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Hepatoprotective effect of ethanolic extract of *Origanum vulgare* against doxycycline toxicity

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ABSTRACT

The current study evaluates the ameliorative action of *Origanum vulgare* ethanolic extract against the hepatotoxic effects of experimental doxycycline over-dose in rats. Forty male Sprague-Dawley rats with an average weight (160–200 g) were divided into four groups; group (1) the control group, administered distilled water (10 ml/kg b. wt.); group (2) administered doxycycline (39.2 ml/kg b. wt.) in distilled water; group (3) administered O. vulgar ethanolic extract (20 mg/kg b. wt.) +doxycycline (39.2 ml/kg b. wt.) in distilled water and group (4) administered O. vulgar ethanolic extract (60 mg/kg b. wt.) + doxycycline (39.2 ml/kg b. wt.) in distilled water. All rats were administrated once per day for 4weeks. Doxycycline administrated rats showed alteration in histological structure of the liver as vacuolization, necrosis, inflammation, and sinusoidal dilatations. Also increased Kupffer cells population and congestion and dilatation of central vain with fibrin thrombi filling their lumen were shown. Ultra-structurally destructed cell membrane of hepatocyte, Pyknotic and karyolitic nuclei, losses in some cytoplasmic organelles, swollen mitochondria, fragmentation of rER and discarded lysosomes were observed. All of those lesions were improved by ethanolic extract of *Origanum vulgare*.

1. INTRODUCTION

Laboratory rats are more widely used especially in toxicology, understanding the pathophysiology of diseases, cure and improvement of human and animal diseases (Nadzirah and Oduola , 2014).

Liver is the biggest gland in the body and has many functions (Benjamin et.al., 2018). The primary function of the liver is detoxification of absorbed substances via the digestive system before their distribution into the blood stream (Moawad et.al., 2014).

Liver injury induced by antibiotics shows degeneration and necrosis of the hepatocytes and accumulation of some exudates filled with inflammatory cells inside the portal triad area and also characterized by cholestasis (Rajesh, 2016).

Doxycycline is a widely used antibiotic. It is considered as bacteriostatic tetracycline and facilitates growth (Fiori et al., 2004). Tetracyclines are associated with acute symptomatic hepatitis resulting in hospitalization (Jeffrey et al., 1993). The hepatotoxicity rate of doxycycline or tetracycline was 3% of the treated people (Heaton et al.2007). Doxycycline overdose causes fatty degeneration or congestion in the liver of calves (Brihoum et al., 2010) and induces dilatation of central vein, degenerative changes in the portal tissue cells and inflammatory response in the triad area of rats' liver (Zeinab et al., 2018).

Origanum vulgare (O. vulgare) (Oregano) is part of the Lamiaceae family (Rechinger and Druk 1982) which is distributed throughout Asia, Europe and North America (Aslam Khan et al., 2011). O. vulgare is used in traditional medicine as diuretic, stimulant, antimicrobial and antioxidant (Duka 2002; Gustavo et al., 2007; Nayan and Namrata, 2016). Also, it has anti-inflammatory and hepatoprotective activities (Antonia et al., 2007).

Thymol and carvacrol represent the main constituents. The antibacterial and antioxidant characters of the Oregano are due to its content of thymol and carvacrol (Adam Figiel et al., 2010). This study aimed to verify the effect of O. vulgare ethanolic extract against toxic effect of doxycycline on rats' liver using histological examination.

2. MATERIAL AND METHODS

2.1. Chemicals:

2.1. Doxycycline obtained from ATCO Pharma For Pharmaceutical Industries, Egypt in the form of yellow powder at 100% concentration.

2.1.2. Dried aerial parts of O. vulgare powder were purchased from Carrefour Egypt. 300 gm samples of the dried plant powder were extracted with 3000 ml of ethanol (75%) for 72 h. After evaporating the solvent under vacuum at a temperature below 50 °C, 35 gm of dried powder was obtained.

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2.2. Experimental design:
Forty male Sprague-Dawley rats with an average weight (160–200 gm) obtained from animal house of National Organization for Drug Control and Research (NODCAR), Egypt. Rats were housed in stainless steel wire mesh cages with bedding of ground saw dust under the artificial light/dark cycle 12:12. Pelleted food and water were available ad libitum. The animals were kept in the animal facility of the (NODCAR), at a controlled temperature (23–24 °C), humidity (60 ± 5%) and ventilation (10–15 air changes/h).The animals were divided into four groups: the control group, administered distilled water (10 ml/kg b. wt.); group (2) administered doxycycline (39.2 ml/kg b. wt.) in distilled water according to Fatima et al. (2013); group (3) administered *O. vulgare* ethanolic extract (20 mg/kg b. wt.) + doxycycline (39.2 ml/kg b. wt.) in distilled water; and group (4) administered *O. vulgare* ethanolic extract (60 mg/kg b. wt.) + doxycycline (39.2 ml/kg b. wt.) in distilled water. All rats were administrated once per day for 4 weeks then animals were sacrificed. O. vulgare dose according to Nema and Omimah (2013).

2.3. Histological examination:
Livers were removed after the rats were sacrificed in humanity method. Small pieces of the hepatic tissues were taken and processed for histopathological sections according to (Bancroft et al., 1996).

2.4. Transmission electron microscope:
Very small hepatic tissue specimens were collected for TEM, the desired techniques were done according to Bozzola and Russell (1999). The pictures were photographed at Al Azhar University, Cairo, Egypt (electron microscope unit).

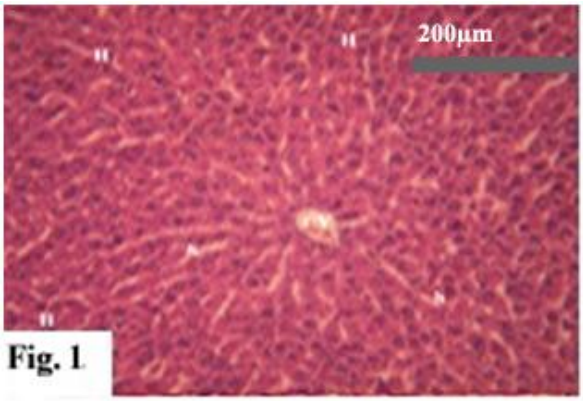
3. RESULTS

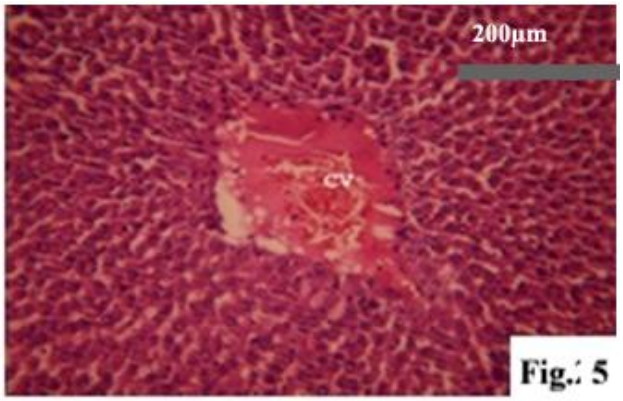
Light microscope
Histological examination of control hepatic tissues in male Sprague-Dawley rat revealed that the liver is built up of columns of hepatocytes which radially arranged from the central vein to the margin of the portal lobule. Hepatic cells contained homogenous cytoplasm and a large nucleus. The blood sinusoids were located between the hepatic cords and taken the same radial direction as the hepatocytes (Fig. 1).The portal area was region of connective tissue containing the portal veins, the hepatic artery, bile duct and lymph vessels. This area called triad area (Fig. 2).
Histological examination of hepatic tissues in male Sprague-Dawley rat treated with doxycycline revealed variable degrees of alterations in response to the time of the treatment and compared to control group. It showed light foamy cytoplasm, enlarged cell , more condensed nuclear chromatin, necrotic hepatocyte with loss of cellular detailes, numerous kupffer cells and enlargedsinusoids (Fig. 3). Additionally, leukocytic infiltration, pyknotic nuclei (Fig. 4) and congested central veins with fibrin thrombi filling their dilated lumen were seen (Fig. 5). Also, portal vein accompanied with periportal and periductal fibrosis with newly formed bile ductules (Fig. 6).
Histological examination of hepatic tissues in male Sprague-Dawley rat treated with doxycycline and O. vulgare at dose (20 Mg/Kg B. WT.) showed lymphocytic infiltration between hepatocytes , and periportal lymphocytic infiltration (Figs.7 & 8) .
Histological examination of hepatic tissues in male Sprague-Dawley rat treated with doxycycline and *O. vulgare* at dose (60 mg/Kg b. wt.) revealed nearly normal hepatic architectures with intact hepatocytes and central vein with mild congestion in sinusoids and few lymphocytic infiltration (Fig. 9).

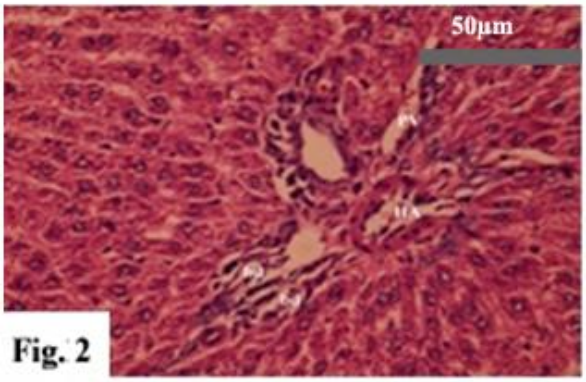
Transmission electron Microscopy
Electron micrograph of liver in control rats showed normal hepatic cells of round nuclei with both dense and light euchromatin and two nucleoli. The granular cytoplasm appeared containing different forms of mitochondria (rounded and elongated), Golgi apparatus, rER, lysosomes, glycogen deposits and lipid droplet (figs.10, 11).
Electron micrograph of liver in rat treated with doxycycline showed destructed cell membrane of hepatocyte, Pyknotic and karyolitic nuclei, lack of numbers of cellular organelles, swollen mitochondria, cytoplasmic vacuoles and fragmentation of rER, and discarded lysosomes (figs. 12, 13, 14).
Electron micrograph of hepatic tissues in rat treated with doxycycline and O. vulgare at dose (20 mg/Kg b. wt.), revealed numerous hepatocytes with eccentric nuclei. The nucleolus appeared in non-central position (Fig. 15)
The fine structure picture in the rat hepatocyte administered doxycycline and O. vulgare at dose (60 mg/Kg b. wt.) displayed hepatocyte with rounded nucleus containing one or two nucleolus. The nucleus was surrounded by intact nuclear envelop. The chromatin consisted of dense clumping heterochromatin and lightly stained euchromatin. The cytoplasm appeared granular containing rounded and elongated mitochondria, rER, glycogen particle and Golgi apparatus (Figs. 16 & 17).

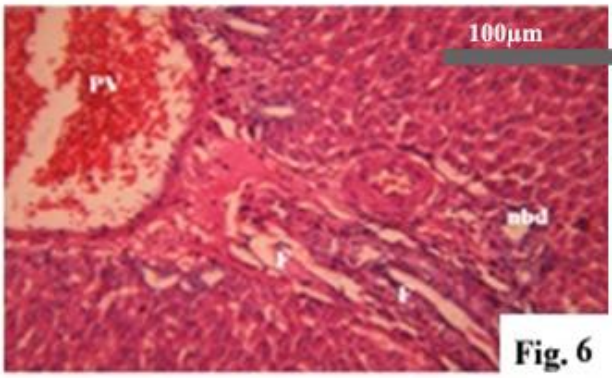
4. DISCUSSION

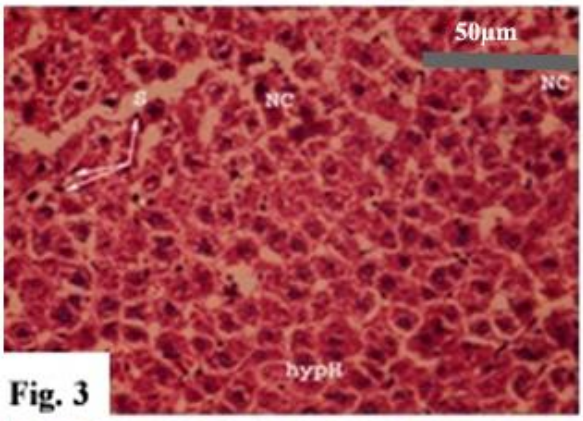
In the present study, histological examination of control liver observed that the hepatic lobule is built up of columns of hepatocytes which radially arranged in the form of cords. The hepatocytes were polygonal in shape; each cell possesses a distinct limiting membrane. Nuclei of hepatocytes were spherical or ovoid with a regular surface. Occasionally, binucleated cells were present. The columns of hepatocytes are separated by blood sinusoids which take the same radial direction as the hepatocytes. Similar observations were recorded by El- Naggar (1989) and Shabana et. al. (2012). Blood sinusoids are scattered between the hepatocytes, so that, their walls are formed by the hepatocytes that was similar to results of Boshra *et.al.* (2008). The portal area consisted of the portal veins, hepatic artery, and bile duct and along with lymph vessels. Similar observation is showed by Janie *et.al.* (2009). Concerning the ultrastructure results of control rat liver, nucleus with two normal nucleoli with euchromatic character, mitochondria, Golgi apparatus, rER, lysosome and glycogen granules were observed by Abeer, *et. al.* (2007) and Sakr *et. al.* (2015).The current data showed that doxycycline induced rats' hepatotoxicity and numerous hazards. As mentioned by Zeinab *et al.* (2018), who noticed that doxycycline induces dilatation of central vein associated with degenerative changes in the neighboring hepatocytes with inflammatory cells infiltration in the portal area in rats. The same observed in mice treated with tetracycline by Angelico *et.al.* (2016).

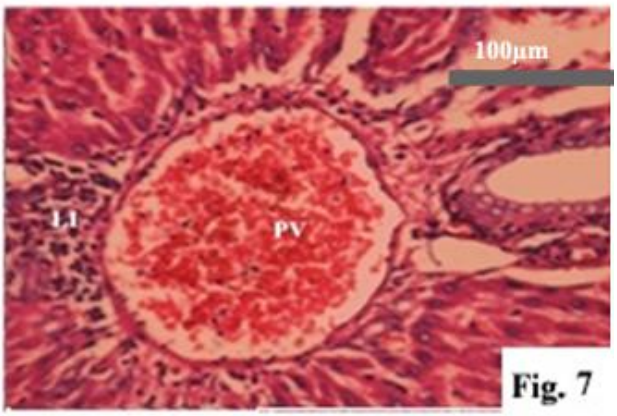


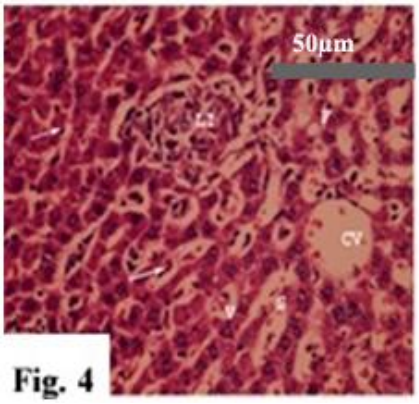


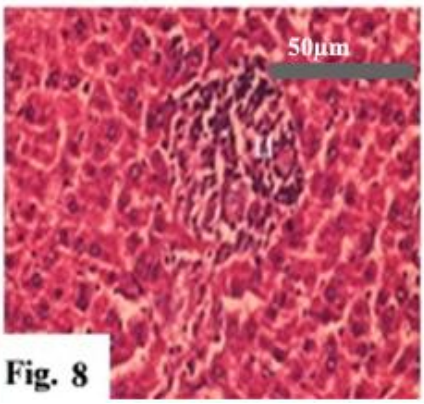












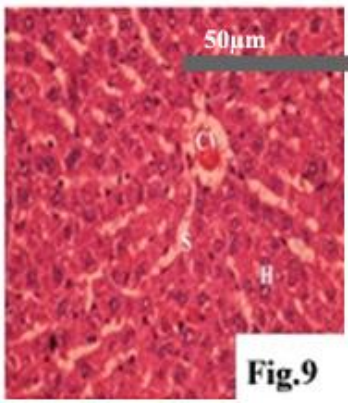


Fig.1. A photomicrograph of rat hepatic tissues in control group showing normal liver architectures with intact liver cell in form of cords (H) radiated from central vein (CV) with normal sinusoids(S). H&E Scale bar = 200µm. Fig.2. A photomicrograph of rat hepatic tissues control group showing normal portal triads containing normal bile duct (bd), hepatic artery (HA) and portal vein (PV). H&E Scale bar = 50µm. Fig.3. A Photomicrograph for rat hepatic tissues in group 2, displaying hypertrophied hepatocytes (hyp H). light, foamy cytoplasm and filled with vacuoles (v) the cell size enlarged and nuclear chromatin is more condensed (NC),necrotic hepatocyte with loss of cellular detailes (N), numerous kupffer cells (arrows) and the sinusoids enlarged and oozing blood (s). H&E Scale bar = 50µm. Fig.4. A Photomicrograph for rat hepatic tissues in group 2, displaying mass of leukocytic infiltration (Li) with hepatocellular necrosis,fatty infiltration (F), cytoplasmic vaculation of hepatocytes with pyknotic nuclei (V), the sinusoids filled with blood(S) and appeared with activated Kupffer cells (arrows). H&E Scale bar = 50µm. Fig.5. A Photomicrograph for rat hepatic tissues in group 2, displaying congestion and dilatation of central vain (CV) with fibrin thrombi filling their lumen. H&E Scale bar = 200µm. Fig.6. A Photomicrograph for rat hepatic tissues in group 2, displaying congestion and dilatation of portal vein (PV) accompanied with periportal and periductal fibrosis (F) with newly formed bile ductules (nbd). H&E Scale bar = 100µm. Fig.7. A Photomicrograph for rat hepatic tissues in group 3, displaying periportal lymphocytic infiltration (LI) .H&E. Scale bar = 100µm. Fig.8. A Photomicrograph for rat hepatic tissues in group 3, displaying lymphocytic infiltration (LI) between hepatocytes.H&E Scale bar = 50µm. Fig.9. A Photomicrograph for rat hepatic tissues in group 4, displaying normal hepatic architectures with intact hepatocytes (H) radiated from central vein (CV), mild congestion in sinusoids(S) and low lymphocytic infiltration. H&E Scale bar = 50µm.

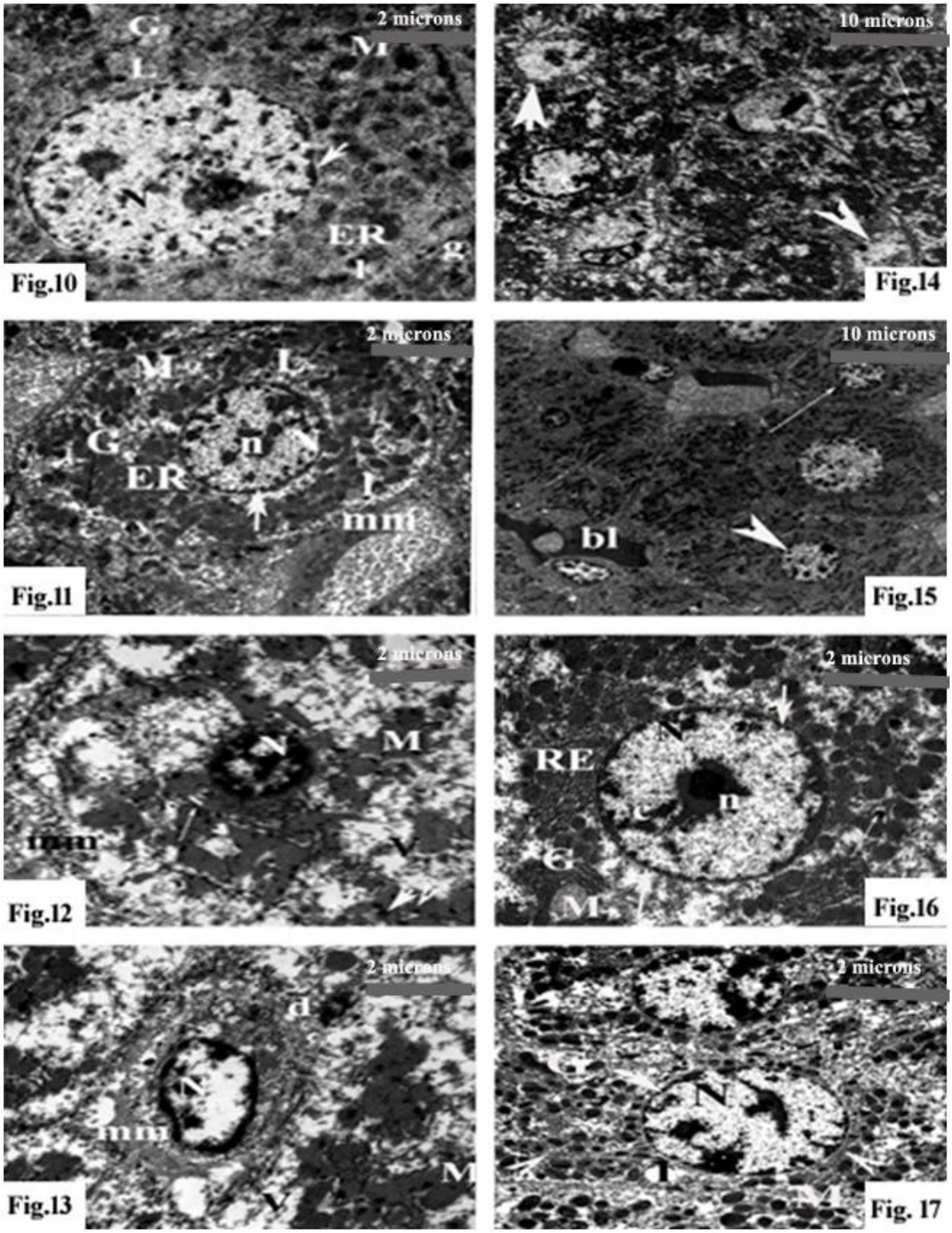


Fig. 10. Electron micrograph for a control liver of rat showing, normal hepatic cell composed of round nucleus (N) contain dense heterochromatin and light euchromatin with two normal nucleoli (n), and surrounded by nucleus envelope (arrow).The cytoplasm appeared granular containing rounded and elongated mitochondria (M), Golgi apparatus (G), rough endoplasmic reticulum (ER), primary lysosomes (L), glycogen deposits (g) and lipid droplet (l). Scale bar = 2 microns. Fig.11. Electron micrograph of hepatocyte of a control rat displaying intact cell membrane (MM), normal round nucleus (N) containing two nucleoli (n) surrounded by nuclear envelope(arrow). Many mitochondria (M), rough endoplasmic reticulum (ER), Golgi apparatus (G), many lysosomes (L) and lipid globules (l).Scale bar =2 microns**.** Fig.12. Electron micrograph for rat hepatic tissues in group 2, displaying destructed cell membrane (mm) of hepatocyte, Pyknotic nucleus (N), lack of numbers of cellular organelles, swollen mitochondria (M), cytoplasmic vacuoles (V) and fragmentation of rough endoplasmic reticulum (long arrow), discarded lysosomes (short arrow). Scale bar = 2 microns**.** Fig.13. Electron micrograph for rat hepatic tissues in group 2, displaying destructed cell membrane (mm) of hepatocyte, karyolitic nucleus (N), lack of numbers of cellular organelles, swollen mitochondria (M), cytoplasmic vacuoles (V) and fragmentation of rough endoplasmic reticulum (arrow), discarded lysosomes (L) and Large lipid droplet (l).Scale bar = 2microns**.** Fig.14. Electron micrograph for rat hepatic tissues in group 2, displaying, Pyknotic (long arrow) and karyolitic nucleus (short arrow).Scale bar = 10 microns**.** Fig.15. Electron micrograph for rat hepatic tissues in group 3, displaying, and different hepatocytes have circular nuclei, lost the central situation in most cells (long arrow). The nucleolus appears in non-central position (short arrow).Invasion of blood (bl). Scale bar = 10 microns**.** Fig.16. Electron micrograph for rat hepatic tissues in group 4, displaying, and each hepatocyte have nuclei which are round in appearance (N) one to two nucleoli was prominent (n), nuclear envelop (short arrow).light and dense chromatin (c), granular cytoplasm with different shape mitochondria rounded and elongated (M), rough ER (RE), glycogen granules (long arrow) and Golgi complex (G). Scale bar = 2 microns**.** Fig.17. Electron micrograph for rat hepatic tissues in group 4, displaying each hepatocyte characterized by round shape nuclei (N) distinct 2 nucleolus (two long arrows), nuclear envelop (short arrow). granular cytoplasm with different shape mitochondria rounded and elongated (M), Golgi apparatus (G), rough endoplasmic reticulum (ER) and lipid particles (l). Scale bar = 2 microns**.**

While Ana Lourdes *et.al.* (2003) showed that tetracycline causes histopathological changes such as dilatation in the blood sinusoids, vacuolization and necrosis in the portal hepatic cells and inflamed hematopoietic cells in the livers of newborns rats. Also, Asha *et.al.* (2007) noticed that tetracycline induced steatosis or fat accumulation in liver of rats. Tetracycline and oxytetracycline administrations showed hypertrophied hepatocytes, cytoplasmic vacuolation, abundance of Kupffer cells and massive lymphatic aggregation inside the hepatic tissue and hepatocellular necrosis observed by Shabana *et. al*. (2012); Samah *et al.* (2018). Additionally, Samira *et.al.* (2012) reported that liver of embryos at 20th days in tetracycline treated group showed congestions of portal vein and sinusoids along with acute inflammation around the portal vein with appearance of giant cells, fatty infiltration, and increased number of Kupffer's cells. The fine results in the current study revealed that doxycycline induced many histopathological hazards in liver tissue, similar to Samira, *et. al*. (2012), who reported few numbers of hepatocytes organelles, mitochondrial swelling and fragmentation of rER in tetracycline treated rat embryos. The nuclei showed pyknosis or karyolysis in some hepatocytes also, irregular nuclear membrane, large fat drops were noted. The current study spot the light on the effective effect of ethanolic extract of O. vulgare leaves against doxycycline induced hepatotoxicity that was similar to previous results obtained by Sikander *et. al.* (2013) on carbon tetrachloride (CCl4) hepatotoxicity Maximum protection was found in CCl4 and O. vulgare (150 mg/kg body weight). The ultrastructure revealed the hepatoprotective effect of O. vulgare against doxycycline induced hepatotoxicity in agreement with results of Sakr *et. al.* (2015) when used rosemary against cadmium chloride (CdCl2) induced hepatotoxicity in rats.

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