover, oleic acid from coffee oil and olive oil exhibited impressive association with xanthine oxidase (XO). The current finding showed that coffee oil and olive oil could be appraised as possible and a likely deterrence component against nephrotoxicity brought about by MAL.

© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords:
Malathion
Arabica coffee oil
Olive oil
Nephrotoxicity
Immunohistochemistry

1. Introduction

The use of organophosphate pesticides (OP) remains largely common globally despite increasing stern control initiatives in cultivation, public gardens, or families, constituting an everyday minimal dose exposure (Cobilinuchi et al., 2020). The occurrence of a large proportion of necrotic cells in the blood of intoxicated individuals is due to organophosphate cytotoxicity (Gundogan et al., 2018). Concerning the OP toxicokinetic characteristics, the most perturbed body parts are the liver and the kidneys. The exact actions of OP-associated renal dysfunction are not completely documented, with the most affected procedure being harmful oxidative stress (Cakici and Akat, 2013). It has been largely credited that unrestricted subjection to OP has damaging renal tissue and, consequently, renal function. Despite the magnitude of this worldwide concern, little information exists on the impact of these chemicals on human well-being. Therefore, novel and wide research are necessary to widen our understanding and ascertain appropriate directives regarding the application of organophosphates...
and safeguarding against their negative repercussions (Georgiadis et al., 2018).

Malathion is an effective pesticide that works by inhibiting the enzyme acetylcholinesterase (AChE). The MAL leads to the formation of harmful reactive oxygen species (ROSs), which accumulate excessively to the level that cells cannot detoxify. This phenomenon is termed oxidative stress, which can alter the body and kidney weights and distort their biochemical markers. MAL brings about the exhaustion of the antioxidant enzymes system and also causes histopathological alterations (Selmi et al., 2018). Organophosphorus toxins attack a variety of organs, including the kidney (Mansour and Mossa, 2010; Sivapriya and Venkatraman, 2006).

Medicinal plants have had a major significance in the therapeutics of many ailments. Medicinal plants are ideal for human beings as they result in reduced after-effects than synthetic medications (Almalki et al., 2019). One of the tropical crops, Arabica coffee, is widely grown in developing nations. However, it has not been fully utilized much in food studies and its usage (Al-Asmari et al., 2020). Arabica coffee (Coffea arabica L.) contains several bioactive components. Recent studies published that coffee oil has several antioxidants that have awesome therapeutic effects on diabetes and cancer (Al-Megrin et al., 2020; Bulldak et al., 2018) and inflammation (Khattisinsin et al., 2019). Olive (Olea europaea L.) oil is also largely used in Mediterranean dishes, which aids in the deterrence of various ailments. The oil obtained from olive is laden with bulk antioxidative markers. XXII. The illustration of antigen in tissue sections by immunostaining was used to distinguish antigen epitopes (Cattoretti et al., 1992). The standard immunohistochemical methods were adopted (Wadhwa et al., 1999). Microwave treatment of tissue sections was used to distinguish antigen epitopes (Cattoretti et al., 1992). The standard immunohistochemical methods were adopted (Wadhwa et al., 1999). Microwave treatment of tissue sections was used to distinguish antigen epitopes (Cattoretti et al., 1992). The standard immunohistochemical methods were adopted (Wadhwa et al., 1999).

2.2. Animals model

Male rats weighing 110–144 g were used in this study. Rats were caged in sets of six polypropylene plastic cages and were kept on a 12 h blackness/day schedule (6:00 p.m. to 6:00 a.m.) at 20 ± 1 degrees Celsius. The rats had ad libitum gain to nutrition (ordinary chow pellets) together with water. All investigations were carried out in conformity to the stipulations published by the Animal Care and Use Committee (ACUC) of King Abdulaziz University concerning rights for animals. In addition, every investigative setting was carried out in accordance with the ARRIVE requirements and conformed to the European Union requirement 2010/63/EU concerned with beast experimentation.

2.3. Experimental design

The rats were allowed one week to aclimatize and separated spontaneously into six sets, each of ten rat. The investigations will last for seven weeks. Rats in Group I (Control) will not receive any treatment. Group II (MAL) rats acquired MAL (100 mg/kg body weight, daily) orally. Group III rats received MAL (identical dose as Group II) and Arabica coffee oil (400 mg/kg body weight daily) via the oral route. Group IV acquired MAL (same dose as Group II) + olive oil (400 mg/kg body weight, daily). Groups V and VI were supplemented with the same dose of Arabica coffee oil and olive oil given to group 3. As the investigation progressed, the rats’ weight changes were monitored and recorded. After seven weeks of investigation, the blood samples and specimens of kidneys were obtained.

2.4. Blood sampling and analysis

Blood was obtained from orbital venous plexus, centrifuged at 2500 rpm for 15 min. Blood serum was preserved at - 80 degrees Celsius. Serum creatinine (Larsen, 1972), BUN (Patton and Crouch, 1977), uric acid (Young et al., 1975), albumin (Doumas et al., 1973) and TP (Peters, 1968) were evaluated.

2.5. Histopathological examination

The procedure of Al-Asmari et al. (2020) was used for the preparation, preservation, staining, and evaluation of kidney tissues. Processing and photomicrographing of histological sections were applied at Alborg Laboratory, Jeddah, KSA.

2.6. Immunohistochemistry examination

The standard immunohistochemical methods were adopted (Wadhwa et al., 1999). Microwave treatment of tissue sections was used to distinguish antigen epitopes (Cattoretti et al., 1992). The illustration of antigen in tissue sections by immunostaining involves two stages. The initial stage involves the association of the primary antibody to the target antigen. The reaction is then seen using a secondary or link antibody to which different enzyme systems are connected, collectively known as the universal. The primary antibody controls the reaction’s specificity, whilst the secondary antibody, in conjunction with its associated enzyme, produces amplification of the response, increasing the test’s sensitivity. The markers were seen using the Biotin-Streptavidin (BSA) technique (Hsu et al., 1981). The chromogen was diaminobenzidine (DAB), which allows for a permanent preparation. Hematoxylin counterstain was done. Processing and photomicrographing of histological sections were applied at Alborg Laboratory, Jeddah, KSA.

2.7. Bioinformatics analysis

2.7.1. Selection of enzymes for docking study

Cyclooxygenase-2 (COX 2) was selected for docking analysis due to its huge significance in the etiology of nephrotoxicity. Xanthine oxidase (XO) speeds up the transformation of hypoxanthine to xanthine together with xanthine to uric acid, giving hydrogen peroxide and ROSs as by-products. This phenomenon is largely sig-
significant in the pathophysiology of tissue deterioration (Bove et al., 2017; Osukoya et al., 2021).

2.7.2. Molecular docking study

The Protein Data Bank website was used to obtain the crystal structures of Cyclooxygenase-2: COX-2 (PDB ID: 6COX) and xanthine oxidase: XO (PDB ID: 3NVY). The main components of test oils were downloaded from the PubChem website as shown in (Table 1 and Table 2) (Barbaro et al., 2014; Calligaris et al., 2009; Covas et al., 2015; Deiana et al., 2018; Manna et al., 2002; Marcelino et al., 2019; Martin-Peláez et al., 2017; Martín et al., 2001; de Oliveira et al., 2014; Wagemaker et al., 2011). The Maestro program from Schrödinger Suite 2021-3 was used to investigate molecular docking. The Maestro program’s LigPrep, Receptor grid production, and SiteMap interfaces were also utilized to prepare ligands, proteins, and active site prediction (Schrödinger, LLC, New York, NY, 2021). Proteins remained stationary, whereas ligands were mobile. The ligands bound (Indomethacin and Allopurinol) to COX-2 and XO receptors were docked for a second time to validate the docking protocol.

2.8. Statistical assessment

Statistical assessment: IBM SPSS version 24 of the Statistical Package for Social Sciences was the computer application of choice for the analysis of statistical data. The outcomes indicated the averages ± standard error (SE). The assessment was mainly meant to differentiate the sets. A comparison could be between all the sets, all among sets 2, 3, and 4. The six sets of rats were compared using one-way ANOVA. The LSD (least significant difference) was then applied as a post-hock check after the considerable divergence among sets was calculated using ANOVA. P values of ≤ 0.05 were appraised to indicate statistically significant variability.

3. Results

3.1. Assessment of biochemical markers

When compared to the normal control group, the serum concentration of creatinine (mol/L) in rats exposed to MAL (G2) exhibited a significant (p< 0.001) increase. In comparison to the control group, MAL-poisoned rats treated with Arabica coffee oil (G3) and olive oil (G4) in doses of 400 mg/kg body weight and showed a significant (p<0.05) increase in serum creatinine levels. When compared to G2, there was no significant difference in serum creatinine levels in the MAL-poisoned rats fed Arabica coffee oil (G3) and olive oil (G4) (Fig. 1a).

The serum levels of BUN (mmol/L) in the rats poisoned with MAL (G2) was markedly up (p<0.001) when measured against the normal control G1 group. Likewise, while taking into comparison with the normal controlled G1 group of rats, the serum levels of BUN showed a marked increase (p<0.01 and p<0.05) in the G3 and G4 groups, i.e., the MAL-intoxicated rats fed daily with the oils of Arabica coffee oil (G3) and olive oil (G4) at a dosage of 400 mg/kg body weight. As compared to MAL-intoxicated G2 rats, the serum BUN levels markedly declined (p<0.05) in rats poisoned

| Table 1 |
| --- |
| XP GScore, glide energy and glide emodel of Arabica coffee oil and olive oil compounds on COX-2. |
| Name | PubChem CID | XP GScore | glide energy | glide emodel |
| COX-2 (6COX) | Arabica coffee oil compounds | | |
| Oleic acid | 445639 | -6.368 | -25.28 | -22.109 |
| Linoleic acid | 5280450 | -5.633 | -35.649 | -28.989 |
| Caffeine | 2519 | -4.883 | -31.735 | -38.815 |
| Palmitic acid | 985 | -4.754 | -25.366 | -19.891 |
| Chlorogenic acid | 1794427 | -2.666 | -31.964 | 4.471 |
| olive oil compounds | | | | |
| Hydroxytyrosol | 82755 | -7.331 | -25.243 | -29.836 |
| Oleic acid | 445639 | -6.189 | -23.278 | -23.151 |
| Tyrosol | 10393 | -5.951 | -26.732 | -30.549 |
| Linoleic acid | 5280450 | -5.633 | -35.649 | -28.989 |
| Palmitic acid | 985 | -4.825 | -25.369 | -22.676 |
| Serric acid | 5281 | -3.449 | -24.373 | -20.51 |
| Cont. | 3715 | -10.3 | -39.5 | -24.3 |

| Table 2 |
| --- |
| XP GScore, glide energy and glide emodel of Arabica coffee oil and olive oil compounds on XO |
| Name | PubChem CID | XP GScore | glide energy | glide emodel |
| XO (3NVY) | Arabica coffee oil compounds | | |
| Oleic acid | 445639 | -11.586 | -32.898 | -34.966 |
| Linoleic acid | 5280450 | -9.87 | -34.779 | -43.207 |
| Chlorogenic acid | 1794427 | -8.855 | -42.03 | -51.22 |
| Palmitic acid | 985 | -8.738 | -30.846 | -36.53 |
| Cafestol | 108052 | -5.084 | -18.697 | -9.45 |
| Kahweol | 114778 | -4.952 | -16.176 | -18.179 |
| Caffeine | 2519 | -4.696 | -37.165 | -41.347 |
| olive oil compounds | | | | |
| Oleic acid | 445639 | -11.586 | -32.898 | -34.966 |
| Linoleic acid | 5280450 | -9.87 | -34.779 | -43.207 |
| Oleuropein | 5281544 | -8.575 | -57.885 | -55.938 |
| Serric acid | 5281 | -9.435 | -34.314 | -35.086 |
| Palmitic acid | 985 | -7.388 | -31.405 | -34.531 |
| Tyrosol | 10393 | -6.052 | -24.908 | -33.671 |
| Hydroxytyrosol | 82755 | -5.999 | -32.518 | -43.508 |
| Squalene | 618072 | -2.303 | -39.342 | -51.289 |
| Allopurinol | 135401907 | -6.4 | -32.4 | -43.5 |

K.M. Al-Asmari, H.N. Altayb, A.M. Al-Attar et al. Saudi Journal of Biological Sciences 29 (2022) 103307
with MAL and treated with Arabica coffee oil (G3) and olive oil (G4) (Fig. 1b).

When the experiment concluded, the serum levels of uric acids (μmol/L) recorded in the rats intoxicated with MAL (G2) was significantly high in comparison with the normal control group of rats (G1). The daily administration of Arabica coffee oil (G3) and olive oil (G4) in rats poisoned with MAL at a dosage of 400 mg/kg body weight demonstrated non-significant variation in the serum levels of uric acid when measured after 49 days (7 weeks) of feeding as compared to G2 group (MAL-intoxicated). Likewise, administration of Arabica coffee oil (G3) and olive oil (G4) to the MAL-poisoned rats resulted in no significant in the serum levels of uric acid, measured against the normal control G1 rats (Fig. 1c).

In comparison with G1 group of rats, the serum levels of albumin (g/L) considerably declined in the G2 (poisoned with MAL), G3 (MAL-intoxicated rats fed with Arabica coffee oil), and G4 (MAL-intoxicated rats fed with olive oil) rats (p < 0.001; p < 0.05, respectively) when treated with a dosage of 400 mg/kg body weight of oil. However, measured against the MAL-intoxicated G2 group of rats, the serum levels of albumin significantly increased (p < 0.001) in the G4 group (MAL-poisoned rats treated with olive oil) in the measures of 400 mg/kg body weight (Fig. 1d).

The serum levels of total protein (g/L) significantly (p < 0.01) decreased in animals poisoned with MAL (G2 group), as measured against the G1 group. Likewise, the daily ingestion of Arabica coffee oil (G3) and olive oil (G4) in rats poisoned with MAL at a dosage of 400 mg/kg body weight demonstrated a considerable (p < 0.05) decline in the serum levels of total protein when correlated with the G1 group of rats (Fig. 1e).
Fig. 2. Photomicrograph of G1 rat’s kidney (normal control rats): Photo 1 showed bowman’s capsule (BC), capillaries (C) and podocytes (PC) the cells that responsible for glomerular filtration as indicated by curved green arrow with well-preserved tubules i.e., single tubule (T). Photo 2 represents normal kidney with normal and intact Bowman’s capsule (BC) (blue arrows), podocytes (PC), and tubules (T). Photo 3 showed normal well preserved Bowman capsule (BC) as indicated by blue arrows, tubules (T) and healthy capillaries (C). Photo1, 2, 3 (H&E x400).

Fig. 3. Photomicrograph of G2 rat’s kidney (MAL-intoxicated rats): Photo 1 Bowman’s capsule (BC) MAL caused 3-dimension aggregation and overlap of podocytes (PC) with dark chromatin (pyknosis and karyorrhexis), wrinkled and disorganized tubules (T). Photo 2 showed podocytes (PC) with extreme effect of MAL, the cells were wrinkled and as a result of water influx effect. Photo 3 of Bowman’s capsule (BC) indicated by (thick blue arrow), showed clear effect on podocytes (PC) and distorted cells that indicate cell death as shown by the presence of karyolysis and debris. Photo1,2 (H&E x400), Photo3 (H&E x1000).
Fig. 4. Photomicrograph of G2 rat's kidney (MAL-intoxicated rats): Photo 4 showed Bowman's capsule (BC) (blue arrows) including podocytes (PC) devoid of nuclei or complete karyolysis that indicate necrosis which present also in tubular cells (white arrow head). Photo 5 showed Bowman's capsule (BC) (blue arrows) with detachment of podocytes (PC) leaving a clear space. The nuclei of these distorted podocytes showed pyknosis and karyorhexis. Photo 6 showed a complete cytolysis that characterized by a complete disappearance of podocytes leaving empty Bowman's capsule with ruminant podocyte at the edge (blue arrow head). Photo 4, 5, 6 (H&E x400).

Fig. 5. Photomicrograph of G3 rat's kidney (MAL-intoxicated rats + Arabica coffee oil): Photo 1 showed Bowman's capsule (BC) indicated by (green arrows) and podocytes (PC) with normal restored cells, architecture and vascular supply (red blood cells indicated by blue thick arrows) with intact tubules (T). Photo 2 showed Bowman's capsule (BC) indicated by blue arrows with well-organized podocytes and good blood supply (red blood cells indicated by blue thick arrows) with blood vessels labelled (V) as well. Photo 3 showed Bowman's capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells and architecture with intact tubules (T). Photo 1, 2, 3 (H&E x400).
Fig. 6. Photomicrograph of G4 rat's kidney (MAL-intoxicated rats + olive oil): Photo 1 showed Bowman’s capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells, architecture and intact tubules (T). Photo 2 also showed Bowman’s capsule (BC) indicated by blue arrows with well-organized podocytes and good blood supply (red blood cells indicated by blue thick arrows) and tubules (T). Photo 3 showed Bowman’s capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells, architecture and intact tubules (T). Photo 1,2,3 (H&E x400).

Fig. 7. Photomicrograph of G5 rat’s kidney (Rats treated with Arabica coffee oil): Photo 1 showed Bowman’s capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells, and intact tubules (T). Photo 2 showed Bowman’s capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells, architecture and intact tubules (T). Photo 3 high power showed Bowman’s capsule (BC) indicated by blue arrows with well-organized podocytes and good blood supply (red blood cells indicated by blue thick arrows) with blood vessels filled with blood as indication of excellent and enhanced blood supply, labelled (BV). Photo 1,2,3 (H&E x400).
Fig. 8. Photomicrograph of G6 rat’s kidney (Rats treated with olive oil): Photo 1 showed 2 Bowman’s capsules (BC) indicated by (blue arrows) and podocytes (PC) with normal cells, architecture and intact tubules (T). Photo 2 also showed 2 Bowman’s capsule (BC) indicated by blue arrows with well-organized podocytes and good blood supply (red blood cells indicated by blue thick arrows) and tubules (T). Photo 3 showed Bowman’s capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal cells, architecture and intact tubules (T). Photo 1,2,3 (H&E x400).

Fig. 9. Photomicrographs of G1 rat’s kidney (normal rats) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed moderate (+++) positive reaction (yellow arrows) on the tubules (T); faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman’s capsule (yellow cross) which was expressed as (+). Photo (3) showed a faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman’s capsule (yellow cross) which was expressed as (+) with moderate reaction on tubules (T). Photo (1,2) x400, Photo (3) x200.
Fig. 10. Photomicrographs of G2 rat's kidney (MAL-intoxicated rats) immune-stained with the apoptotic marker ki67: Photo (1, 2) both showed strong (+++) positive reaction (yellow arrows) on the tubules (T) and Bowman's capsule cells (podocytes) (yellow arrow head). Photo (3) showed strong (+++) positive reaction (yellow arrows) on the tubules (T) and Bowman's capsule cells (podocytes) (yellow arrow head). Photo (1,2) x200, Photo (3) x400.

Fig. 11. Photomicrographs of G3 rat's kidney (MAL-intoxicated rats + Arabica coffee oil) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed strong to moderate (+++/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman's capsule (yellow cross) which was expressed as (+). Photo (3) showed a faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman's capsule (yellow cross) which was expressed as (+) with strong to moderate (+++/++) reaction on tubules (T) (yellow arrows). Photo (1,2) x200, Photo (3) x400.
Fig. 12. Photomicrographs of G4 rat’s kidney (MAL-intoxicated rats + olive oil) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed strong to moderate (+++/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman’s capsule (yellow cross) which was expressed as (+). Photo (3) showed a faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman’s capsule (yellow cross) which was expressed as (+) with strong to moderate (+++/++) reaction on tubules (T) (yellow arrows). Photo (1,2) x200, Photo (3) x400

Fig. 13. Photomicrographs of G5 rat’s kidney (Rats treated with Arabica coffee oil) immune-stained with the apoptotic ki67: Photo (1,2) both showed strong to moderate (+++/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman’s capsule (yellow cross) which was expressed as (+). Photo 3 showed a faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman’s capsule (yellow cross) which was expressed as (+) with strong to moderate (+++/++) reaction on tubules (T) (yellow arrows). Photo (1,2) x200, Photo (3) x400
3.2. Histopathological studies

The kidney’s sections of normal control rats (Group 1) showed maintained architecture, normal cellular components in epithelial lining or tubular epithelial cells, with healthy glomeruli, Bowman’s capsules and podocytes, with healthy capillaries, and blood vessels. The cells showed normal nucleus and nuclear activities manifested by presence of nuclei. The cytoplasm exhibited normal granules and activity at all microscopic magnifications (Fig. 2).

In rats orally administrated with MAL (Group 2), the histological sections showed extreme and frank architectural distortions as wrinkling of Bowman’s capsule basement membrane structure. The Bowman capsule showed membrane syncytial or merging of cells closely to dissolvability and disappearance. Also, many cells
were fragmented with evidence of dystrophic calcifications. The detached cells were aggregated forming degenerative capsule in 3-dimension arrangement which was characterized by cell overlapping over each other. Other changes as shrinkage due to digestion were observed that attributed to osmotic imbalance. Cellular wrinkling and disorganization forming wavy pattern were also as observed. Another change includes capsule podocyte vacuolization. Nuclear loss or karyolysis with debris was also evident. Podocyte digestion, capsule wrinkling and blood vessel distortion were also noticed. There was also extreme cytoplasmic clearing seen with cells disorganization. Also, a complete capsule loss with blood clot and aggregations leading to vascular occlusion forming clot like were seen with evidence of tubular damage (Fig. 3 and Fig. 4).

After MAL administration, rats given Arabica coffee oil (Group 3) orally demonstrated a powerful protection against MAL toxicity, resulting in a complete restoration, which was characterized by intact tubules and cells with well-preserved basement membrane. The sections also showed that Bowman capsule was completely protected as indicated by appearance of fresh blood reflecting vascular maintenance with healthy cells bearing well preserved nucleoli. The capillaries were also excellently preserved compared to group 2, where capillary coagulative changes were observed (Fig. 5).

There is a complete restoration of histological structures in MAL-intoxicated rats orally treated with olive oil (Group 4) as evidenced by intact tubules and cells as well as a well-preserved basement membrane of Bowman’s capsule. Moreover, The Bowman capsule was completely protected, fresh blood was observed indicating vascular maintenance with healthy cells bearing well preserved nucleoli, and the capillaries were preserved as compared as compared to group 2 (Fig. 6).

In rats orally supplemented with Arabica coffee oil (Group 5), the histological sections showed normal intact tubules and cells with well-preserved basement membrane. The Bowman capsule was completely maintained with fresh blood demonstrating vascular maintenance with healthy cells bearing, well preserved nucleoli, indicating excellent vasculature and blood supply, identical to the normal control group (Fig. 7). Similar to control group, the histological sections of rats orally supplemented with olive oil (Group 6) showed intact tubules and cells with well-preserved basement membrane. The Bowman capsule was also well maintained (Fig. 8).

3.3. Immunohistochemical studies

The randomly selected histological sections of Group 1, revealed mild immunoreactivity reactions to Ki67 in the cytoplasm of podocytes cells in Bowman’s capsule (C) as well as kidney tubules, which was expressed as only one cross (+) (Fig. 9).

The kidney tubules and Bowman’s capsule of the experimental animals exposed to MAL (Group 2) showed the strongest immunohistochemical reaction in the cytoplasm of podocytes cells in Bowman’s capsule (C), which was expressed as three crosses (+++) that corresponding strong positivity with highest reactivity level. The sections showed cytoplasmic reaction in a wide area at low magnification which justified the strongest reactivity as compared to normal control group (Group 1) especially the reactions of Bowman capsule (Fig. 10).

The histological sections obtained from rats that exposed to pesticide and supplemented with Arabica coffee (Group 3) exhibited immunoreactivity that observed in Bowman’s capsule as weak reactivity in podocytes, which was expressed as two crosses (++), while tubules exhibited strong reaction that expressed as three crosses (+++); indicating high expression of Ki67. In contrast to group 2, which exhibited a strong reaction in both tubules and Bowman capsules, this group showed a moderate reaction in Bowman capsules and a strong reaction in tubules, showing that this oil has a profound effect on capsules. The tubular reaction was remained significant in tubules as compared to (G1), but the Bowman’s capsular reaction was mild (Fig. 11).

The animals in group 4 were orally given olive oil following MAL administration showed weak reactivity in Bowman’s capsule podocytes (+) but moderate reactivity in tubules (++). Olive oil had a noticeable potent effect as compared to group 3 (Fig. 12).

The histological sections of Group 5, in which rats were given Arabica coffee oil orally, revealed weak reactivity in podocytes of Bowman’s capsule (+), but strong reactivity in tubules (+++). The result was similar to group 1, except tubular cells had higher Ki67 expression. This most trustworthy finding supported the powerful activity of Arabica coffee oil on Bowman’s capsule podocytes (Fig. 13). The histological sections of rats in group 6, which were given olive oil orally, revealed weak (+) reactivity in Bowman’s capsule podocytes and tubule cells. These alterations were similar to those seen in (G1) (Fig. 14).
3.4. Molecular docking study

The docking of Arabica coffee oil and olive oil compounds on COX-2 proteins showed good binding energy ranging from -7.331 to -2.666 kcal/mol. The COX-2 (6COX) protein showed the best interaction with oleic acid (-3.686 kcal/mol) and hydroxytyrosol (-7.331 kcal/mol) (Table 1). The oleic acid from Arabica coffee oil bind to the COX-2 protein by interacting with one hydrogen bond Hie 90 (Fig. 15 A and B). The hydroxytyrosol from olive oil showed good binding energy to the COX-2 protein by interacting with two hydrogen bonds Tyr 385, Ser 530 (Fig. 15 C and D). The redocking of the attached ligand (Indomethacin) showed a -10.3 kcal/mol docking score.

The oleic acid in Arabica coffee oil and olive oil demonstrated the best binding energy with XO protein with docking scores - 11.586 kcal/mol, respectively (Table 2). Two hydrogen bonds were formed with Arg 880 and Thr1010 (Fig. 16 A and B). The redocking of co-crystalized ligand (Allopurinol) of XO protein showed a -6.4 kcal/mol docking score.

4. Discussion

Malathion was once thought to be a genotoxic and carcinogenic pesticide (Deka and Mahanta, 2012). Findings have indicated oxidative stress to be a major consequence of pesticide usage. Oxidative stress is the main mode of destructiveness linked to human beings and animals and is a credible source of data in monitoring studies. Oxidative stress comes about when there is a disparity in the balanced levels of ROS and antioxidant defenses, causing modifications in the levels of several lipid peroxidation and antioxidant enzymes (Medithi et al., 2021).

Usage of herbal medicine has been on the rise due to ease of accessibility without prescription, reduced expenses, and minimal after effects (Grandjean and Landrigan, 2014). Coffee is a resourceful origin of antioxidants, including components obtained from hydroxycinnamic acid derivatives (caffeine, chlorogenic, coumaric, ferulic, and sinapic acids), flavonoids, and polyphenols (Manach et al., 2004). Olive oil is majorly incorporated in Mediterranean dishes. Olive oil seems to be an important nutritional material with several inclusions, including monounsaturated fatty acids (MUFA), which could carry nutritional advantages. Olive oil also contains phytochemicals such as phenolic compounds, which are beneficial for health (Al-Attar et al., 2018; Radd-Vagenas et al., 2017).

The kidney is one of the organs of interest in this experimentation (Al-Attar, 2010; Mansour and Mossa, 2010). The kidney function parameters showed an upward trajectory in rats exposed to MAL. Earlier findings suggest a considerable up regulation in blood creatinine, urea, and uric acid levels in animals treated with MAL and other pest control chemicals (Al-Attar, 2015; Baiomy et al., 2015; Kanbur et al., 2016; Kaya et al., 2018; Li et al., 2016; Muhammad et al., 2019). Failure of the kidney function reduces excretion of creatinine, constituting higher blood borne creatinine. Therefore, creatinine gauges give an estimation of the glomerular filtration capacity. Increased creatinine levels are directly linked to renal dysfunction (Lopez-Giacomani and Madero, 2015).

In this experiment, rats exposed to MAL also showed considerably lesser albumin gauges than the ordinary trial rats. Albumin, the most plentiful plasma protein in the blood, is manufactured in the liver. Several findings have suggested that organophosphates can hamper its generation (Abdel-Daim et al., 2020; Kalender et al., 2010; Ogutcu et al., 2008; Youssef et al., 2006). Albumin aids in diagnosing kidney and liver conditions. Reduced albumin levels in the blood point to a liver or kidney disorder. It is quite probable that organophosphates like MAL modify protein and free amino acid metabolism and their production in the hepatocytes (Abdel-Daim et al., 2020; Ncibi et al., 2008; Park et al., 2017). Low serum albumin is connected to mortality in patients with chronic kidney disease (CKD) in part because of its relationship to systemic inflammation (Alves et al., 2018).

The current findings showed that exposure to MAL caused a reduction of serum TP. This result conforms to previous studies, which showed that MAL and similar chemicals induced serious disturbance of TP (Abdel-Daim et al., 2020; Khalifa and Alkalaf, 2020). The decline of serum TP indicates the presence of paraproteins or decreased antibody production. Moreover, this change of serum TP level could be caused by modification in the intracellular protein manufacture mechanism and the level of oxidative enzymes in liver (Kalender et al., 2010). The authors agreed that the consequent hepatic damage could explain the notable reduction in serum levels of TP in MAL-inoculated mice. An alternative description of the latter outcome is the capacity of MAL to induce pancreatic inflammation or block the exocrine role of the pancreas, yielding in defective breakdown and assimilation of proteins and lipids consumed in the diet from the gut (Abdel-Daim et al., 2020; Firdikli et al., 2018).

The outcomes of the current analysis showed that Arabica coffee oil and olive oil administered orally halted the extensive alterations in the studied parameters within normal ranges in MAL-intoxicated male rats. The present results demonstrated the efficacy of consumption of Arabica coffee oil on improving creatinine, BUN, uric acid, albumin, and TP levels. Coffee oil contains highly effective antioxidants (Brezoiva et al., 2008; Calligaris et al., 2009). Antioxidants in coffee could elicit protective effects in clear cell renal cell carcinoma and renal control function (Huang et al., 2014).

Olive oil substitution ameliorated all constituents of interest, i.e. (creatinine, BUN, uric acid, and albumin). The kidney histology ascertained the biochemical characteristics and the advantages of olive oil. Olive oil confers advantages against kidney damage by clearing reactive species and by its powerful anti-oxidizing capacity when added to the diet. Earlier findings indicated convergent outcomes in experimental animals subjected to acrylamide (Ghorbel et al., 2017). This influence may be due to the anti-oxidative polyphenols, oleic acid, and MUFA, in olive oil (Covas et al., 2015). The findings of this research suggest that olive oil could protect the kidney from destruction through oxidation by scavenging free radicals as well as deterring their creation. Earlier findings suggest that olive oil promotes the resistance to lipid and protein oxidation, as well as the antioxidant defense system due to its significant composition of components laden with phenols (JL, 1997; Ghorbel et al., 2015a; 2015b). Furthermore, olive oil increases serum albumin amounts (Al-Seeni et al., 2016; Ghorbel et al., 2015a). These results conform to the findings of the experiment at hand.

The current investigation on the kidney sections of MAL-induced rats showed dystrophic calciifications of Bowman’s capsule, cell detachment, 3D aggregation and overlap of podocytes, which were characterized by cell overlapping. Other changes reported in this study include pathological hydropic swelling with water influx with evidence of calcification, shrinkage in tissues, and digestion of podocytes. Cellular wrinkling and disorganization with nuclear loss (karyolysis) were also observed. Some glomeruli showed wrinkled capsule with distorted blood vessels; while others showed complete loss of capsule with blood clots and aggregation. This was confirmed by elevation of kidney function parameters. Previous research found that experimental rats exposed to MAL and other pesticides developed a kidney histological alteration (Castro et al., 2014; Kata, 2020; Mamun et al., 2015; Selimi et al., 2018; Zidan, 2015).

This study showed that rats orally supplemented with Arabica coffee oil and olive oil after MAL administration, showed a protec-
tive effect which characterized by intact tubules with well-preserved basement membrane. The sections also showed that Bowman capsule is completely protected as indicated by appearance of fresh blood reflecting vascular maintenance with healthy cells bearing and well preserved nuclei. The capillaries were also excellently preserved compared to MAL-intoxicated rats. Several experimental studies investigated the effects of Arabica coffee and olive oils on histopathological and oxidative alterations caused by chemical toxicants, indicating that they may play a therapeutic role in free radical mediated antioxidant defense mechanisms against the toxicity of these chemicals (Amamou et al., 2015; Mohammed et al., 2014; Necib et al., 2014; Saber et al., 2015; Wen et al., 2004).

The probable mechanism of Arabica coffee oil and olive oils as deterrence components could be caused by their outcomes against oxidative compounds responsible for impeding MAL induction into a form that can react. Ingesting antioxidant foods could stop or slow down the oxidation of susceptible cellular substrates so prevent stress associated with oxidation (Calligaris et al., 2009; Covas et al., 2015; Rice-Evans et al., 1996).

The monoclonal antibody Ki67 is consider as the most widely used immunohistochemical cell-cycle markers (Burger et al., 1986; Gerdès et al., 1983; Shibata and Burger, 1987). In the present study, the tubules and capsule of rats exposed to MAL showed strongest immunohistochemical reaction in the cytoplasm of podocytes cells in Bowman’s capsule with highest level of reactivity. The sections showed cytoplasmic reaction in a wide area at low magnification which justified the strongest reactivity as compared to normal control group. These results are conformity with previous study by Baiomy et al. (2015) who reported the effects of MAL-induced changes in rat kidney, additionally numerous ki67 positive cells in the collecting tubules were observed. This confirms and extends the previous observations of Celik et al. (2015) and Rahimi Anbarkeh et al. (2020).

The current findings showed that treating rats with Arabica coffee oil and olive oil reduced the histopathological changes caused by MAL poisoning. In the histological sections obtained from rats in group 3 which were orally supplemented with Arabica coffee oil and exposed to MAL; the immunoreactivity of Bowman’s capsule showed weak reactivity in podocytes, while tubules exhibited strong reaction; indicating high expression of Ki67 in the tubules. Moreover, in comparison to group 2, rats of group 3 showed moderate reaction in Bowman capsule and strong reaction in tubules indicating the potent effect of this oil. However, group 4 rats, which orally treated with olive oil after MAL administration showed weak reactivity in podocytes of the Bowman’s capsule while tubules displayed moderate reaction, the effect of olive oil was noticeable and having a potent effect. These results agreed with the previous research works (Domitrovic´ et al., 2014; KHALATBARY, 2013; Mokhtari et al., 2020; QU et al., 2020). Green coffee and olive oil were reported to have a potent polyphenolic antioxidant and anti-carcinogenic effects as they reduce the levels of apoptosis related proteins, including Ki67, caspase-3 and Bax (Abdelaal et al., 2019; Khalatbary, 2013).

The in silico studies illustrated the presence of two components from Arabica coffee oil (oleic acid) and olive oil (hydroxytyrosol); these compounds exhibited good interaction with the COX-2 enzyme that is significant in the pathophysiology of nephrotoxicity. Attaining notable down regulation in the expression of COX-2 enzyme at the time of oxidative stress could be a vital technique in controlling nephron destruction (Morsy et al., 2013; Osukoya et al., 2021). This experimentation also illustrates the derrentence capacity of bioactive compounds originating from Arabica coffee oil and olive oil (oleic acid) against the XO enzyme-linked to uric acid synthesis (Osukoya et al., 2021). Xanthine oxidase is responsible for speeding up the transformation of hypoxanthine to xanthine as well as xanthine to uric acid. The byproducts of this process are hydrogen peroxide and reactive oxygen species, which have been purported to be an important factor in the etiology of tissue destruction (Bove et al., 2017). The oleic acid had a better docking score than known XO protein inhibitor (Allopurinol), while hydroxytyrosol had a redocking score similar to the known anti-inflammatory and nephrotoxic medication (Indomethacin). Other studies support the findings of the present research, which showed the antiinflammatory and nephrotoxic effect of oleic acid and hydroxytyrosol (Chashmi et al., 2017; Foscolou et al., 2018; Ghorbel et al., 2017; Sales-Campos et al., 2013; Tejada et al., 2017).

5. Conclusions

This study indicated that MAL dosing causes apoptosis, inflammation, oxidative stress, and nephrotoxicity in rats. The test oils improved renal function markers, kidney tissue damage, and reduced inflammation and apoptosis, in MAL-induced rats. However, more research is needed to better understand the exact mechanisms of the beneficial role of these oils against MAL-induced kidney injury. Moreover, computational analysis shows cessation of COX-2 enzyme by Arabica coffee oil (oleic acid) and olive oil (hydroxytyrosol). Additionally, computational analysis shows cessation of XO enzyme by Arabica coffee oil and olive oil (oleic acid). More in vivo investigations are needed to validate our findings, followed by safety and short and long-term trials in individuals with kidney disorders.

Funding: This research received no external funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdelaal, S., E. Mousa, H., Ahmed, S., 2019. Effect of Silymarin versus Silymarin and green coffee extract on Thioacetamide induced liver injury in adult male albino rats (histological and Immunohistochemical study). Egypt. J. Histol. 42 (1), 133–146.

Abdel-Daim, M.M., Aboushouk, A.L., Bungalu, S.G., Bin-Jumah, M., El-kott, A.F., Shati, A.A., Aleya, L., Alkahtani, S., 2020. Protective effects of thyroquinone and diallyl sulphide against malathion-induced toxicity in rats. Environ. Sci. Pollut. Res. 27 (10), 10228–10235.

Al-Asmari, K.M., Zeid, I.M.A., Al-Attar, A.M., 2020a. Medicinal Properties of Arabica coffee (Coffee arabica) Oil: An Overview. Adv. Life Sci. 8, 20–29.

Al-Asmari, K.M., Zeid, I.A., Al Thobaiti, S., 2020b. Sciatic nerve crush in rats and its impact on soleus muscle. World Appl. Sci. J. 38, 195–203.

Al-Attar, A.M., 2010. Physiological and histopathological investigations on the effects of α-lipoic acid in rats exposed to malathion. J. Biomed. Biotechnol. 2010, 1–5.

Al-Attar, A.M., 2015. Effect of grapeseed oil on diazinon-induced physiological and histopathological alterations in rats. Saudi J. Biol. Sci. 22 (3), 284–292.

Al-Attar, A.M., Elnagar, M.H.R., Almaliki, E.A., 2018. Physiological study on the influence of some plant oils in rats exposed to a sublethal concentration of diazinon. Saudi J. Biol. Sci. 25 (4), 786–796.

Almaliki, D.A., Alghamdi, S.A., Al-Attar, A.M., 2019. Comparative study on the influence of some medicinal plants on diabetes induced by streptozotocin in male rats. Biomed. Res. Int. 2019, 1–11.

Al-Megrin, W.A., El-Khadragy, M.F., Hussein, M.H., Mahgoub, S., Abdel-Mohsen, D.M., Taha, H., Bakkar, A.A., Abdel Moneim, A.E., Amin, H.K., 2020. Green Coffee arabica extract ameliorates testicular injury in high-fat diet/streptozotocin-induced diabetes in rats. J. Diabetes Res. 2020, 1–13.

Al-Senisi, M.N., El Rabey, A.A., Zannami, M.A., Alnafaseeh, A.M., 2016. The hepatoprotective activity of olive oil and Nigella sativa oil against CCl 4 induced hepatoxicity in male rats. BMC Complement. Altern. Med. 16 (1), 1–14.

Alves, F.C., Sun, J., Qureshi, A.R., Dai, L., Su, L., Suadela, S., Barany, P., Heimbürg, O., Lindholm, B., Stenvinkel, P., Shearer, G., 2018. The higher mortality associated with low serum albumin is dependent on systemic inflammation in end-stage kidney disease. PLoS One 13 (1), e0190410.
Ghorbel, I., Elwej, A., Fendri, N., Mnif, H., Jamoussi, K., Boudawara, T., 2018. Olive oil consumption and human health. Food Funct. 9 (8), 4253–4262.

Kallistratos, V., 2002. Protective effect of the phenolic fraction from virgin olive oils subjected to refining process. Nutr. Hosp. 12, 309-311.

Kim, Y., Oh, K.M., Kim, J., Lee, S., 2017. Protective effect of extra virgin olive oil on the hepatotoxicity induced by co-exposure of adult rats to acrylamide and aluminum. Food Funct. 6 (4), 1126–1135.

Khorbel, I., Khemakhem, M., Boudawara, O., Marrechelli, R., Jamoussi, K., Ben Amar, A., Boudawara, T., Zeghal, N., Grati Ramoun, N., 2013b. Effects of dietary extra virgin olive oil and its fractions on antioxidant status and DNA damage in the heart of rats co-exposed to aluminum and acrylamide. Food Funct. 6 (9), 3098–3108.

Grandjean, P., Landrigan, P.J., 2014. Neurobehavioral effects of developmental toxicity. Lancet Neurol. 13 (3), 330–338.

Gundogan, K., Donnev-Altuntas, H., Hamurcuz, A., Akbudak, I.H., Sungur, M., Bitgen, M., 2016. Protective effect of extra virgin olive oil on myocardial damage caused by organophosphorus intoxication in rats. Pestic. Biochem. Physiol. 126, 11–17.

Hohmann, C.D., Kessler, C., Fleischer, G., Beck, H., Zou, J., Kohn, K., 2015. Effects of high phenolic olive oil on cardiovascular risk factors: A systematic review and meta-analysis. Phytomedicine 22 (6), 631–640.

Hsu, S.M., Raine, L., Fanger, H.A., 1981. Comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am. J. Clin. Pathol. 75, 734–738.

Huang, T.-B., Guo, Z.-F., Zhang, X.-L., Zhang, X.-P., Liu, H., Geng, J., Yao, X.-D., Zheng, J.-H., 2014. Coffee consumption and urolologic cancer risk: A meta-analysis of cohort studies. Int. J. Urol. Nephrol. 46 (8), 1481–1493.

Ji, M.R., 1997. Variations in fatty acids, tocopherol and other quality parameters of virgin olive oil subjected to refined process. Nutr. Res. 19, 309-311.

Kaledinov, F., Uzun, F.G., Duncanson, A., 2015. Protective effect of extra virgin olive oil subjected to refining process in rats: the effects of vitamins C and E and Food Chem. Toxicol. 48 (2), 633–638.

Kanbur, M., Silig, Y., Eraslan, C., Kavarac, M., Soyar Sarac, Z., Sahin, S., 2016. The role of trimethylsilyl amine, amides and combinations of trimethylsilyl in rats. Environ. Sci. Pollut. Res. 23 (6), 5212–5224.

Kata, F., 2020. Short-time effects of malashi protein on functional and histological changes of liver and kidney in female mice. PLoS Biol. 23 (9), e3001212.

Kaya, Y., Bas, O., Hanci, H., Cankaya, S., Yalanci, I., Odaci, E., Avni Uydu, H., Aslan, A., 2018. Acute renal involvement in organophosphate poisoning: histological and immunohistochemical investigations. Res. Fetal (41), 410–415.

Khalatbar, A.R., 2013. Olive oil phenols and neuroprotection. Nutr. Neurosci. 16 (6), 327–330.

Khalifa, F.K., Alkalhalal, M.I., 2020. Effects of black seed and thyme leaves dietary supplements on the antioxidant activity in experimental rat model. J. Neurol. Sci. 423 (1), 109494.

Kilinc, T., Tunc, R.C., Cobelinski, C.O., Neagu, T.P., Becheanu, G., Sinescu, R.-D., Becheanu, V., 2014. Protective effects of ginger and zinc chloride mixture on the liver and kidney alterations induced by co-exposure of adult rats to acrylamide and aluminum. Food Funct. 6 (9), 3098–3108.

Koehnlein, M., Fiedler, S., Wiesel, F., Hoffmeister, G., 2013. Acute renal involvement in organophosphate poisoning: histological and immunohistochemical investigations. Res. Fetal (41), 410–415.

Kohler, J., Schwab, U., Lemke, H., Stein, H., 1983. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int. J. Cancer. 31 (1), 13–20.

Khan, M.A., Van der Kwast, T.H., Sudaryo, M.K., Mathewa, R.B., van den Heuvel, M.V., D'Aray, R., 2004. Evaluation of the human ovarian DNA damage, cytoplastosis, oxidative DNA damage and their relationship with endocrine hormones in patients with acute organophosphate poisoning. Mutat. Res. Toxicol. Environ. Mutagen. 825, 1–7.

Khalatbary, A.R., 2013. Olive oil phenols and neuroprotection. Nutr. Neurosci. 16 (6), 327–330.

Koegel, R., Töpel, G., Arciello, M., Tarocco, M., Galli, A., Balansa, C., 2014. Effects of the olive-derived polyphenol deurosin on human health. Int. Mol. Sci. 15, 18085–18024.

Koerle, I., Khemakhem, M., Boudawara, O., Marrechelli, R., Jamoussi, K., Ben Amar, A., Boudawara, T., Zeghal, N., Grati Ramoun, N., 2013b. Effects of dietary extra virgin olive oil and its fractions on antioxidant status and DNA damage in the heart of rats co-exposed to aluminum and acrylamide. Food Funct. 6 (9), 3098–3108.

Koegel, R., Töpel, G., Arciello, M., Tarocco, M., Galli, A., Balansa, C., 2014. Effects of the olive-derived polyphenol deurosin on human health. Int. Mol. Sci. 15, 18085–18024.

Koerle, I., Khemakhem, M., Boudawara, O., Marrechelli, R., Jamoussi, K., Ben Amar, A., Boudawara, T., Zeghal, N., Grati Ramoun, N., 2013b. Effects of dietary extra virgin olive oil and its fractions on antioxidant status and DNA damage in the heart of rats co-exposed to aluminum and acrylamide. Food Funct. 6 (9), 3098–3108.

Koegel, R., Töpel, G., Arciello, M., Tarocco, M., Galli, A., Balansa, C., 2014. Effects of the olive-derived polyphenol deurosin on human health. Int. Mol. Sci. 15, 18085–18024.

Koerle, I., Khemakhem, M., Boudawara, O., Marrechelli, R., Jamoussi, K., Ben Amar, A., Boudawara, T., Zeghal, N., Grati Ramoun, N., 2013b. Effects of dietary extra virgin olive oil and its fractions on antioxidant status and DNA damage in the heart of rats co-exposed to aluminum and acrylamide. Food Funct. 6 (9), 3098–3108.

Koegel, R., Töpel, G., Arciello, M., Tarocco, M., Galli, A., Balansa, C., 2014. Effects of the olive-derived polyphenol deurosin on human health. Int. Mol. Sci. 15, 18085–18024.

Koerle, I., Khemakhem, M., Boudawara, O., Marrechelli, R., Jamoussi, K., Ben Amar, A., Boudawara, T., Zeghal, N., Grati Ramoun, N., 2013b. Effects of dietary extra virgin olive oil and its fractions on antioxidant status and DNA damage in the heart of rats co-exposed to aluminum and acrylamide. Food Funct. 6 (9), 3098–3108.

Koegel, R., Töpel, G., Arciello, M., Tarocco, M., Galli, A., Balansa, C., 2014. Effects of the olive-derived polyphenol deurosin on human health. Int. Mol. Sci. 15, 18085–18024.
Morsy, M.A., Ibrahim, S.A., Amin, E.F., Kamel, M.Y., Rifai, R.A., Hassan, M.K., 2013. Curcumin ameliorates methotrexate-induced nephrotoxicity in rats. Adv. Pharmacol. Sci. 2013, 1–7.

Muhammad, Y., Nazia, E., Rehman, Q.U., Abid, A., Ali, K.W., Azizullah, K., 2019. Effect of organophosphate pesticide (chlorpyrifos, fipronil, and malathion) on certain organs of Rattus rattus. GSC biol. pharm. sci. 6, 11–15.

Nebi, S., Ben Othman, M., Akacha, A., Krifi, M.N., Zourgui, L., 2008. Opuntia ficus indica extract protects against chlorpyrifos-induced damage on mice liver. Food Chem. Toxicol. 46 (2), 797–802.

Necib, Y., Bahi, A., Zerizer, S., Abdennour, C., Boulakoud, M.A., 2014. Protective effect of virgin olive oil (Olea europeus L.) against oxidative damage induced by mercuric chloride in rat albinos Wistar. J. Stress Physiol. Biochem. 10, 45–58.

Ogutcu, A., Suludere, Z., Kalender, Y., 2008. Dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamins C and E. Environ. Toxicol. Pharmacol. 26 (3), 355–361.

Osukoya, O.A., Oyinloye, B.E., Ajiboye, B.O., Olokode, K.A., Adeola, H.A., 2021. Nephroprotective and anti-inflammatory potential of aqueous extract from persimma americana seeds against cadmium-induced nephrotoxicity in wistar rats. Biometals 34 (5), 1141–1153.

Park, J.I., Baek, H., Kim, B.R., Jung, H.H., Tayo, B.O., 2017. Comparison of urine dipstick and albumin: creatinine ratio for chronic kidney disease screening: A population-based study. PLoS ONE 12 (2), e0171106.

Patton, G., Crouch, S., 1977. Determination of urea (urease modified Berthelot reaction). Anal. Chem. 49, 464–469.

Peters Jr, T., 1968. Proposals for standardization of total protein assays. Clin. Chem. 14, 1147–1159.

Qu, S., Dai, C., Hao, Z., Wang, H., Wang, J., Zhao, H., 2020. Chlorogenic acid prevents vancomycin-induced nephrotoxicity without compromising vancomycin antibacterial properties. Phytother. Res. 34 (12), 3189–3199.

Radd-Vagenas, S., Kouris-Blazos, A., Singh, M.F., Flood, V.M., 2017. Evolution of Mediterranean diets and cuisine: concepts and definitions. Asia. Pac. J. Clin. Nutr. 26, 749–763.

Rahimi Anbarkeh, F., Jalali, M., Nikravesh, M.R., Soukhtanloo, M., 2018. Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice. Toxicol. Rep. 5, 189–195.

Shen, J., Wilmut, K.A., Ghasezmazdeh, N., Molloy, D.L., Burkmann, G., Mekonnen, G., Gongora, M.C., Quyumi, A.A., Sperling, L.S., 2015. Mediterranean dietary patterns and cardiovascular health. Annu. Rev. Nutr. 35 (1), 425–449.

Shibata, T., Burger, P.C., 1987. The use of the monoclonal antibody Ki-67 in determination of the growth fraction in pediatric brain tumors. Childs Nerv. Syst. 3 (6), 364–367.

Sivapirya, V., Jayanthisakthisekaran, Venkatraman, S., 2006. Effects of dimethoate (O, O-dimethyl S-methyl carbamoyl methyl phosphorodithioate) and ethanol in antioxidant status of liver and kidney of experimental mice. Pestic. Biochem. Physiol. 85 (2), 115–121.

Sun, X., Kaufman, P.D., 2018. Ki-67: more than a proliferation marker. Chromosoma 127 (2), 175–186.

Tejada, S., Pinya, S., del Mar Bibiloni, M., A Tur, J., Pons, A., Sureda, A., 2017. Cardioprotective effects of the polyphenol hydroxytyrosol from olive oil. Curr. Drug Targets. 18, 1477-1486.

Wadhwa, R., Sugihara, T., Yoshida, A., Duncan, E.L., Hardeman, E.C., Nomura, H., Reddel, R.R., Kaul, S.C., 1999. Cloning and characterization of a novel gene, Strasmin, that interacts with the tumor suppressor protein p53. J. Biol. Chem. 274 (21), 14948–14955.

Wagemaker, T.A.L., Carvalho, C.R.L., Maia, N.B., Baggio, S.R., Guerreiro Filho, O., 2011. Sun protection factor, content and composition of lipid fraction of green coffee beans. Ind. Crops. Prod. 33 (2), 469–473.

Wen, X., Takenaka, M., Murata, M., Homma, S., 2004. Antioxidative activity of a zinc-chelating substance in coffee. Biosci. Biotechnol. Biochem. 68 (11), 2313–2318.

Young, D.S., Pestaner, L.C., Gibberman, V.A.L., 1975. Effects of drugs on clinical laboratory tests. Clin. Chem. 21 (5), 1–432.

Yousef, M.I., Awad, T.I., Mohamed, E.H., 2006. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology 227 (3), 240–247.

Zidan, N.-H., 2015. Hepato-and nephrotoxicity in male albino rats exposed to malathion and spinosad in stored wheat grains. Acta Biol. Hung. 66 (2), 133–148.