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Original article

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

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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 alterations 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.

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 (Cobilinschi 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

Abbreviations: AChE, Acetylcholinesterase; ACUC, Animal care and use committee; BSA, Biotin streptavidin; BUN, Blood urea nitrogen; CKD, Chronic kidney disease; COX 2, Cyclooxygenase-2; DAB, Diaminobenzidine; H2O2, Hydrogen peroxide; LSD, Least significant difference; MAL, Malathion; MUFA, Monounsaturated fatty acids; OP, Organophosphate; ROS, Reactive oxygen species; SE, Standard error; TP, Total protein; XO, Xanthine oxidase.

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and safeguarding against their negative repercussions (Georgiadis et al., 2018).

Malathion is an effective pesticides 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; Sivapiriya 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; Bułdak et al., 2018) and inflammation (Kiattisin 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 amounts of phenols which assist in intercepting the consequences of primary and secondary cardiovascular illness (Hohmann et al., 2015: Shen et al., 2015). In addition, olive oil also keeps blood pressure in arteries in check (Martín-Peláez et al., 2017), improves oxidative stability, and enhances the quality of inflammatory biomarkers (Hohmann et al., 2015).

Ki67 is among the numerous antigenic proteins used as a multiplication identifier for tumor cells (Sun and Kaufman, 2018). The assessment of the existence of cell cycle-related proteins constitutes vital facts about the tumor's tendency (Ghanem et al., 2004). The usage of Ki67 immunohistochemistry to study damage accrued in the kidney can show the preventive outcomes of the coffee and olive regimens. Cyclooxygenase-2 (COX 2) is an important enzyme in nephrotoxicity pathophysiology. The xanthine oxidase (XO) speeds up the transition of hypoxanthine to xanthine as well as xanthine to uric acid. The by-products of this reaction are hydrogen peroxide (H2O2) and ROS, which have a major contribution in explaining the etiology of damage to tissues (Bove et al., 2017; Osukoya et al., 2021). This experimentation aimed to determine the beneficial outcomes of Arabica coffee oil and olive oil against MAL-induced nephrotoxicity in the kidney using biochemical, histological, immunohistochemical, and *in silico* studies.

2. Materials and methods

2.1. Plant oils

Arabica coffee oil was obtained from Fushi, London, United Kingdom, and olive oil was obtained from Aljouf region, Saudi Arabia.

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 acclimatize 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-

nificant 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; Martín-Peláez et al., 2017; Martın 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 ($p \leq 0.001$) 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 ($p \leq 0.05$) 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
	Stearic acid	5281	-3.449	-24.373	-20.51
Cont.	Indomethacin	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	-9.857	-57.885	-55.938
	Stearic 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	638072	-2.203	-39.342	-51.289
Cont.	Allopurinol	135401907	-6.4	-32.4	-43.5

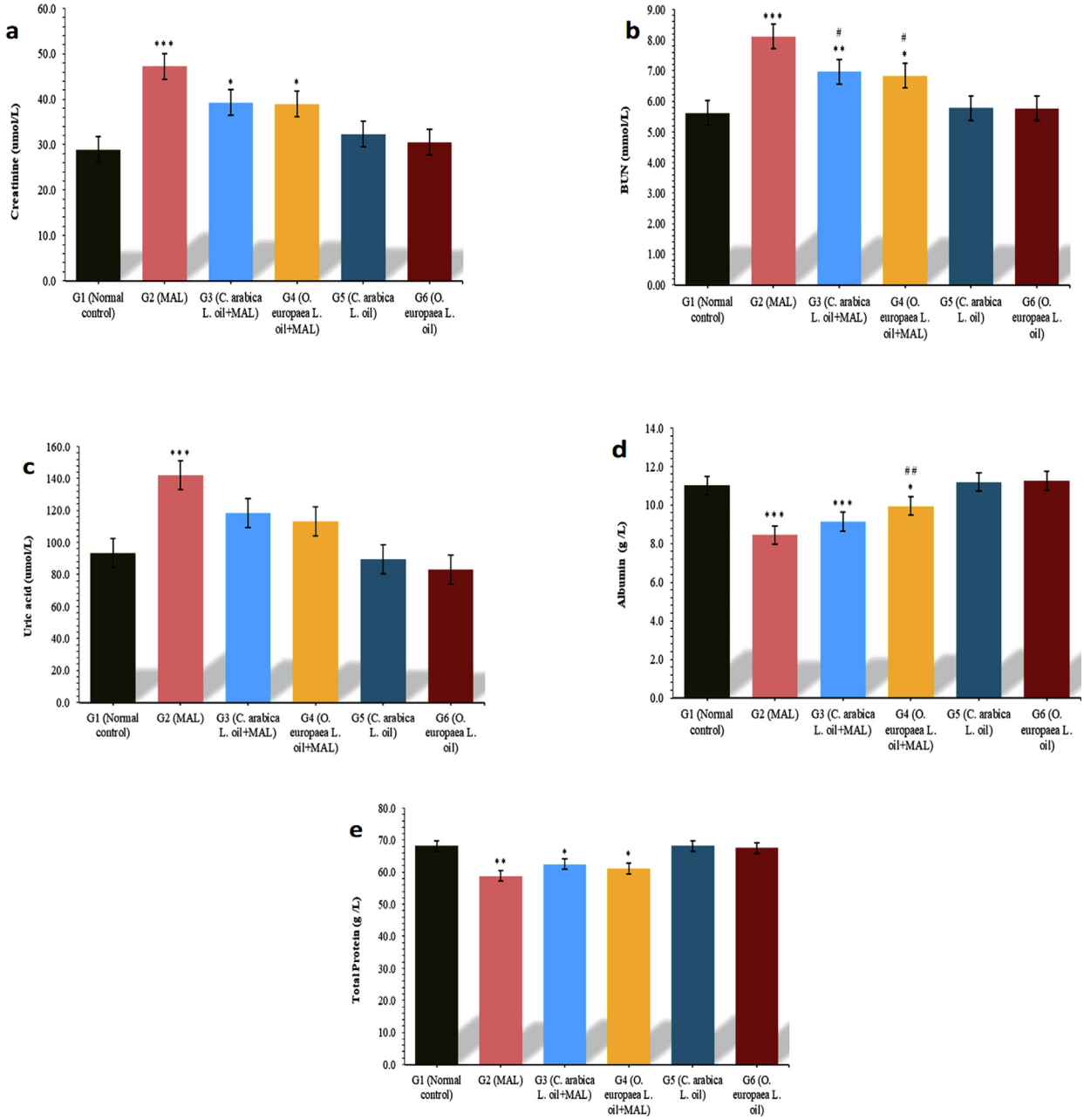

Fig. 1. Levels of serum (a) creatinine, (b) BUN, (c) uric acids, (d) albumin and (e) total protein in all experimental groups after 7 weeks. The acquired data were presented as means ± SE (standard error). * Indicates that the difference between the G1 and other treated groups is significant. # Denotes substantial difference between rats given MAL, MAL plus Arabica coffee (*Coffea arabica* L.) oil, or MAL plus olive (*Olea europaea* L.) oil.

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 ($p \leq 0.001$; $p \leq 0.05$, 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 ($p \leq 0.001$) 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 ($p \leq 0.01$) 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 ($p \leq 0.05$) decline in the serum levels of total protein when correlated with the G1 group of rats (Fig. 1e).

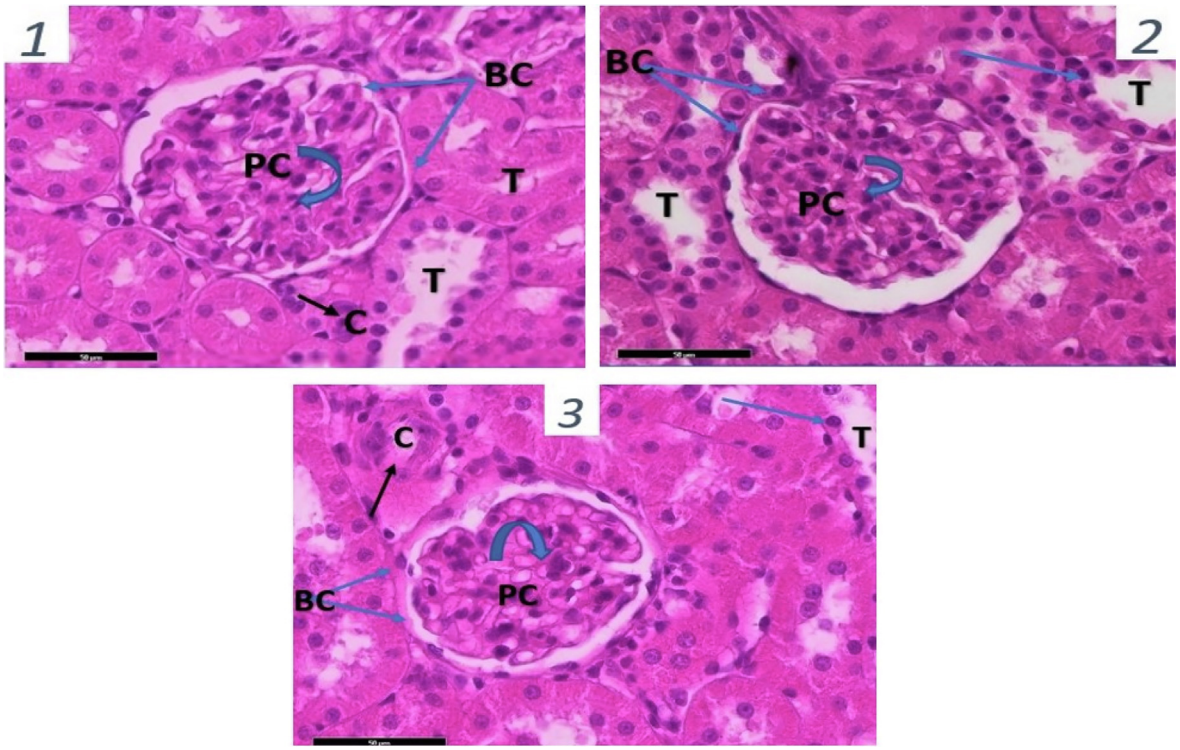

Fig. 2. Photomicrograph of G1 rat's kidney (normal control rats): Photo 1 showed bowman's capsule (BC), capillaries (C) and podocytes (PC) the cells that responsible for glomerular filtration as indicated by curved green arrow with well-preserved tubules i.e., single tubule (T). Photo 2 represents normal 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&Ex400).

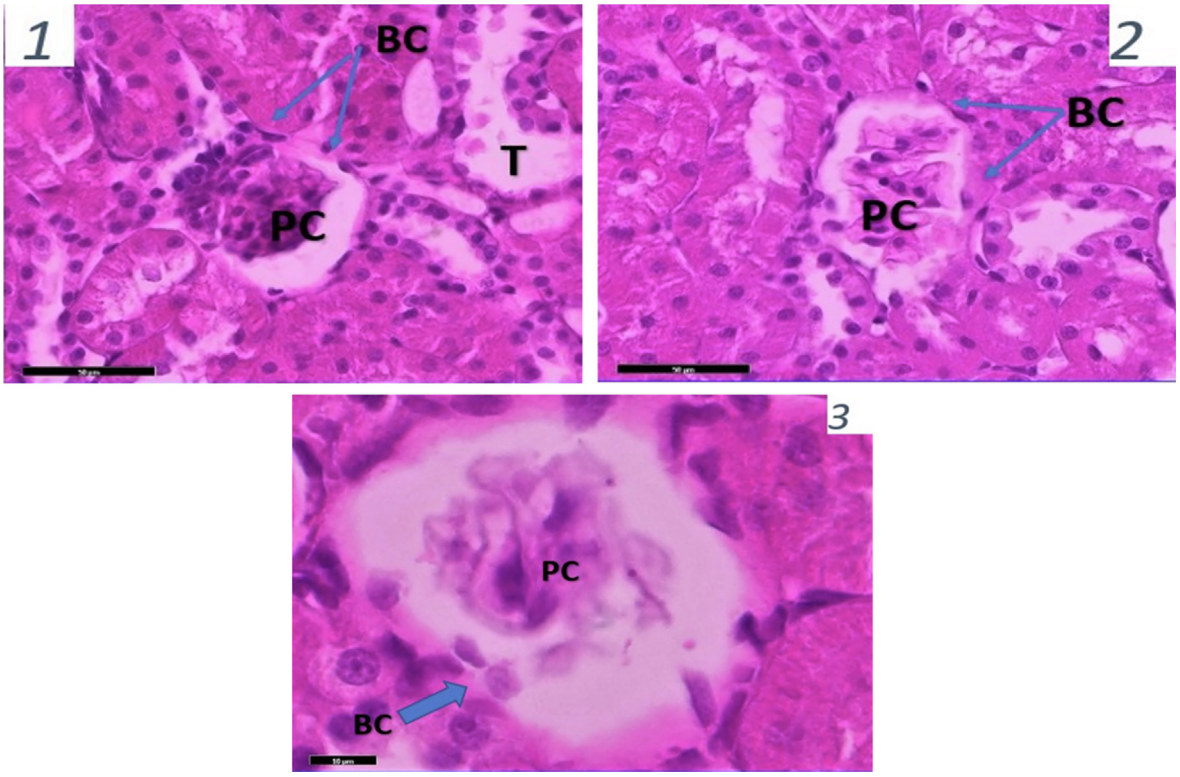

Fig. 3. Photomicrograph of G2 rat's kidney (MAL-intoxicated rats): Photo 1 Bowman's capsule (BC) MAL caused 3-dimension aggregation and overlap of podocytes (PC) with dark chromatin (pyknosis and karyorrhexis), wrinkled and disorganized tubules (T). Photo 2 showed podocytes (PC) with extreme effect of 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&Ex400), Photo3 (H&Ex1000).

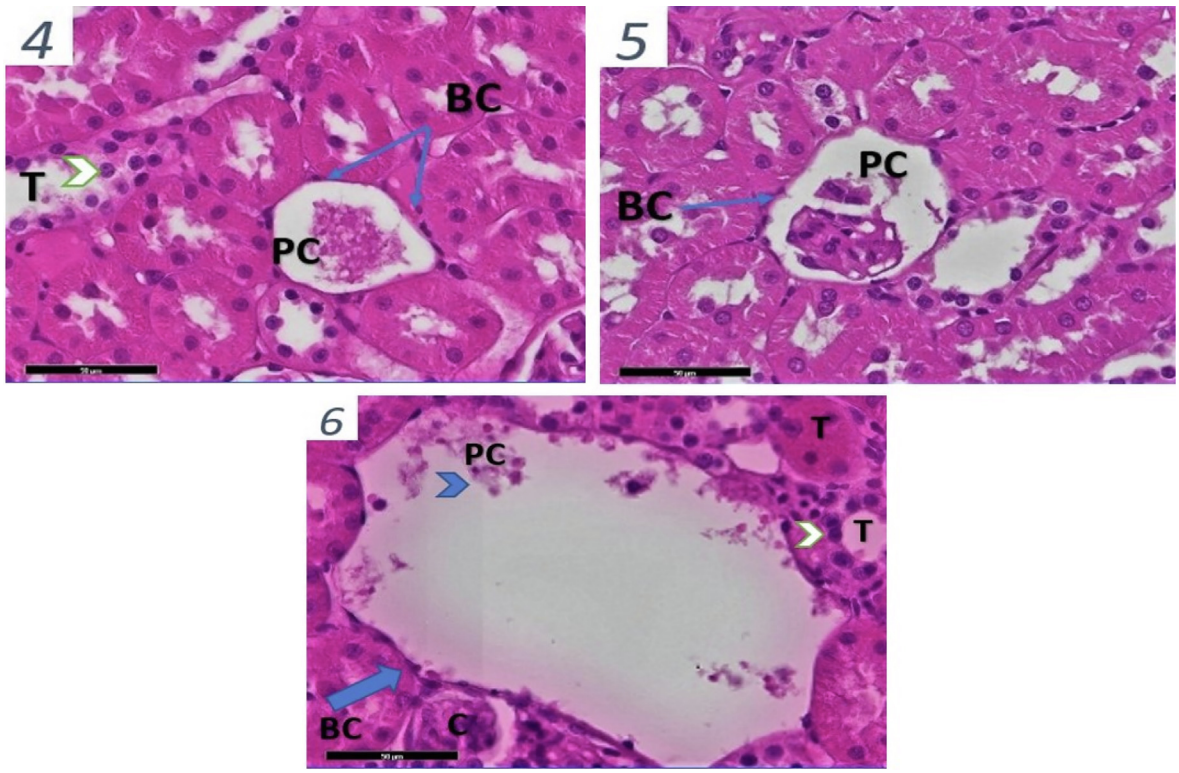

Fig. 4. Photomicrograph of G2 rat's kidney (MAL-intoxicated rats): Photo 4 showed Bowman's capsule (BC) (blue arrows) including podocytes (PC) devoid to nuclei or complete karyolysis that indicate necrosis which present also in tubular cells (white arrow head). Photo 5 showed Bowman's capsule (BC) (blue arrows) with detachment of podocytes (PC) leaving a clear space. The nuclei of these distorted podocytes showed pyknosis and karyorrhexis. 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). Photo4,5,6 (H&Ex400).

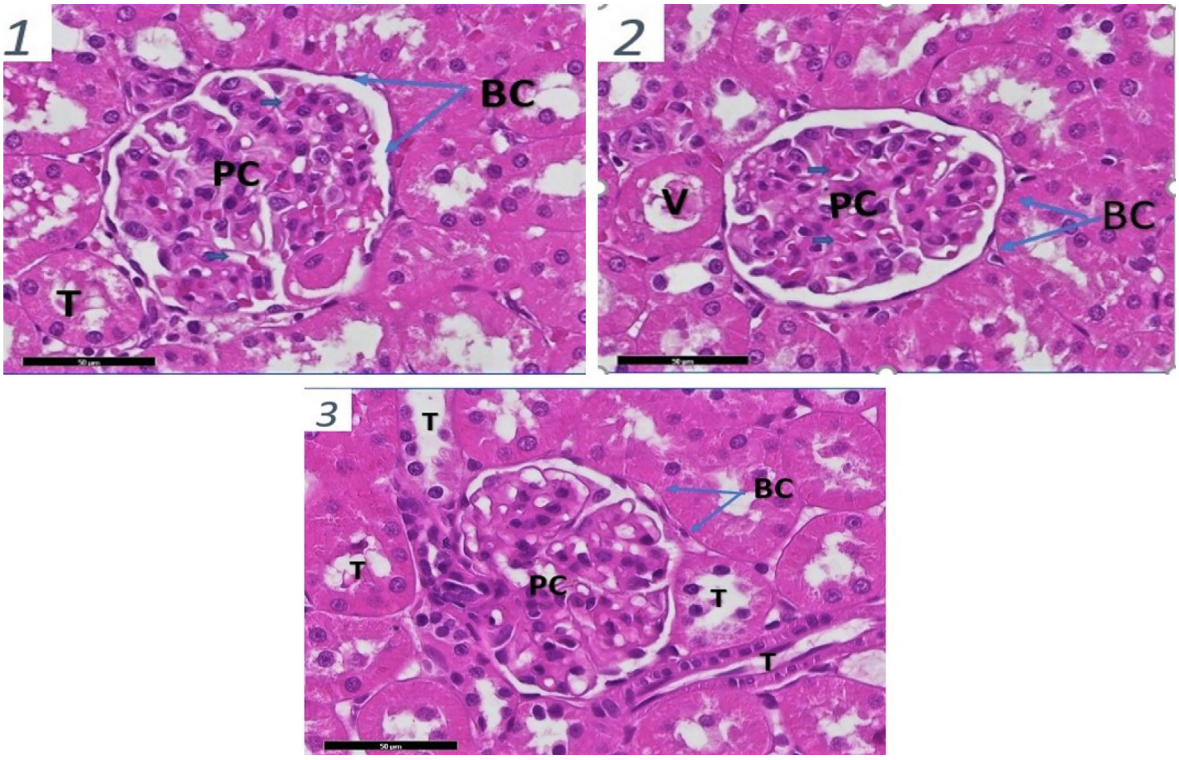

Fig. 5. Photomicrograph of G3 rat's kidney (MAL-intoxicated rats + Arabica coffee oil): Photo 1 showed Bowman's capsule (BC) indicated by (green arrows) and podocytes (PC) with normal restored cells, architecture and vascular supply (red blood cells indicated by blue thick arrows) with intact tubules (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). Photo1,2,3 (H&Ex400).

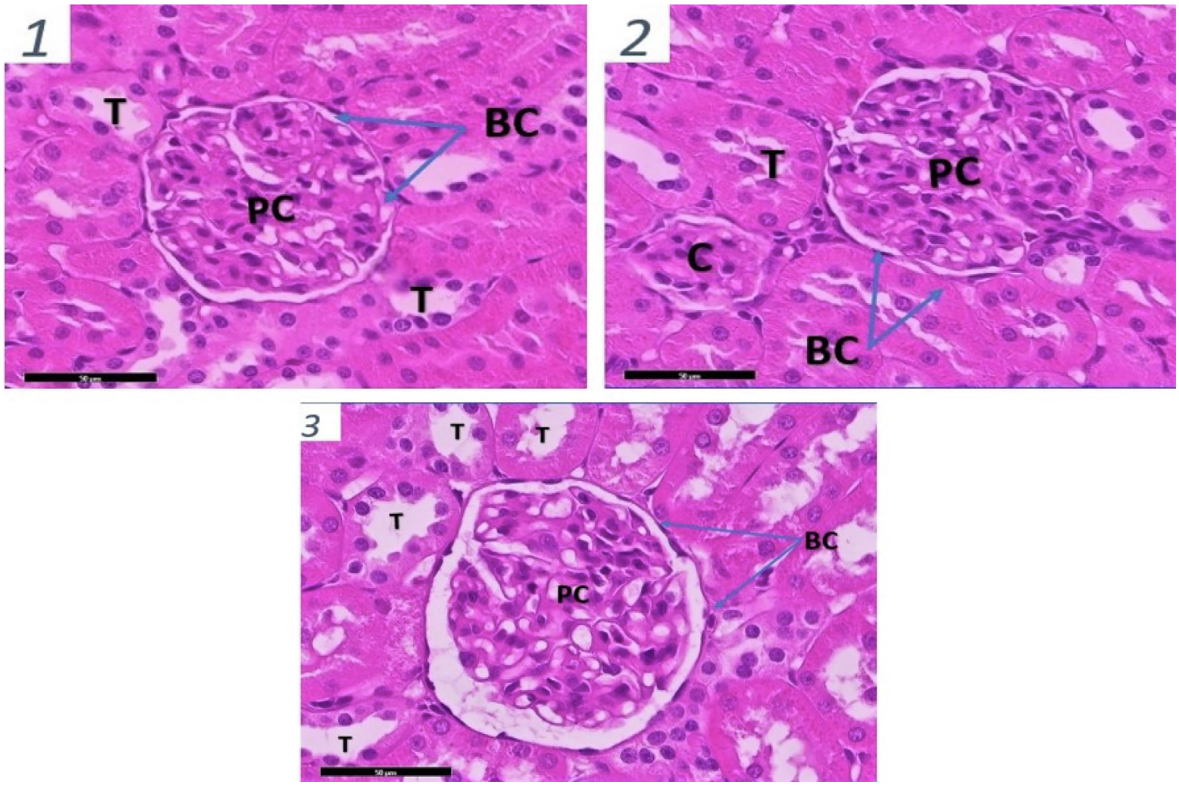

Fig. 6. Photomicrograph of G4 rat's kidney (MAL-intoxicated rats + olive oil): Photo 1 showed Bowman's capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells, architecture and intact tubules (T). Photo 2 also showed Bowman's capsule (BC) indicated by blue arrows with well-organized 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). Photo1,2,3 (H&Ex400).

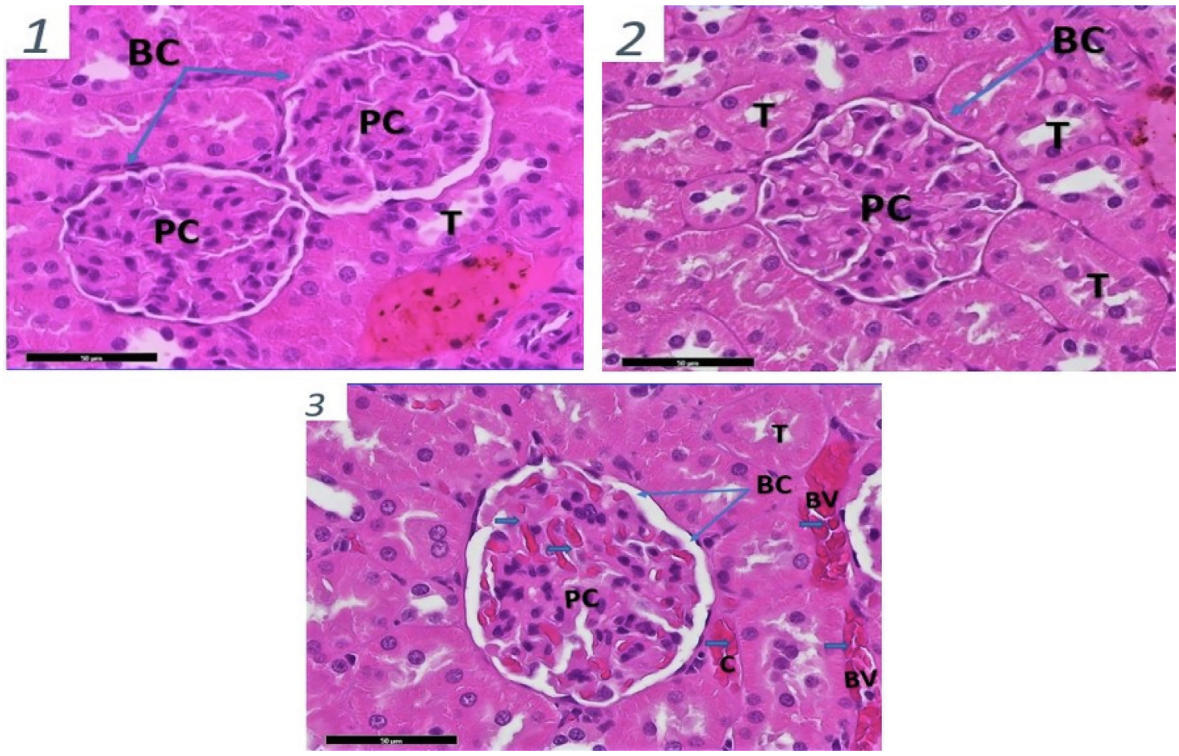

Fig. 7. Photomicrograph of G5 rat's kidney (Rats treated with Arabica coffee oil): Photo 1 showed Bowman's capsule (BC) indicated by (blue arrows) and podocytes (PC) with normal restored cells, and intact tubules (T). Photo 2 showed Bowman's capsule (BC) indicated by (blue arrows) and podocytes (PC) 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). Photo1,2,3 (H&Ex400).

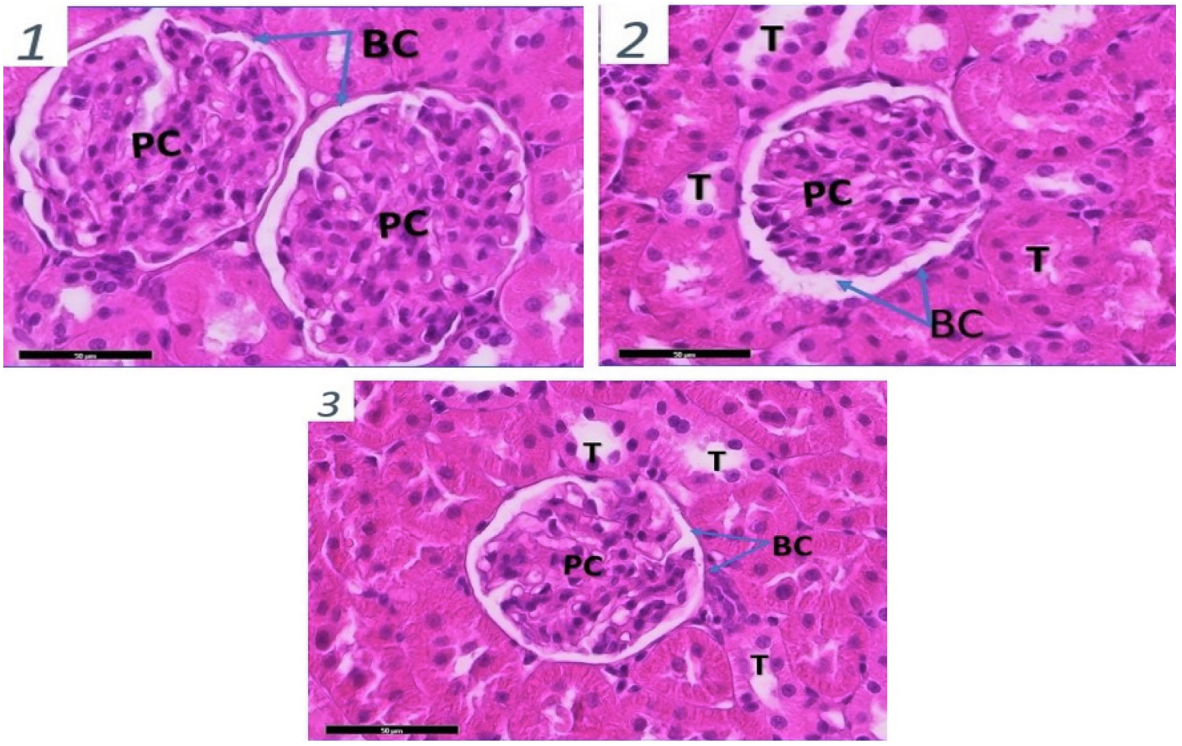

Fig. 8. Photomicrograph of G6 rat's kidney (Rats treated with olive oil): Photo 1 showed 2 Bowman's capsules (BC) indicated by (blue arrows) and podocytes (PC) with normal cells, architecture and intact tubules (T). Photo 2 also showed 2 Bowman's capsule (BC) indicated by blue arrows with well-organized 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). Photo1,2,3 (H&Ex400).

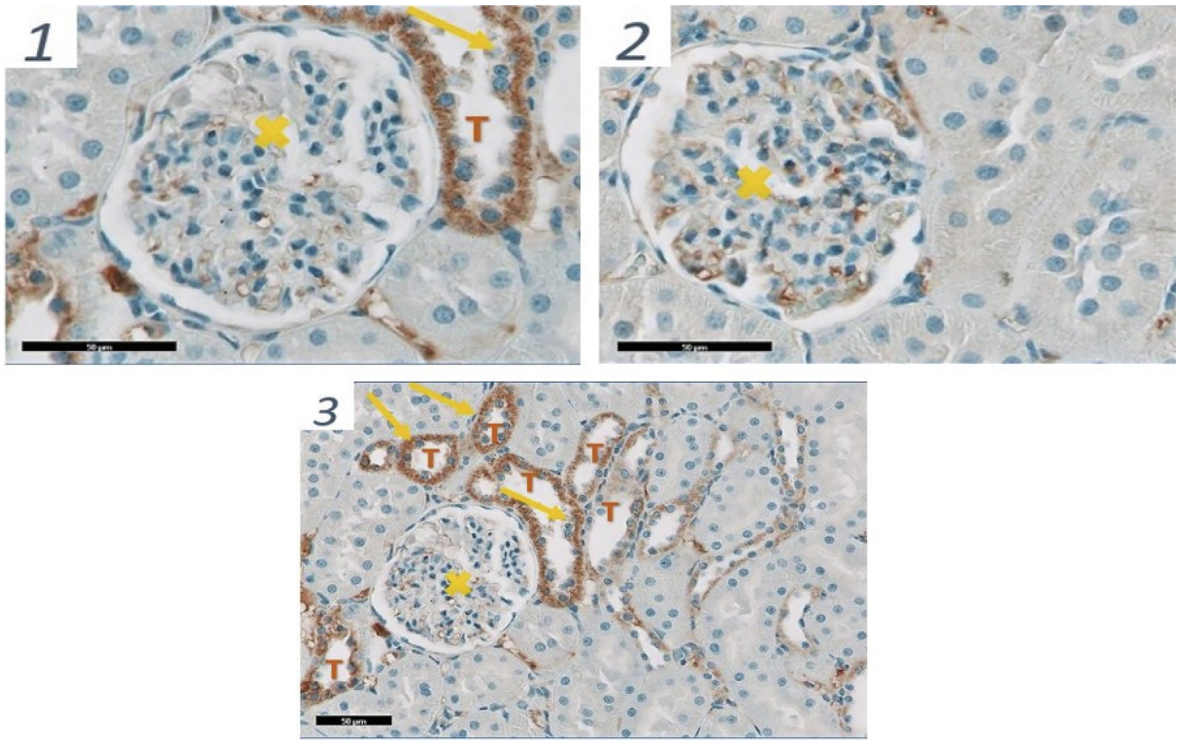

Fig. 9. Photomicrographs of G1 rat's kidney (normal rats) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed moderate (++) positive reaction (yellow arrows) on the tubules (T); faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman's capsule (yellow cross) which 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

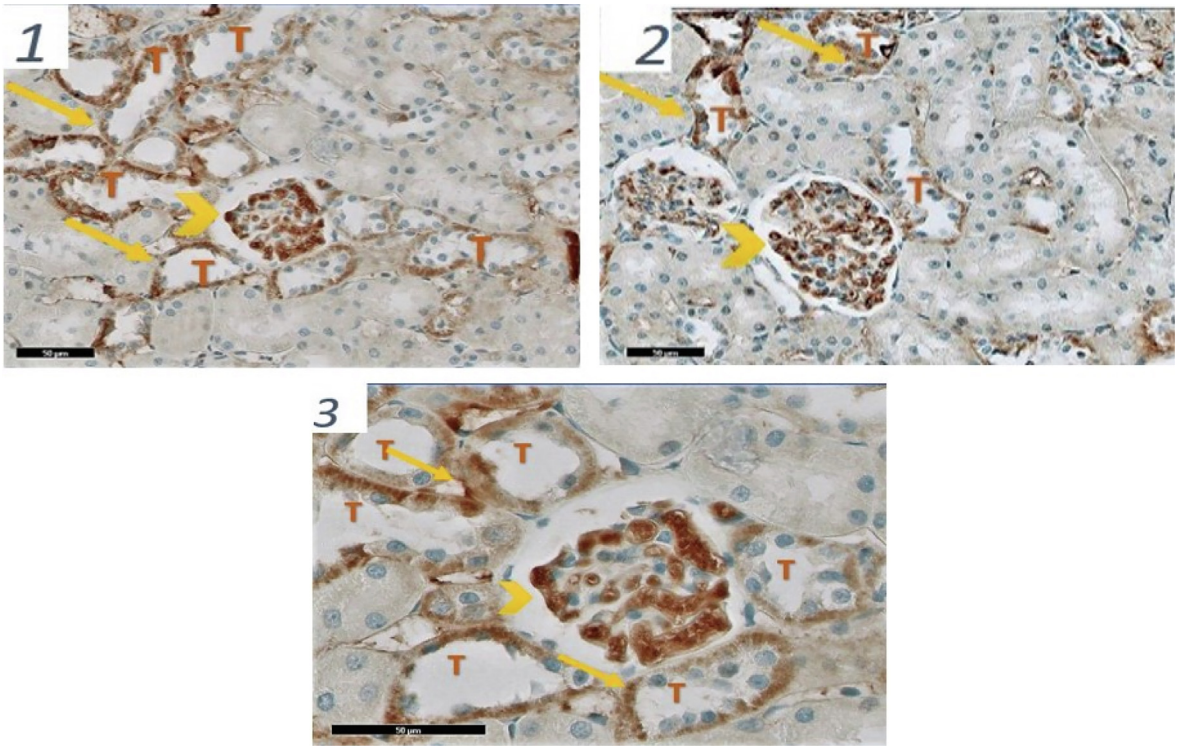

Fig. 10. Photomicrographs of G2 rat's kidney (MAL-intoxicated rats) immune-stained with the apoptotic marker ki67: Photo (1, 2) both showed strong (+++) positive reaction (yellow arrows) on the tubules (T) and Bowman's capsule cells (podocytes) (yellow arrow head). Photo (3) showed strong (+++) positive reaction (yellow arrows) on the tubules (T) and Bowman's capsule cells (podocytes) (yellow arrow head). Photo (1,2) x200, Photo (3) x400.

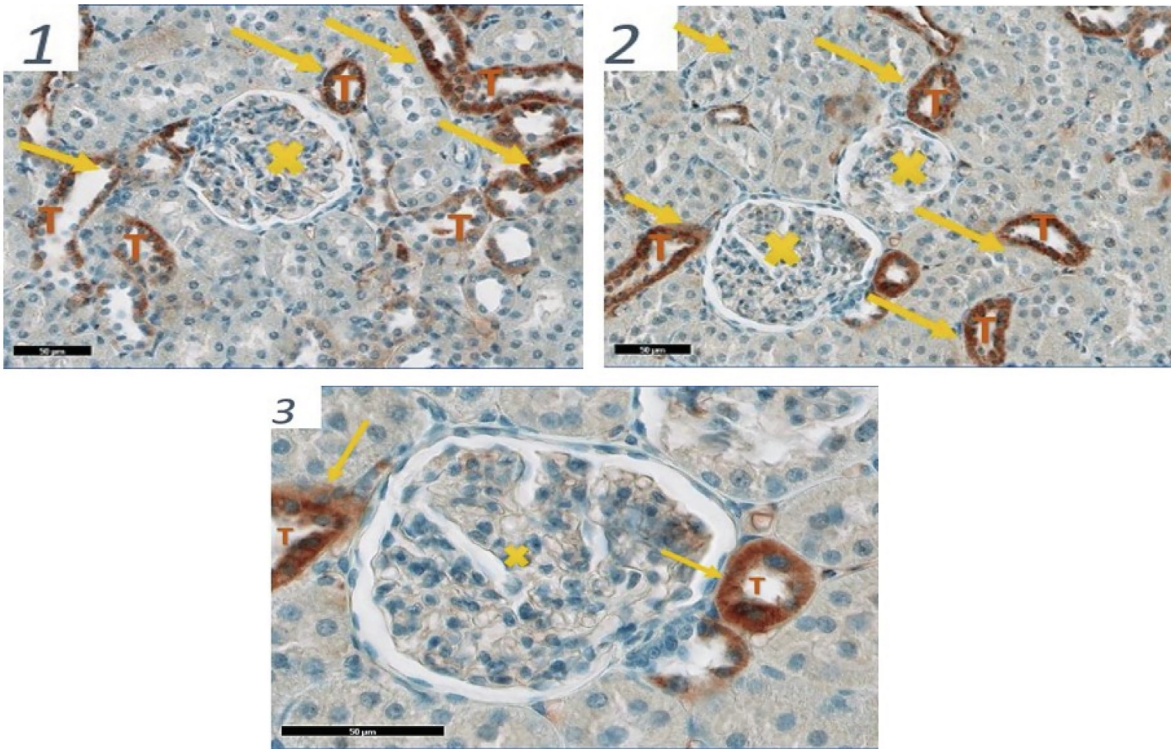

Fig. 11. Photomicrographs of G3 rat's kidney (MAL-intoxicated rats + Arabica coffee oil) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed strong to moderate (+++/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes 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

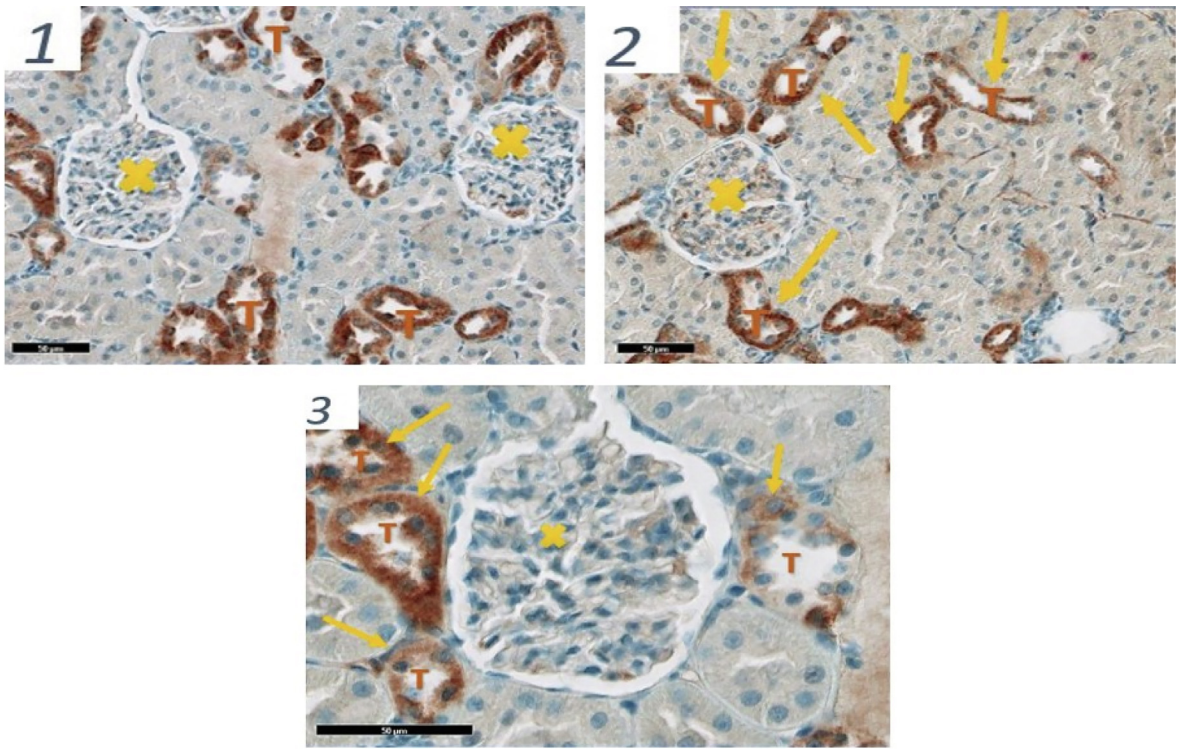

Fig. 12. Photomicrographs of G4 rat's kidney (MAL-intoxicated rats + olive oil) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed strong to moderate (+++/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes cells 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

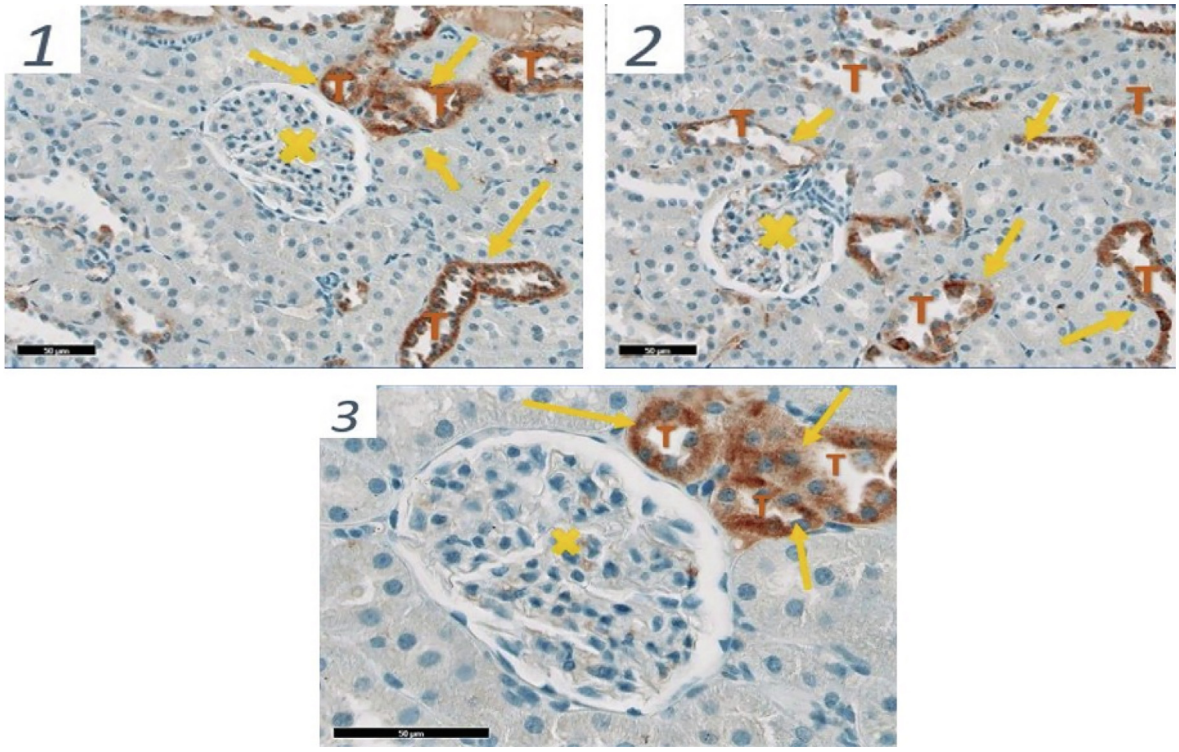

Fig. 13. Photomicrographs of G5 rat's kidney (Rats treated with Arabica coffee oil) immune-stained with the apoptotic ki67: Photo (1,2) both showed strong to moderate (++ +/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes cells in 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

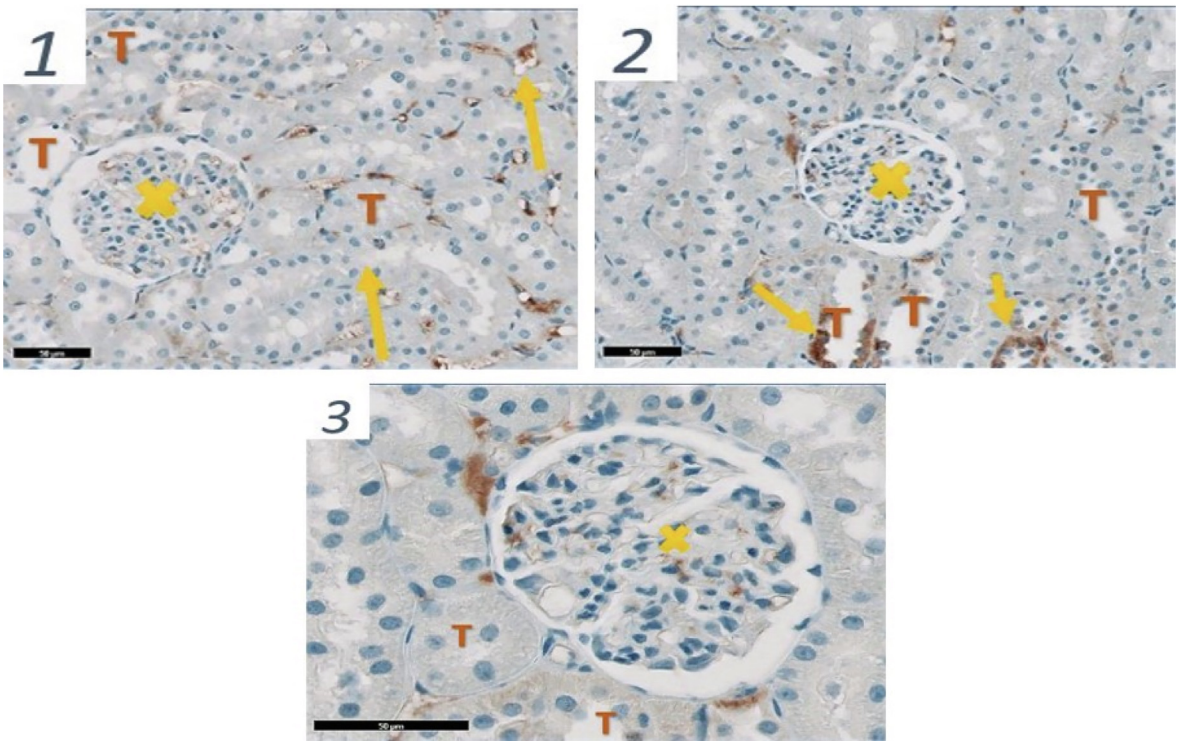

Fig. 14. Photomicrographs of G6 rat's kidney (Rats treated with olive oil) immune-stained with the apoptotic marker ki67: Photo (1,2) both showed strong to moderate (+++/++) positive reaction (yellow arrows) on the tubules (T) and faint immunoreactivity result in the cytoplasm of podocytes cells in Bowman's 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

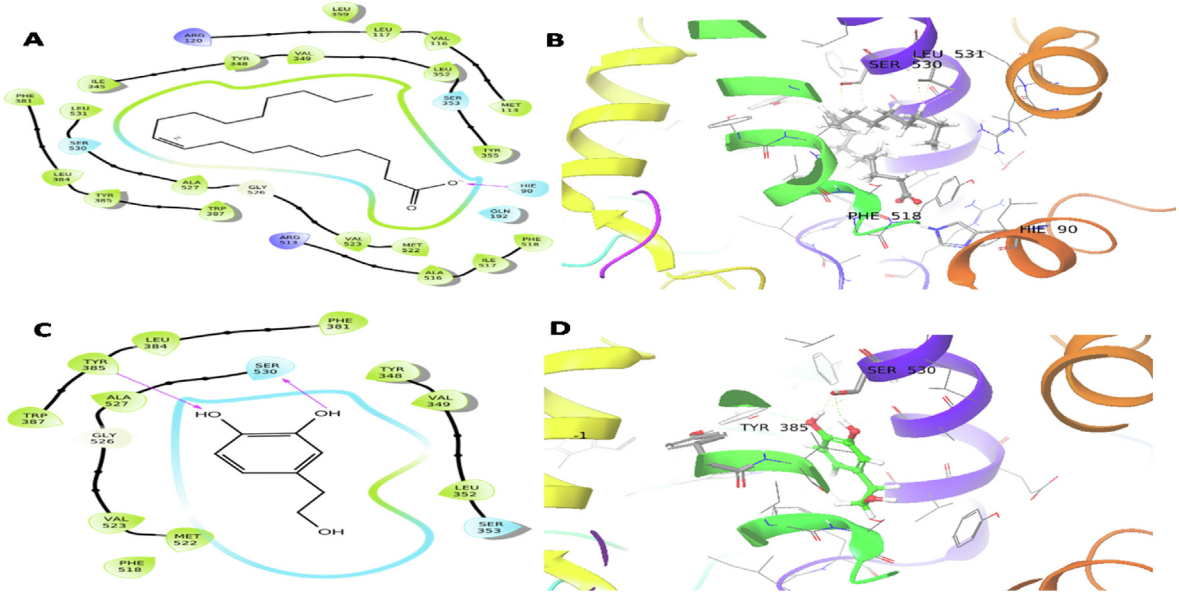

Fig. 15. 3D and 2D interaction of COX-2 macromolecule, the ligands are shown in balls at 3D representation, and the protein backbones are shown in ribbons, hydrogen bonds are shown in purple arrows. A and B 3D and 2D Interaction of oleic acid. C and D. 3D and 2D interaction of COX-2 protein and hydroxytyrosol ligand.

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, Bowmans 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

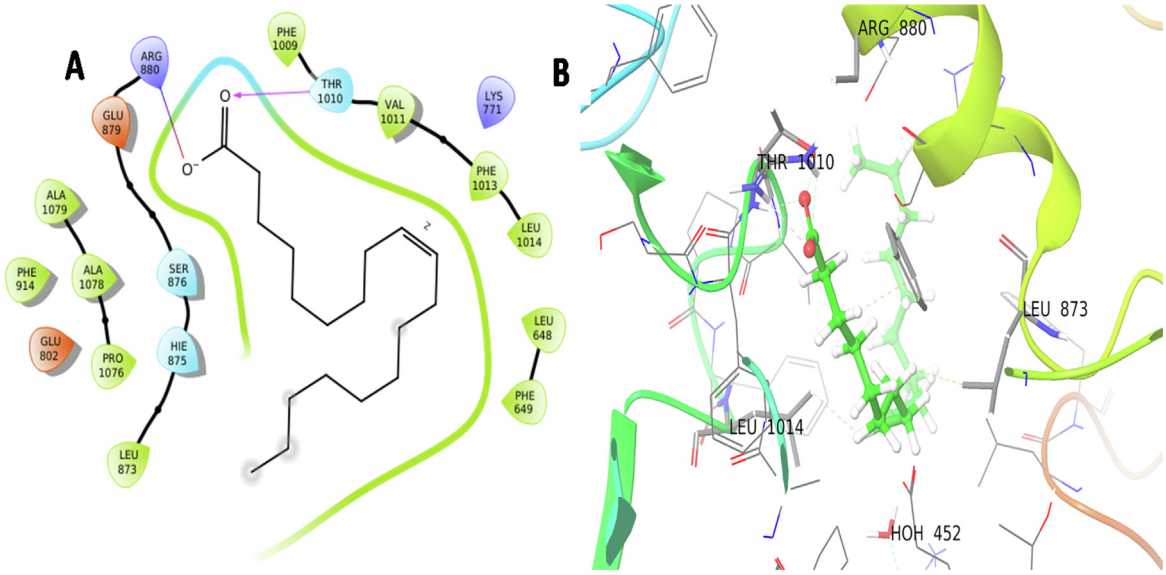

Fig. 16. 3D and 2D interaction of XO protein, the ligands are shown in balls at 3D representation, and the protein backbones are shown in ribbons, hydrogen bonds are shown in purple arrows. A and B 3D and 2D Interaction of oleic acid.

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 capscular 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 -2.666kcal/mol. The COX-2 (6COX) protein showed the best interaction with oleic acid (-6.368 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 bonds 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 (caffein, 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-Giacoman 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; Yousef 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 Alkhalaf, 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; Fındıklı 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 (Brezová et al., 2009; 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 antioxidative 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 (JJ, 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 calcifications 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 hyrdophic 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; Selmi 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 nucleoli. 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; Gerdes 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 (Domitrović 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 deterrence 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 nephrotoxicity medication (Indomethacin). Other studies support the findings of the present research, which showed the antiinflammatory and nephrotoxicity 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.

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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.

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