Evaluate of the efficacy of Extra Virgin Olive Oil against Paracetamol overdose induced renal toxicity in albino rats

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Abstract

Paracetamol has a reasonable safety profile in therapeutic doses, but the overdose cause nephrotoxicity and acute kidney failure. The aim of the present work was to investigate whether Extra-Virgin Olive Oil (EVOO), has the potential to protect from the nephrotoxicity induced by paracetamol overdose. Forty Male Wistar Albino rats were used in this study. The animals were divided into four groups (n=10): Animals in control group (1) were given daily distilled water orally. Group 2 animals were given EVOO orally at the daily rate (2 ml/kg b.wt.). Group 3 animals were given paracetamol orally at the daily rate (650 mg/kg b.wt.). Group 4 animals were given daily paracetamol and EVOO orally using the same dose used in groups 2 and 3. All animals were sacrificed after 15 days. The histological and ultrastructure examination showed several alterations in renal tubules cells such as cells damage, necrotic cells, pyknotic nucleus and destroy some renal tubules in paracetamol comparing to control group. The treatment by EVOO appeared protective effect on renal tubules comparing to paracetamol. Also, the data of plasma biomarker activity showed significant ($p<0.001$) increase in uric acid, urea and creatinine levels in paracetamol compared to the control group. While, the results of paracetamol and EVOO showed a significant decrease ($p<0.001$) in uric acid, urea and creatinine levels activities compared to the paracetamol. In conclusion, the results of present study indicate that EVOO may protect kidney against paracetamol- induced nephrotoxicity and renal tubules cells damaged in rats. The effect may due to EVOO phenolic component and its antioxidant properties.

Key words: Extra Virgin Olive Oil, Paracetamol, nephrotoxicity, kidney failure, antioxidants.

1 Introduction

Paracetamol (acetaminophen or APAP) is the most common used medication for pain and fever in Saudi Arabia, and most of countries such as United States, Europe and Middle East. It is available as a generic medication with trade names such as ***Tylenol*** and ***Panadol*** among others. Paracetamol is available without a prescription and it is one of several first line therapies for treatment of tension, migraine headache or dental pain. Paracetamol also considered as one of several treatment options for people such as arthritis pain of the hip, hand, or knee. Also, Paracetamol has relatively little anti-inflammatory activity, and can relieve pain in mild arthritis, (De Martino *et al.* 2015). Therefor, the misuse of paracetamol by taking overdose or used for long periods may cause damage to some organs.

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Several studies indicated that paracetamol overdose cause nephrotoxicity, hepatotoxicity and damage in other organs (Lorz *et al.* 2004; Naggayi *et al*. 2015; Hassan. and Rahman 2016; Canayakina *et al.* 2016; Dokumacioglu and Iskender 2017). Study by Lorz *et al.* (2004) showed that paracetamol had a phenacetin metabolite which is considered one of the most nephrotoxic analgesics by induction of apoptosis. Also, paracetamol induced several histopathological and immuno-histochemical changes in liver, kidney and brain tissue (Hassan. and Rahman 2016).

Olive oil is a liquid fat obtained from olives fruits in Mediterranean Basin area. The olive oil is usually used in cooking and as a salad dressing. It is also used in cosmetics, pharmaceuticals, and soaps, and as a fuel for traditional oil lamps. (Riley 2002).

Virgin olive oil is produced from the first and second pressings of the olive fruit by the cold pressing method (no chemicals) and is composed of a glycerol fraction (making up 90–99% of the olive fruit) and a non-glycerol which contains phenolic compounds (making up 0.4–5% of the olive fruit) (Cicerale *et al.* 2010)

EVOO is an important daily diet in Mediterranean Basin area because of its significant health benefits. These benefits include reducing DNA oxidation and positively influencing cholesterol regulation and oxidation of low-density lipoprotein. In addition, EVOO has anti-inflammatory, antithrombotic, antimicrobial, anticarcinogenic, antihypertensive, and vasodilatory effects. (Covas *et al.* 2000; Visioli *et al.* 2002; Covas *et al.* 2006; Covas 2007; Cicerale *et al.* 2012; Hernáez *et al.* 2014; Berrougui *et al.* 2015). EVOO is effective at ameliorating some of the DNA and kidney damage induced by hexavalent chromium toxicity in rats (Saber *et al.* 2015).

Several studies have investigated the role of EVOO as one of the active constituents in complementary medicine and consider as natural sources contain a variety of molecules with potent biological activities. In animal study reported that rats fed on a diet containing EVOO were protected the liver against paracetamol overdose-induced hepatotoxicity. (Khayyat 2016). In vivo study have shown that the antioxidant properties of phenolic compounds (oleuropein and hydroxytyrosol) derived from Olive oil are linked with inhibition of lipid peroxidation and free radical scavenging activity (Tuck *et al.* 2001). Other study suggests that EVOO is effective at ameliorating some of the DNA and kidney damage induced by hexavalent chromium toxicity in rats (Saber *et al.* 2015).

Therefore, the objective of present study was to evaluate the efficacy of EVOO against paracetamol overdose induced renal toxicity in albino rats based on renal function markers in the serum, histopathological and ultrastructural changes in the kidney.

2 Materials and methods

Animals:

Male Wister albino rats (weight 146–156 g) were used in the present study. Forty adult rats maintained in special clean standard metallic cages (5 rats per cage) under standard laboratory conditions an air conditioned room (24 ± 2°C) with relative humidity of 50 ± 5% and 12-h light/12-h dark cycle and acclimatized for 1 week prior to the study. They fed with free access to

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a commercial balanced stock diet and water. The experiments were done in compliance with the Guide for the Care and Use of Laboratory animals.

Drug and Oil used:

The Paracetamol was purchased from local pharmacy at Makkah, Saudi Arabia (Panadol 665, GlaxoSmithKline Australia Pty Limited, Australia**).** Extra virgin olive oil was obtained from a local market in Makkah, Saudi Arabia.

Experimental design:

Rats were randomly divided into 4 groups (n= 10). Control group (group1), animals were given distilled water orally at the daily rate of 2ml/kg b.wt. for 15 days. Group 2 animals were given extra virgin olive oil orally at the daily rate (2ml/kg b.wt.) through gastric tube for 15 days (served as positive control group). (Yousaf *et al.* 2012). Group 3 nimals were given paracetamol orally at the daily rate (650mg/kg b.wt.) through gastric tube for 15 days. (Basu, et al. 2014). Group 4 animals were given paracetamol orally at the daily rate (650mg/kg b.wt.) and extra virgin olive oil orally at the daily rate (2ml/kg b.wt.) through gastric tube for 15 days.

After 15 days, rats from both control and experimental groups were killed by cervical dislocation. Small samples of Kidney were quickly removed and fixed for light- and electron microscopical studies.

Biochemical testes:

For biochemical study, blood samples were collected from each rat via cardiac puncture method and allowed to clot. The serum was rapidly separated by centrifuging the clotted blood at 3000g for 10 min in a Beckman Model T-6 refrigerated centrifuge and processed for determination into clean and dry tubes. Sera were stored at -20 0C until assayed for the biochemical parameters. Creatinine, urea and uric acid were estimated. (Henry 1974; Potton and Crouch, 1977; Caraway (1955).

2-Histological and Ultrastructural study:

Kidneys of all groups were fixed in Bouin's solution then dehydrated, cleared, embedded in paraffin wax. Sections of 5µ were stained with hematoxylin and eosin for histological examination.

For transmission electron microscopy, small pieces of kidney were immediately fixed in 4F1G in phosphate buffer (pH 7.2) for 3 hours at 4°C, then post-fixed in 2% OSO4 in the same buffer at 4°C for 1-2 hours. The specimens were dehydrated through graded series of ethanol, embedded in epon-araldite mixture and polymerized at 60°C. Ultrathin sections (50 nm) from selected areas were cut with glass knives on LKB ultra microtome, double stained with uranyl acetate and lead citrate and examined by jeol 100CX electron microscope.

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3-Statistical Analysis:

Data were presented as mean ± SD of ten replicates and were analyzed by one way ANOVA and followed by student's T test. Results considered to be statistically significant when $p<0.05$.

3 Main Results

Histological results:

Examination of kidney sections from the rats in control group showed basic structure of kidney with normal glomeruli and renal tubules (Fig. 1a &1b).

Also, the histological examination of kidney sections from animals given EVOO were observed normal glomeruli and renal tubules (Fig. 2).

However, histological changes were observed in the kidney sections from rats treated with paracetamol compared to control group. The changes include shrinkage and lobulated glomerular capillaries with appears of cavitation area and necrotic lesion in some glomeruli and renal tubules (Fig. 3a &3b). Also, the membrane of tubular epithelial cells injury in apical surfaces and degeneration in the basal membrane of cells (Fig. 3b).

The light microscopic examination of kidney sections from animals given paracetamol and EVOO showed that most glomeruli had normal glomerular capillaries and normal renal tubules epithelial cells with normal basal membrane compared to control group (Fig. 4).

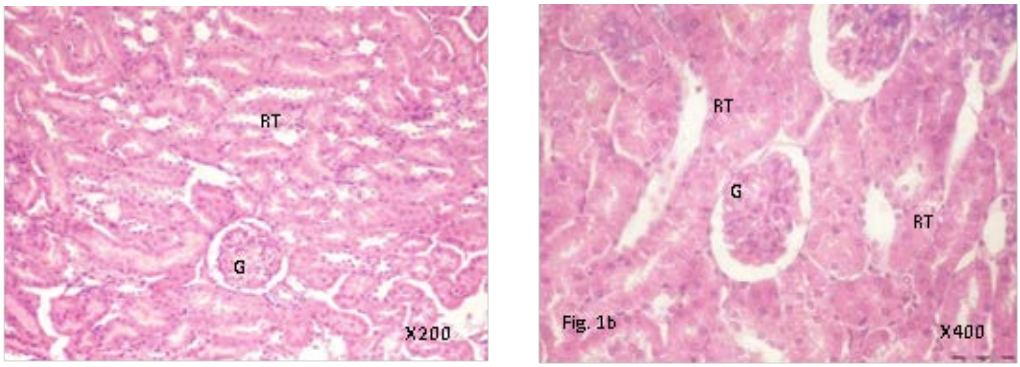

Fig.1a &1b: light micrograph of kidney section of rat from control group showing normal glomeruli

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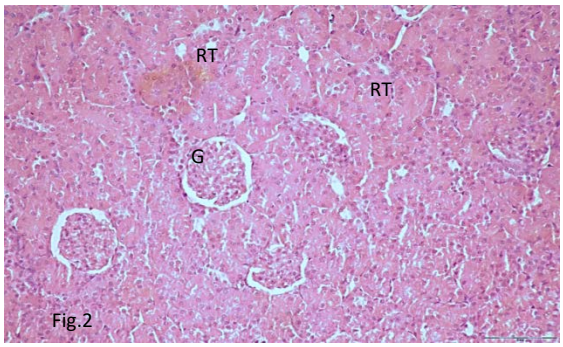
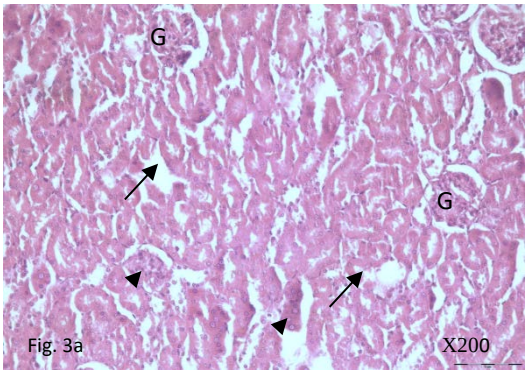


Fig.2: light micrograph of kidney section of rat from EVOO treatment group showing normal structure of glomerular (G) and renal tubules (RT). H&E.



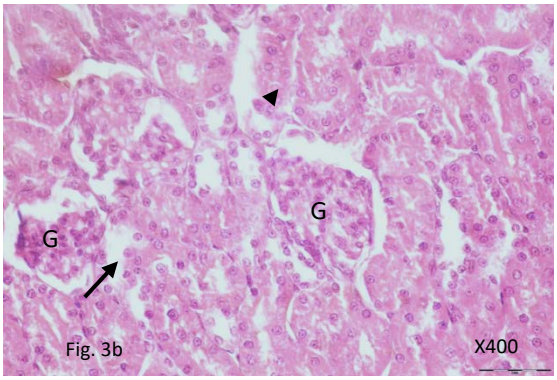


Fig.3a &3b: light micrograph of kidney section of rat from paracetamol treatment group showing, shrinkage, lobulated in some glomeruli capillaries (G). Cavitation area (arrows) and necrotic lesion in some glomeruli and renal tubules (head arrows). The membrane of tubular epithelial cells injury in apical surfaces and degeneration in the basal membrane of cells .H&E

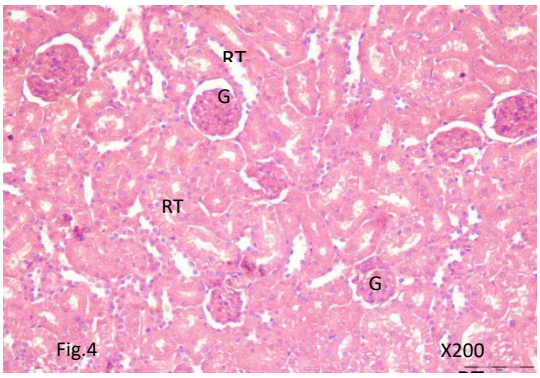


Fig.4: light micrograph of kidney section of rat from EVOO and paracetamol treatment group showing normal structure of most glomerular capillaries (G) and renal tubules (RT). H&E.

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Ultrastructural study:

The electron microscopic examination of the proximal tubular cells in control group appeared with normal nuclei, regular basement cell membrane, apical microvilli formed a brush border, numerous electron dense mitochondria and rough endoplasmic reticulum microvilli. (Fig.5a). The distal tubular cells of the control rats showed with regular basement cell membrane, microvilli zone is less developed than in the proximal and the lumen is wider comparing to proximal lumen. Nucleus appeared with a central or peripheral electron-dense nucleolus. Mitochondria appeared with rounded-shaped (Fig.5b).

The ultrastructure of the proximal tubular cells in EVOO rats demonstrate regular basement cell membrane with well-developed basal infoldings. The nuclei appeared large spherical, with one or more dense nucleoli and surrounded by numerous electron dense mitochondria. Lysosomes, pinocytotic vesicles, rough endoplasmic reticulum cisternae can be observed throughout the cytoplasm (Fig.6a). The distal tubular cells of the EVOO rats showed spherical apical nucleus with a central or peripheral electron-dense nucleolus. basement membrane appeared with many basal infoldings. The microvilli zone is less developed comparing to the proximal compartment with wider lumen. (Fig.6b).

Electron microscopic observations showed the proximal tubular cells in rats treated with paracetamol for 5 days. Some cells of proximal tubules showed pyknotic nuclei and the others appeared with irregularly-shaped nuclei with loose chromatin materials, (Fig.7a & Fig. 7b). In addition, irregular mitochondria, lysosomes containing dense material, pinocytotic vesicles were appeared. (Fig.7a). The cytoplasm of distal tubular cells appeared with pyknotic nuclei and the other with swollen nuclei, irregular mitochondria and pinocytotic vesicles. Also, the distal tubular showed with unnormal microvilli and the lumen appeared wider (Fig. 7b).

In the EVOO and paracetamol group the proximal tubular cells appeared with nuclei and regular chromatin materials, regular mitochondria, lysosomes, basement membrane and microvilli (mv). The cytoplasm appeared with pinocytotic vesicles. (Fig.8a). While the distal tubular cells appeared with irregular nuclei. Their cytoplasm showed contained numerous elongated mitochondria and many electron-lucent vacuoles and there was regular basement membrane. Also, some pinocytotic vesicles appeared in the cells. (Fig. 8b).

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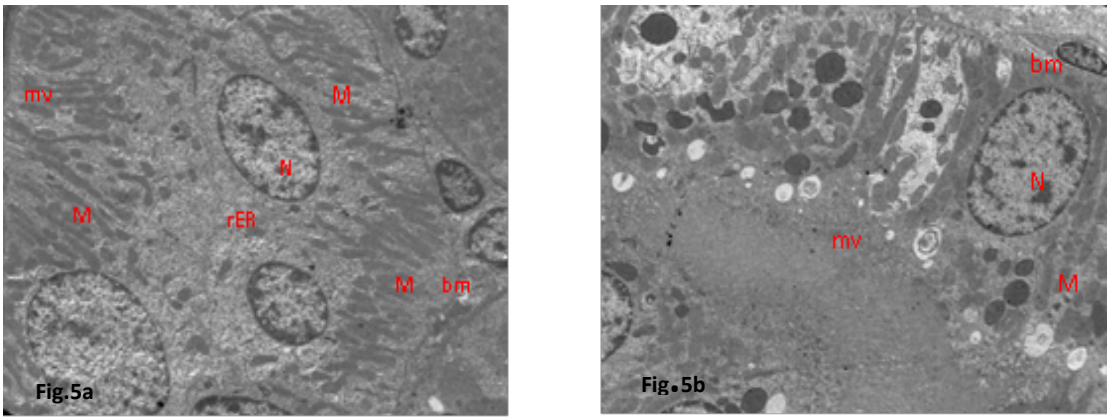

Fig. 5a &5b: Electron micrograph of kidney rat in control group showing the proximal tubular cells with normal nuclei (N), regular basement cell membrane (bm), apical microvilli formed a brush border (mv), numerous electron dense mitochondria (M) and rough endoplasmic reticulum (rER) microvilli (mv) (Fig. 5a). the distal tubular cells with regular basement cell membrane (bm), nuclei (N), microvilli (mv) and mitochondria (M) (Fig. 5b). (X2000)

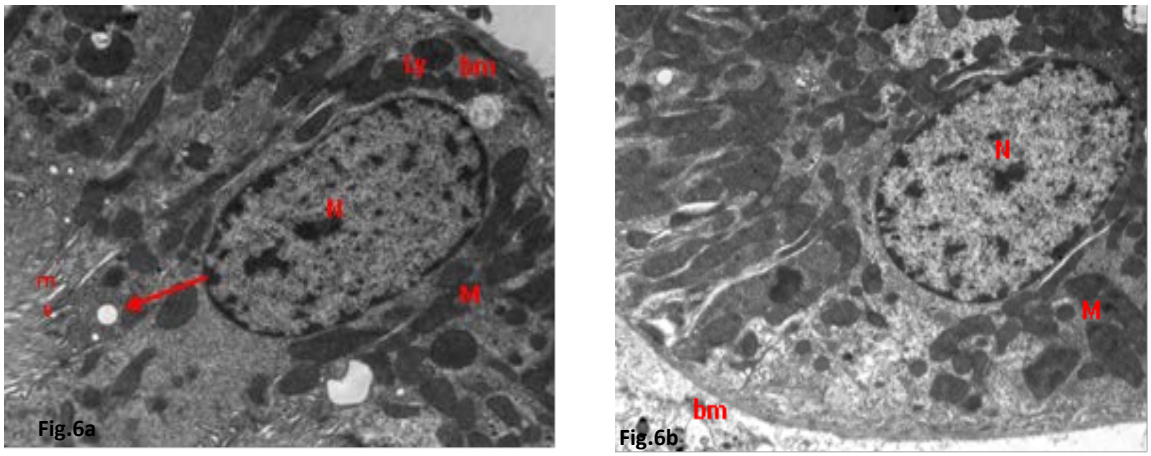

Fig. 6a & 6b: Electron micrograph of kidney rat in EVOO group showing the proximal tubular cells with normal nuclei and regular chromatin materials (N), regular mitochondria, lysosomes (Ly), basement membrane (bm)and microvilli (mv). Some pinocytotic vesicles appeared (arrow) (Fig. a) (X3000). The distal tubular cells with normal nuclei (N), regular mitochondria and basement membrane (bm) (Fig.6b). (X2500)

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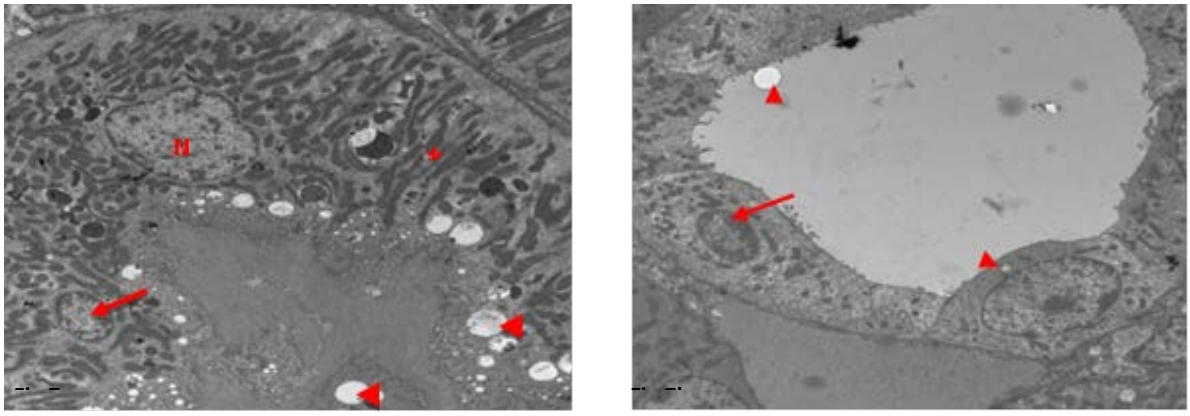

Fig. 7a & 7,b: Electron micrograph of kidney rat in paracetamol group showing the proximal tubular cells with pyknotic nuclei (arrow) and the others appeared with irregularly-shaped nuclei with loose chromatin materials (N), irregular mitochondria, lysosomes containing dense material (*), pinocytotic vesicles (head arrow)(Fig. 7a) (X1500). The distal tubular cells with pyknotic nuclei (arrow) and the other with swollen nuclei (N), irregular mitochondria (M) and pinocytotic vesicles (head arrow). The microvilli irregular (mv) and the lumen appeared wider (L) (Fig.7b) (X 2000).

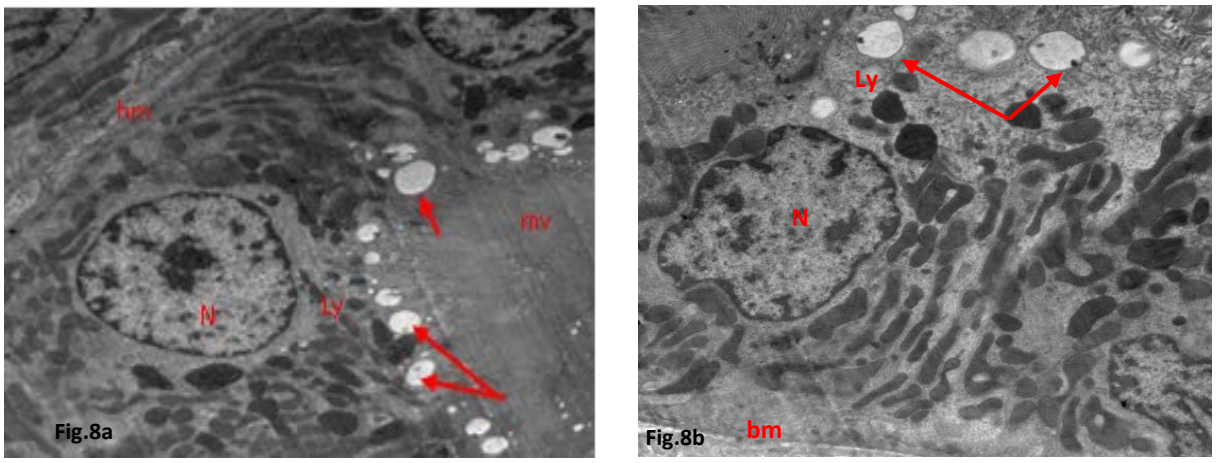

Fig 8a & 8b: Electron micrograph of kidney rat in EVOO and paracetamol group showing the proximal tubular cells with nuclei and regular chromatin materials (N), regular mitochondria, lysosomes (Ly), basement membrane (bm)and microvilli (mv). The cytoplasm appeared with pinocytotic vesicles (arrow)(Fig 8b). Some of the distal tubular cells with irregular nuclei (N), regular mitochondria and basement membrane (bm). Some pinocytotic vesicles appeared (arrow) (Fig 8b). (X2000).

Biochemical results:

Table 1 shows that treatment with paracetamol resulted in a significant ($p<0.001$) increase in the activity of plasma uric acid, urea and creatinine levels after 15 days of treatment compared to the control group. On the other hand, animals treated with paracetamol and EVOO had a significant decrease ($p<0.001$) in uric acid, urea and creatinine levels activities after 15 days of treatment when compared to the paracetamol treated group.

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Table (1): Effect of administration of paracetamol and/or olive oil on urea, uric acid and creatinine in male Wistar rats.

	Contol	**Paracetamol**	**Olive oil**	**Paracetamol + Olive oil**	**P**
Urea (mg/d)	$22.60^{a} \pm 2.29$	$53.88^{b} \pm 5.71$	$24.20^{a} \pm 2.52$	$40.13^{c} \pm 1.26$	$<0.001^{*}$
Creatinine (mg/dl)	$0.34^{a} \pm 0.05$	$0.99^{b} \pm 0.14$	$0.42^{a} \pm 0.07$	$0.63^{a} \pm 0.07$	$<0.001^{*}$
Uric acid (mg/dl)	$2.28^{a} \pm 0.25$	$5.63^{b} \pm 0.49$	$2.46^{a} \pm 0.34$	$3.65^{c} \pm 0.16$	$<0.001^{*}$

Data represented as mean ± SE.

Different subscripts are significant

p: p value for F test (ANOVA) and significant between groups using Post Hoc Test (LSD)

*: Statistically significant at $p \leq 0.05$

4 Discussion

The chronic used or overdose of paracetamol may induce renal toxicity and acute renal failure (Fored *et al*. 2001). The histological and ultrastructure results obtained in present study showed that paracetamol overdose cause damage in renal tubular and nephrotoxicity. These results agreement with several studies indicated that the overdose of paracetamol can cause nephrotoxicity, renal tubular damage and renal failure (Ucheya 2006; Canayakin 2016; Dokumacioglu 2017). Also, the present study showed that paracetamol overdose increases the activity of plasma uric acid, urea and creatinine levels after 15 days of treatment compared to the control group. The current results are agreed with the results obtained by Mathur *et al.* (2016). They showed that the proximal tubular damage by using paracetamol overdose leading to apoptotic cell death through decrease in Nuclear factor erythroid 2-related factor (2Nrf2), and this explain the rise in plasma uric acid, urea and creatinine and the apoptosis detected in the renal tubular cells in present study. Also, Abdul Hamid *et al.* (2012) showed that paracetamol overdose induced nephrotoxicity and oxidative stress in rats. The DNA damage may result of nephrotoxicity, and this damage can lead to cancer development (Salvini *et al.* 2006). The present results indicated to the protective effects of EVOO on kidney in a model of toxicity induced by paracetamol overdose in rats. The histological and ultrastructure results obtained showed improvement in renal tubular cells in paracetamol and EVOO comparing to paracetamol. Also, the EVOO showed in Group 4 significant decrease in levels activities in uric acid, urea and creatinine after 15 days of treatment when compared to the paracetamol. This improvement may possibly be due to EVOO which is have unique chemical structure beneficial to health and protects from damage by free radical oxidation. It consists of highest concentration of polyphenols compared to other types of virgin olive oils (Bawazir 2012). Many studies in human and animal, both in vivo and in vitro demonstrated that olive oil phenolic compounds have

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positive effects on various physiological biomarkers. (Visioli and Gallil, 2002; Carluccio *et al.* 2003). A more investigation demonstrated that intake of phenol rich virgin olive oil decreases oxidative DNA damage by up to 30% compared to a low phenol virgin olive oil (Salvini *et al.* 2006). Support to these findings, in vivo studies have also showed that a diet enriched with olive oil phenolic compounds has a protective effect against DNA damage (Jacomelli *et al.* 2010, Quiles *et al.* 2002). More studies showed the beneficial effect of olive oil phenolic compounds on lipid oxidation (Jacomelli *et al.* 2010). However, in humans the ingestion of olive oil phenolic compounds increases the total plasma antioxidant activity (Bogani *et al.* 2007). An additional study also indicated that after consumption of phenol rich virgin olive oil there was decreased urinary excretion of 8-oxo-deoxygyuanosine (8oxodG) a systemic marker of DNA oxidation (Machowetz *et al.* 2007).

In conclusion, the current data demonstrated that EVOO have a protective action against toxicity damage in renal cells induced by paracetamol overdose and this protective process is probably due to its phenolic compounds and its antioxidant properties.

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