Possible Protective Role of Ginger Extract on Diclofenac Induced Hepatotoxicity in Adult Male Albino Rats (Histological and ultrastructural studies)

Eman A. El-Kordy* and Madiha M. Makhlouf**

Histology Department, Faculty of Medicine, Tanta* and Assiut** University, Egypt.
emanalimah@yahoo.com

Abstract: Introduction: Diclofenac is one of the most frequently prescribed non-steroidal anti-inflammatory drugs (NSAIDs) and have been reported to cause multiple organs damage. Ginger extract has been used as an antioxidant and preventive agent against a number of diseases. **Aim of the Work:** This work aimed to study the possible histological and ultrastructural changes of liver induced by diclofenac treatment and to evaluate the possible protective effect of ginger extract. **Materials and Methods:** Forty adult male albino rats were divided into 4 main groups. Group I: served as control. Group II: received ginger extract orally in a dose of 250 mg/kg /day. Group III: animals received diclofenac intramuscularly at a dose 150 mg/kg /day. Groups IV: animals received ginger extract then diclofenac after two hours in the previous doses. The treatments were given for rats for 7 days, then, rats were sacrificed by ether anesthesia and specimens from liver were taken for light and electron microscopic examination. **Results:** Light microscopic examination of the liver treated with diclofenac revealed disorganized hepatic architecture. The affected lobules appeared with dilated congested central veins and blood sinusoids, vacuolated hepatocytes with dark nuclei, cellular infilteration and fibrosis. Ultrastructurally, hepatocytes showed disintegration of cellular organelles, destructed mitochondria and pyknotic nuclei. Coadministration of diclofenac with ginger extract showed a slight improvement in some hepatocytes that looked normal in both LM and EM examination but still others were markedly affected and showing signs of degeneration**. Conclusion:** Results obtained in this study demonstrated that high doses of diclofenac induced histological and ultrastructural changes in the liver due to oxidative stress and the use of ginger extract had partially improved the toxic effect of diclofenac.
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Keywords: Diclofenac, hepatotoxicity and ginger extract.

1.Introduction:

The liver is one of the major organs in the body responsible for the removal of toxins, so it can suffer extensive damage before malfunction becomes pronounced. Non-Steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed therapeutic agents, as they have analgesic, antipyretic and anti-inflammatory actions. They are commonly used by more than 30% in developed countries [1&2].

Diclofenac sodium is a phenylacetic acid derivative and is a widely used NSAID for the treatment of a variety of inflammatory conditions such as rheumatoid arthritis, osteoarthritis and acute muscle aches [3&4]. Also, it may be used for the treatment of fetal developmental problems and the prevention of premature birth [5].

Normal therapeutic dose of diclofenac is safe, effective and widely used but extensive use of this agent, leading to toxicity and untoward effects many times especially when therapy of pain, inflammation and fever involves use of higher dose for longer period [6].

NSAIDs especially diclofenac are among the most common drugs associated with drug-induced liver injury with an estimated incidence between 3 and 23 per 100,000 patient [7&8]. It has been reported that oxidative stress through the generation of reactive oxygen species, decreases antioxidant defense system are major alterations in the diclofenac hepatotoxicity [9].

Medicinal plants have continued providing valuable therapeutic agents, both in modern and in traditional medicine [10]. At present, it is estimated that about 80% of the world population relies on botanicals preparation as medicine to meet their health needs [11]. Ginger is example of botanicals which is gaining popularity amongst modern physicians and used for the treatment of rheumatism, gingivitis, toothache, asthma, nausea, vomiting and diabetes. It is considered a safe herbal medicine with only few and insignificant adverse/side effects [12]. Extracts of the ginger contain polyphenol compounds (6-gingerol and shogaols) exhibit antiplatelet, anti-inflammatory, anti-oxidant and anti-tumour proprieties [13&14].

Based on these, this work aimed to study the possible histological and ultrastructural changes of liver induced by diclofenac treatment and to evaluate the possible protective effect of ginger extract in ameliorating these changes.

2. Materials And Methods

Chemicals

Diclofenac sodium (declophen) ampoules (75 mg/3 ml) were obtained from Pharco Pharmaceutical Co. Alexandria, Egypt.

Ginger extract

Fresh ginger (Zingiber Officinale) rhizome (purchased from local market) was washed several times with water. Aqueous extract of rhizome of ginger was prepared by the method as previously described [15]. In brief, the rhizome was dried and crushed into fine powder using an electrical blender. The powder (125 g) was macerated in 200 mL of distilled water for 12 h at room temperature and were then filtered to obtain the final aqueous extract (concentration: 120 mg/ml) for use in the experiment.

Animals

Healthy forty adult male albino rats (average weight 150-200 gm) were housed in clean properly ventilated cages. They were kept under normal conditions, adequately fed and provided with sufficient fresh water. Ethical approval was obtained from the ethical committee of Tanta Faculty of Medicine.

Experimental groups

The rats were randomly divided into 4 groups, each with 10 rats, as follow:

Group I (control group): received distilled water

Group II (ginger extract group): animals received ginger extract by intragastric tube at a dose of 250 mg/kg /day for 7 days [16].

Group III (diclofenac treated group): animals received diclofenac intramuscularly at a dose 150 mg/kg /day for 7 days [17].

Group IV (diclofenac / ginger extract treated group):animals received ginger extract then diclofenac after two hours in the previous doses and methods.

At the end of the experiment, the rats were sacrificed by ether anesthesia, specimens from the liver were taken, put in 10% buffered formalin for fixation, processed for examination by light microscopy to gain 5µm sections of paraffin and stained with haematoxylin and eosin (H & E) to verify histological details and Masson's trichrome to demonstrate the collagen fibers [18].

For electron microscopy, small pieces from the liver were fixed in 5% cold gluteraldehye for at least 24 hours then washed in 3-4 changes of cacodylate buffer (pH 7.2) for 20 minutes in each change and post fixed in cold osmium tetroxide for 2 hours. The specimens were washed in four changes of cacodylate buffer for 20 minutes for each. Dehydration was done by using ascending grades of alcohol (30, 50, 70%) each for 2 hours and then 90%, 100% two changes 30 minute each. Embedding was done in Epon 812 using gelatin capsules for polymerization. The embedded samples were kept in incubator at 35°C for one day, at 45°C for another day and for three days at 60°C. Then semithin sections (0.5-1 microns) were prepared by using LKB ultra microtome. The sections were stained with toluidine blue, examined with light microscope and photographed. Ultrathin sections (50-80 nm) from selected areas of the trimmed blocks were made and collected on copper grids. The ultrathin sections were contrasted with uranyl acetate for 10 minutes, lead citrate for 5 minutes [19]. Finally, the sections were examined and photographed by transmission electron microscopy (Jeol EM- operated at 80 KV) in Tanta University Electron Microscopy Unit.

3. Results

1- Control group (group I) and ginger extract group (group II):

Examination of H & E stained sections of liver of control and ginger extract treated rats showed a normal hepatic architecture, in which the liver of rat was divided into ill-defined classic hepatic lobules formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The hepatocytes were polyhedral in shape with finely granular acidophilic cytoplasm and lightly stained vesicular nuclei. At the periphery of the lobules, branches of portal vein, hepatic artery and bile duct were located in the portal tracts. The hepatic sinusoids were seen as narrow spaces in between the hepatic cords (Figs.1&2). Using semithin sections stained with toluidine blue revealed that large polyhedral hepatocytes with granular cytoplasm and vesicular round central nuclei with prominent nucleolei as well as blood sinusoids lined with Kupffer cells (Fig. 3). Regarding the Masson's trichrome stain, few collagen fibers was observed around the central veins and the portal areas (Fig. 4).

Ultrastructurally, these groups showed normal hepatocytes with nearly rounded euchromatic nuclei with regular structural organization. The cytoplasm appeared crowded with organelles, particularly mitochondria, cisternae of rough and smooth endoplasmic reticulum, in addition to characteristic rosette-shaped α-glycogen granules (Figs. 5&6).

2- Diclofenac treated group (group III):

Treatment with diclofenac led to obvious histological changes in the structure of the liver. Sections stained by H & E showed a disorganized hepatic architecture characterized by focal affection of hepatic lobules. In the affected lobules, the central veins as well as hepatic sinusoids exhibited remarkable dilatation and congestion. The hepatocytes appeared disorganized with cytoplasmic vacuolation and others appeared swollen with deeply

stained acidophilic cytoplasm and dark nuclei (Figs. 7&8). In addition, enlarged portal areas with periportal mononuclear cellular infiltrate, dilated branches of portal vein and proliferated bile ductules with newly formed one were observed (Fig. 9).

Examination of semithin sections stained with toluidine blue revealed ill defined boundaries between the hepatocytes, some of them appeared larger in size with pale vacuolated cytoplasm. Their nuclei showed signs of karyolysis. Few cells exhibited small deeply stained pyknotic nuclei. In addition, dissolution of hepatocytes was observed in some hepatic lobules. The hepatic sinusoids appeared dilated with hypertrophic Kupffer cells (Figs. 10&11).

Collagen fibers, were distinctly accumulated around the central veins as well as at portal tracts (Fig. 12).

Ultrastructurally, treatment with diclofenac showed remarkable changes in the hepatocytes. Some of them appeared with heterochromatic shrunken nuclei and others had irregular nuclear membrane with widenining of perinuclear space (Figs.13-16). Polymorphic mitochondria were observed with destructed cristae and electron-dense granular matrix. Some of them appeared irregular with rupture of their membranes (Figs.13-15). The cytoplasm showed proliferated smooth endoplasmic reticulum (SER) (Fig.14), marked depletion of glycogen granules (Figs.14&15), disrupted rough endoplasmic reticulum (rER) (Fig.15) and multiple lysosomes (Fig.16). Marked accumulation of lipid droplets was also seen (Figs.15-17). Vacuoles in dilated intercellular spaces between the hepatocytes and collagen-like fibrous septa deposition were observed (Fig. 17). Kupffer cells appeared with multiple lysosomes and vacuoles with irregular pyknotic nuclei (Fig. 18).

3- Diclofenac / ginger extract treated group (group IV):

Light microscope examination of the liver of rats of this group showed partial improvement in the histological structure of its cells. Most hepatocytes appeared with vesicular nuclei and granular acidophilic cytoplasm more or less similar to the control group while few one still appeared with vacuolated cytoplasm and pyknotic nuclei. Slightly congested dilated central veins and hepatic sinusoids were seen (Figs. 19&20). Using semithin sections stained with toluidine blue revealed that most of the hepatocytes exhibited vesicular nuclei with granular cytoplasm. However, few hepatocytes still affected and appeared with deeply stained cytoplasm and dense nuclei (Fig. 21). Few collagen fibers were seen around the central vein and at portal areas (Fig. 22).

Ultrastructurally, most of the hepatocytes showed good improvement. They had euchromatic nuclei with prominent nucleoli and their cytoplasm contained mitochondria with intact cristae and parallel stacks of rough endoplasmic reticulum as well as abundant amount of glycogen particles (Fig. 23). However, few hepatocytes showed slight cytoplasmic vacuolation, few lipid droplets and electron dense nuclei (Fig.24).

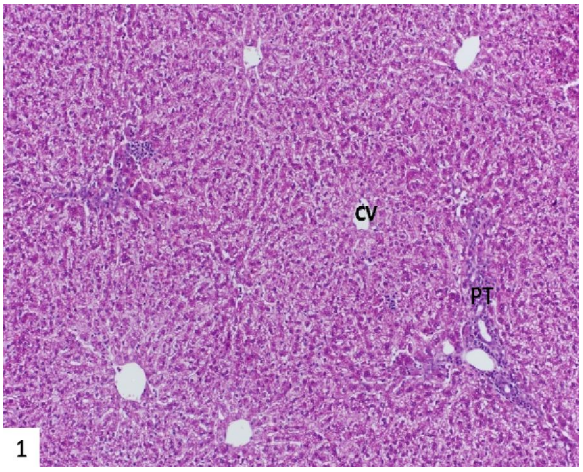

Fig. (1): A section in the liver of the control group stained with H & E showing cords of hepatocytes radiating from the central vein (CV) and a portal tract (PT) with its contents. (x 100)

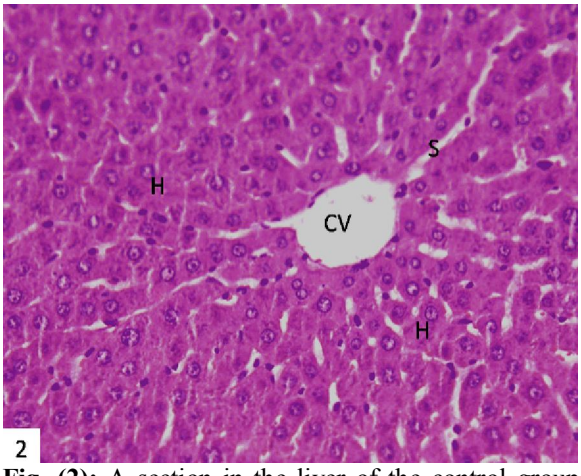

Fig. (2): A section in the liver of the control group stained with H & E showing the hepatocytes with acidophilic granules and vesicular nuclei (H) as well as the blood sinusoids (S). Note, the central vein (CV). (x 400)

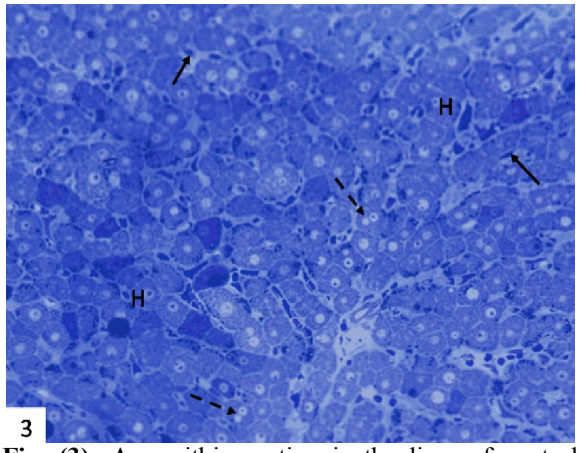

Fig. (3): A semithin section in the liver of control group stained with toluidine blue showing groups of polyhedral hepatocytes had vesicular nuclei with prominent nucleulei (H), some of them are binucleated (dotted arrows) and Kupffer cells lining blood sinusoids (arrows). (x 400)

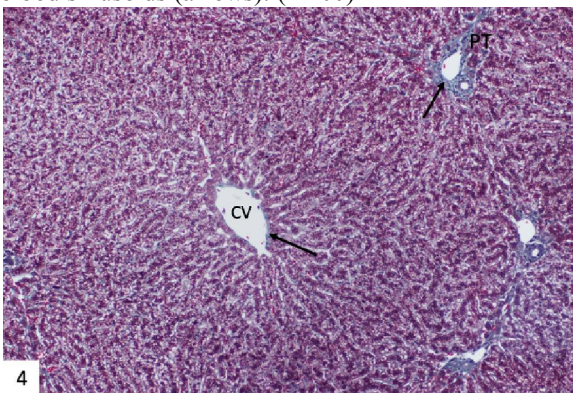

Fig. (4): A section in the liver of the control group stained with Masson's trichrome showing few collagen fibers (arrows) around the central vein (CV) and the portal tract (PT).(x 200)

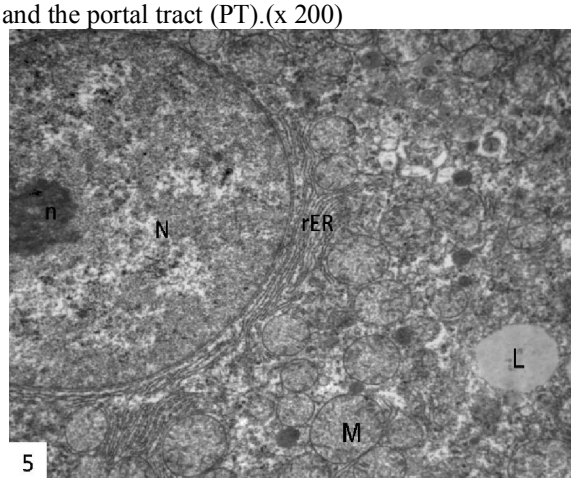

Fig. (5): An electron micrograph of a control hepatocyte showing rounded euchromatic nucleus (N) with prominent nucleolus (n), mitochondria (M), cisternae of rER and lipid inclusion (L). (x 7200)

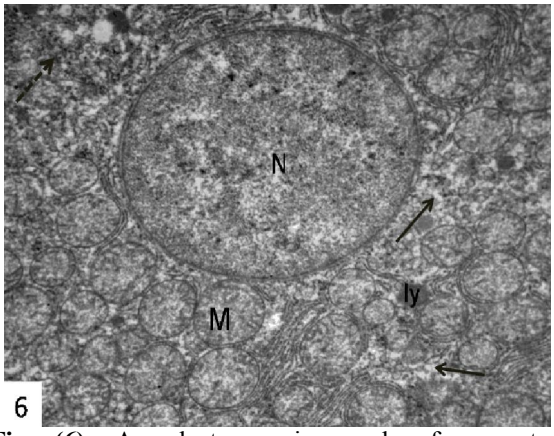

Fig. (6): An electron micrograph of a control hepatocytes showing numerous mitochondria (M), smooth endoplasmic reticulum (arrows), rosette-shaped α-glycogen granules (dotted arrow) and lysosomes (ly). Note, rounded euchromatic nucleus (N). (x 7200)

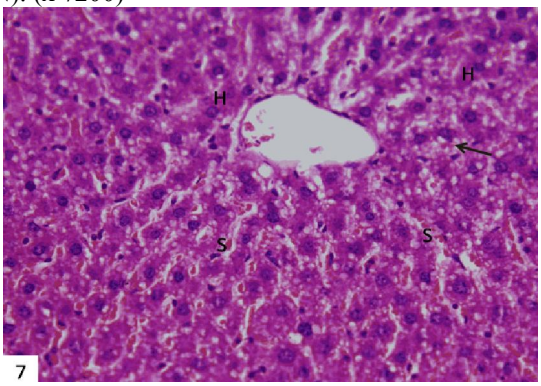

Fig. (7): A section in liver of diclofenac treated group stained with H & E showing swollen hepatocytes with vacuolated cytoplasm and dark nuclei (arrow), others with deeply stained acidophilic cytoplasm (H). Note, congested and dilated blood sinusoids (S). (x 400)

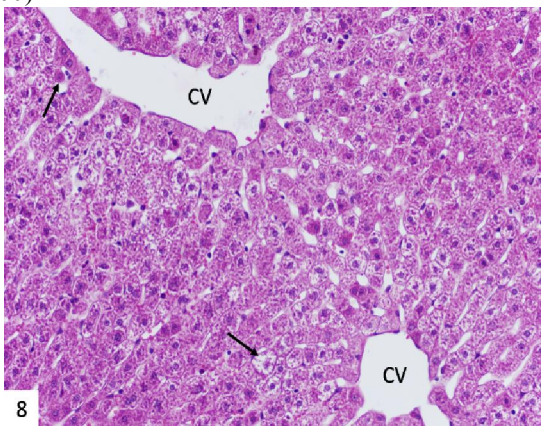

Fig. (8): A section in liver of diclofenac treated group stained with H & E showing swollen vacuolated hepatocytes (arrows) around dilated central vein (CV). (x 200)

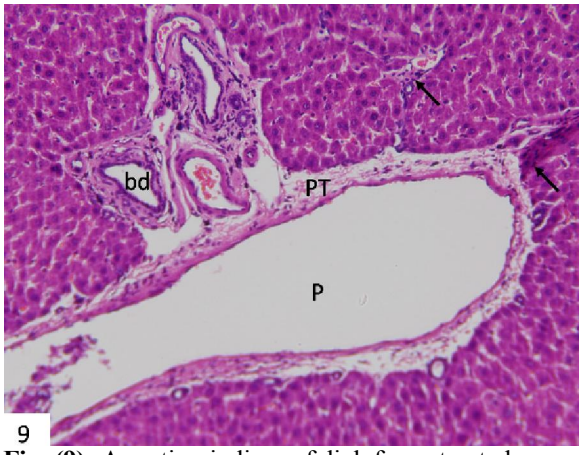

Fig. (9): A section in liver of diclofenac treated group stained with H & E showing enlarged portal tract (PT), dilated portal vein (P), proliferated bile ducts (bd) and cellular infiltration (arrows). (x 200)

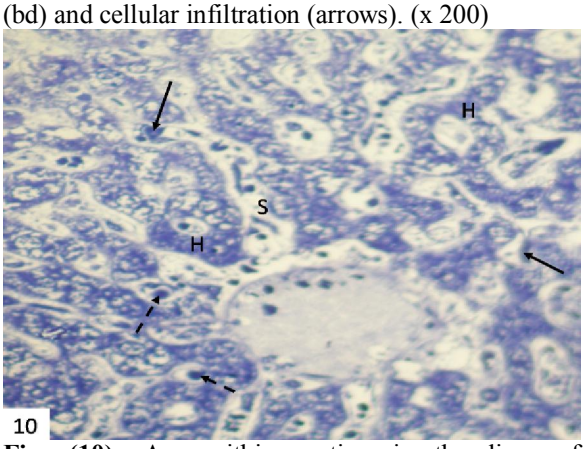

Fig. (10): A semithin section in the liver of diclofenac treated group stained with toluidine blue showing hepatocytes with vacuolated cytoplasm (H), others with deeply stained pyknotic nuclei (dotted arrows). Note, dilated blood sinusoids (S) with hypertrophic Kupffer cells (arrows). (x 1000)

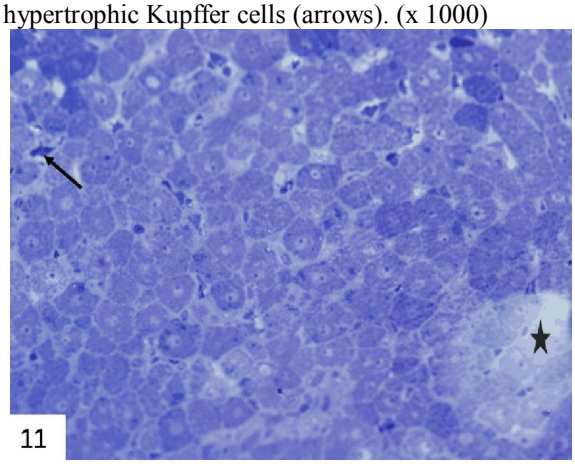

Fig. (11): A semithin section in the liver of diclofenac treated group stained with toluidine blue showing area of hepatocytes dissolution (stars) and hypertrophic Kupffer cells (arrow).(x 400)

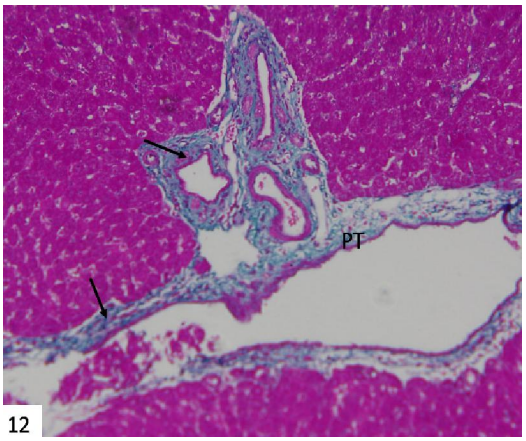

Fig. (12): A section in liver of diclofenac treated group stained with Masson's trichrome showing marked accumulation of collagen fibers (arrows) at portal tract (PT). (x 200)

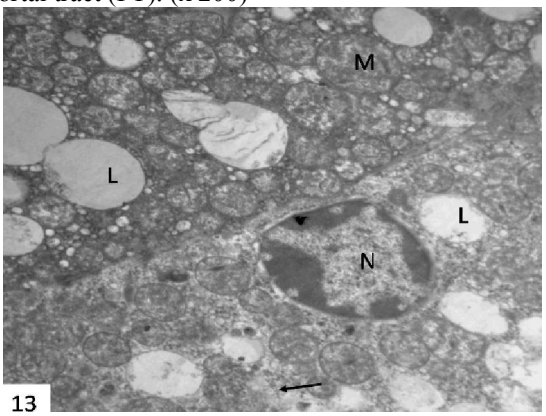

Fig. (13): An electron micrograph of hepatocytes of diclofenac treated group showing polymorphic mitochondria with destructed cristae (M) and rupture membrane (arrow) as well as pyknotic nucleus (N). Note, numerous lipid droplets (L). (x 7200)

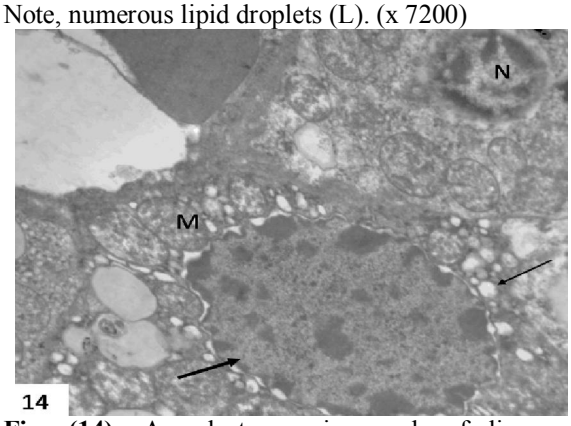

Fig. (14): An electron micrograph of liver of diclofenac treated group showing hepatocytes, one of them with pyknotic nucleus (N) and other with irregular widenining nuclear cisternae (thick arrow). Note,destructed mitochondria (M), proliferated SER (thin arrow) and marked depletion of glycogen granules. (x 7200)

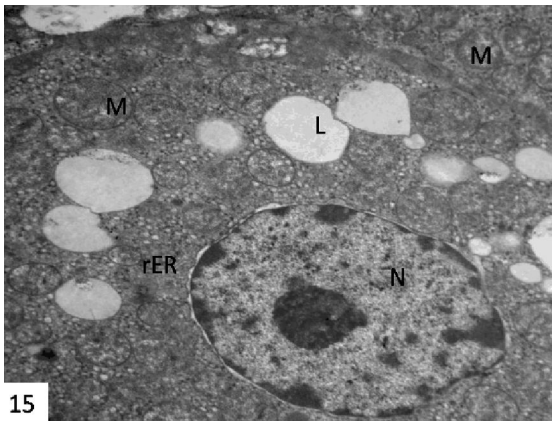

Fig. (15): An electron micrograph of hepatocytes of diclofenac treated group showing destructed mitochondria (M), disrupted rER and marked depletion of glycogen granules. Note, heterochromatic nucleus (N) and multiple lipid droplets (L). (x 7200)

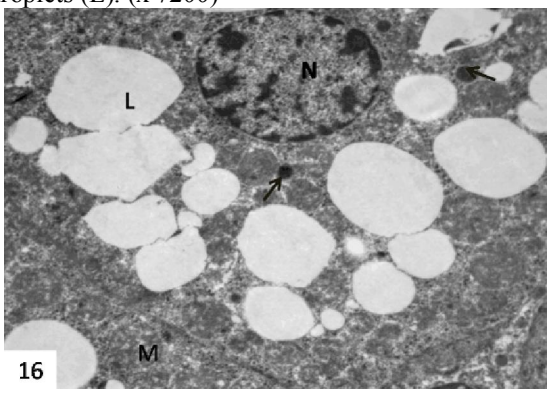

Fig. (16): An electron micrograph of hepatocytes of diclofenac treated group showing multiple lipid droplets (L), multiple lysosomes (arrows) and peripheral heterochromatic condensation of the nucleus (N). Note, destructed mitochondria (M). (x 4800)

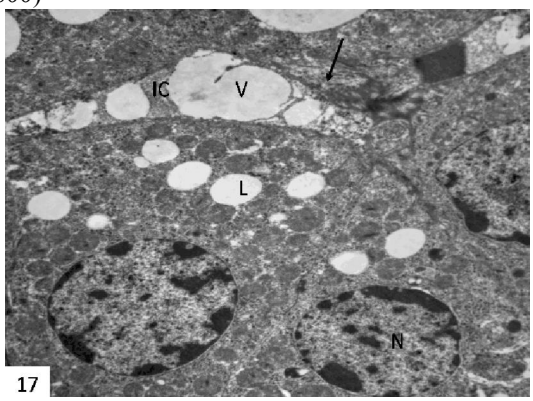

Fig. (17): An electron micrograph of hepatocytes of diclofenac treated group showing multiple vacuoles (V) in dilated intercellular spaces (IC) and collagen fibers deposition (arrow). Note, lipid droplets (L) and pyknotic nucleus (N). (x 4800)

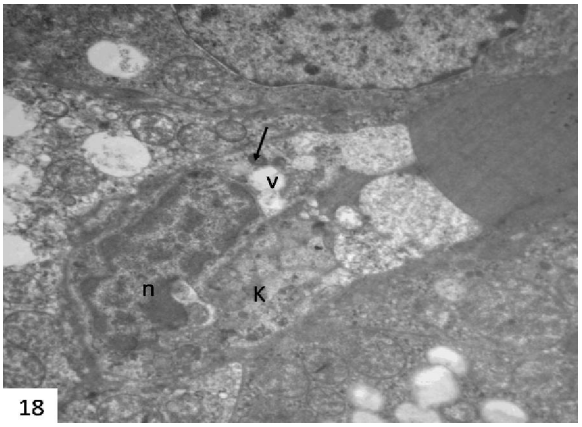

Fig. (18): An electron micrograph of liver of diclofenac treated group showing a Kupffer cell (K) with pyknotic irregular nucleus (n), multiple lysosomes (arrow) and vacuoles (V). (x 7200)

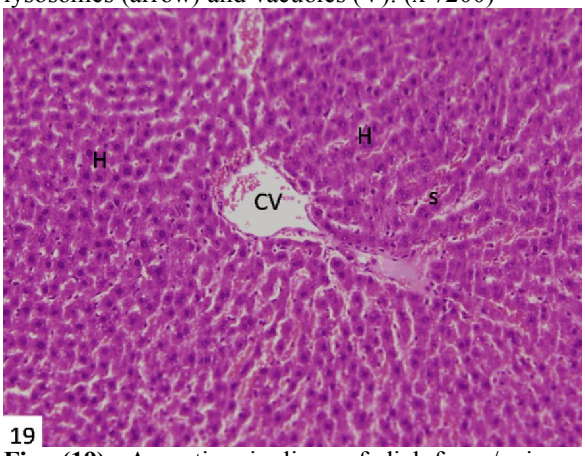

Fig. (19): A section in liver of diclofenac/ ginger extract treated group stained with H & E showing restoration of liver structure, most of hepatocytes (H) appear more or less normal. Note, dilated congested central vein (CV) and blood sinusoids (S). (x 200)

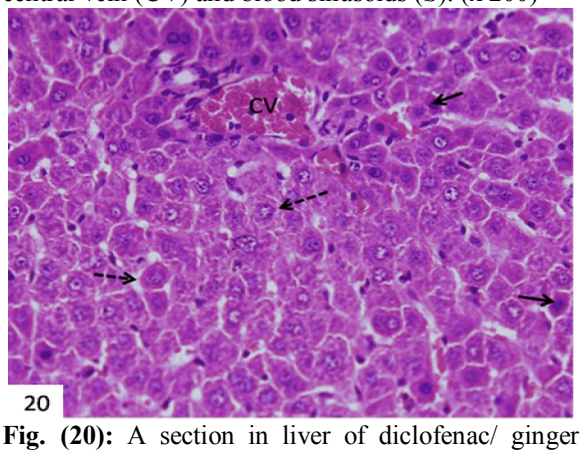

Fig. (20): A section in liver of diclofenac/ ginger extract treated group stained with H & E showing some hepatocytes still vacuolated (dotted arrows) and others with pyknotic nuclei (arrows). Note, dilated congested central vein (CV). (x 400)

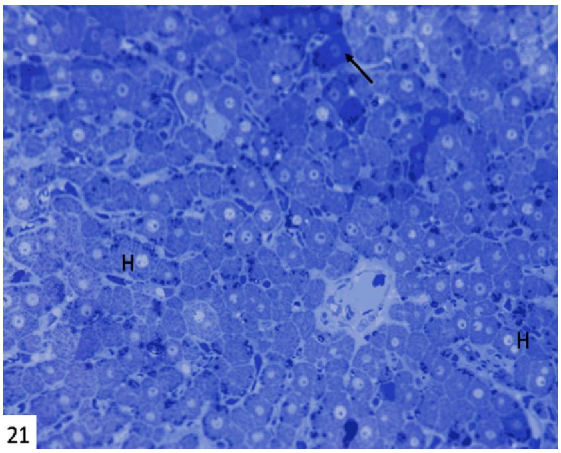

Fig. (21): A semithin section in the liver of diclofenac/ ginger extract treated group stained with toluidine blue showing most hepatocytes appeared normal (H) and few with deeply stained cytoplasm and dense nuclei (arrow).(x 400)

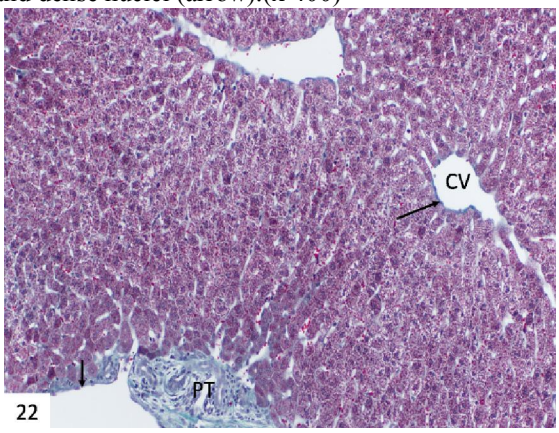

Fig. (22): A section in liver of diclofenac/ ginger extract treated group stained with Masson's trichrome showing few collagen fibers (arrows) around central vein (CV) and at portal tract (PT). (x 200)

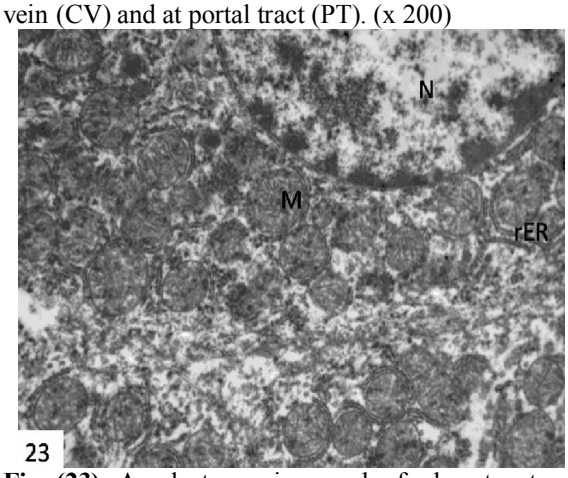

Fig. (23): An electron micrograph of a hepatocyte of diclofenac/ ginger extract treated group showing euchromatic nucleus (N), intact mitochondria (M) and rER. (x 7200)

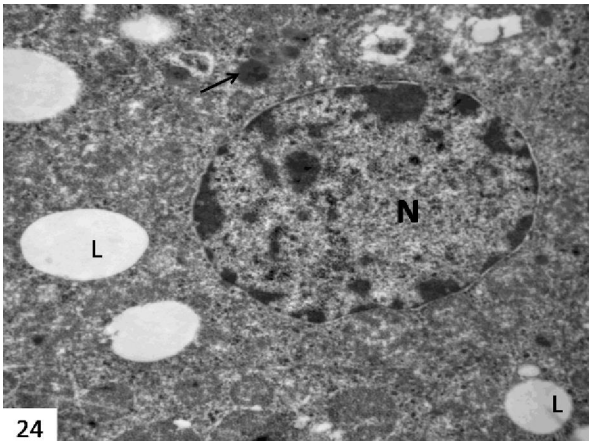

Fig. (24): An electron micrograph of a hepatocyte of diclofenac/ ginger extract treated group showing electron dense nuclei (N) and lipid droplets (L). Arrow points to a lysosome. (x 7200)

4. Disscusion

Liver is a target organ for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed therapeutic agents because they have analgesic, antipyretic and anti-inflammatory actions. There is considerable interest in the toxicity of diclofenac because of its clinical use [20-22].

The present study ascertained that, there is no histological difference between control and ginger extract groups. These results were agreed with observations of previous workers who reported that ginger is safe and well tolerated [12].

In the present study, treatment with diclofenac induced obvious histological and ultrastructural changes in the liver. By light microscope, the changes was manifested by focal affection of hepatic lobules as there were hepatocellular necrosis and swollen vacuolated hepatocytes with nuclear pyknosis as well as congested blood vessels. Increased collagen fibers around central veins and portal tracts was also seen. These changes are in agreement with the results of other researchers who reported that diclofenac in high doses induced hepatotoxic and hepatocellular necrosis in the liver [23-26]. Moreover, our study goes hand in hand with biochemical study of previous work who reported that, high doses of diclofenac produced impairment in the liver and kidney function testes which be taken as a markers of their injuries [22].

As clearly evident from ultrastructural results obtained in this study, diclofenac affects many intracellular targets in hepatocytes. These changes including mitochondrial alterations, disintegration of

cell organelles and accumulation of lipid droplets as well as pyknotic nuclei. Mitochondria have been considered as most important one of these targets. The ultrastructural changes confirmed by El-khishin & Amer [27] and El-Naggar & Hussein [28] who found that intoxication with diclofenac showed damage to mitochondria, fragmentation of rER, fat globules and glycogen depletion.

The hepatotoxic effects of diclofenac sodium in both human and experimental animals have been reported [29&30]. The exact mechanism of these toxic has been attributed to drug metabolism in the liver by cytochrome p-450 and production of active metabolites, namely 4Õ hydroxy 3 diclofenac, 5Õ hydroxy 4 diclofenac and 5Õ hydroxy 6 diclofenac [31&32]. These agents bind extensively with hepatic proteins forming toxic derivates. Both the formation of a toxic metabolite and these derivates have been resulted in direct cytotoxicity, generation of reactive oxygen species (ROS) which propagate mitochondrial injury and cytochrome P450-dependent monooxygenases inhibition as well as oxidation of nicotinamide adenine dinucleotide phosphate(NADPH) [33].

Many investigators [34&35] reported that, there is depletion of ATP synthesis by mitochondria as a result of effect of diclofenac metabolites on the mitochondrial inner membrane and opening of the mitochondrial membrane permeability transition pore as well as uncoupling of respiration by proton shuttling.

The observed nuclear and cytoplasmic changes in hepatocytes in diclofenac treated group might be attributed to the induction of oxidative stress and lipid peroxidative damage of DNA and other cytoplasmic macromolecules which may damage membranes and cause degeneration of the cells. The distorted cells and vacoulation which reported in the present study is in accordance with the other findings [36] where vacuolation and damage of liver cells were observed following exposure to Piroxicam (NSAID).

These hepatic changes have been resulted in impairment of liver function which interferes with the secretion of plasma proteins and subsequently blood osmotic pressure and drainage of tissue fluids were decreased [37]. This may explain the observed congestion of blood vessels.

Cellular Infiltration of portal tract was recorded in the present study and also by other workers [28]. Previous study suggested that this infiltrating cells, leucocytes, in general, and lymphocytes, in particular are a prominent response of body tissues facing any injurious impacts [38]. Leukocytes adherence to endothelium of blood vessels have been suggested to play an important role in the pathogenesis of NSAID associated injury [39].

Furthermore, the observed bile duct proliferation and periportal deposition of collagen fibers in diclofenac treated rats which is the picture of portal fibrogenesis could be attributed to generation of reactive oxygen species. He et al.,[40] mentioned that oxidative stress is relevant to the formation of fibrosis in most chronic liver diseases accompanied by decline of antioxidant abilities and accumulation of lipid peroxidation which have a key role in pathogenesis of liver diseases. Also, intralobular fibrosis were attributed by El-Banhawy et al., [38] to the activation of myofibroblast-like cells that were present normally within the hepatic parenchyma as a result of recruitment of inflammatory cells to the portal field.

Moreover, generation of reactive oxygen species caused lipid peroxidation and oxidation of cellular proteins including those involved in the mitochondrial respiration and those present in endoplasmic reticulum, thus inducing impairment of mitochondrial protein synthesis and disruption of rough endoplasmic reticulum [41&42]. In addition, the effect of the free radicals on the endoplasmic reticulum causes damage to membranes and enzymes necessary for glycogen synthesis [43] and this may explain the observed decreased glycogen granules content in diclofenac treated group in this study.

The presence of lipid vacuoles in the hepatocytes of diclofenac treated rats indicate inability of the cells to catabolize the fat. This is in consistence of other study [44] who reported that, with some drugs, as diclofenac, liver triglycerides accumulate as a large lipid vacuoles and referred this lesion as macrovacuolar steatosis. They added that, this lesion can progress in the long-term to steatohepatitis, which is characterized by necrosis, inflammation and fibrosis. Fromenty [45] attributed that to inhibition of mitochondrial fatty acid β-oxidation, enhanced de novo lipogenesis and reduced secretion of very low density lipoprotein. Damaged Kupffer cells contained multiple lysosomes and vacuoles were seen in this study. The liver is known to be a major immunological organ affecting systemic responses in animals because of the abundance of mononuclear phagocytes [46]. The Kupffer cells and liver phagocytic system produces an array of mediators which protect the body against invasion by xenobiotic chemicals and materials [47].

The present study showed that, co-administration of ginger extract in dose of 250 mg/kg with diclofenac sodium partially ameliorated the histotological changes produced in the liver by diclofenac toxicity. This was clearly evident by light and electron microscopic examination of the liver where the hepatic lobules regained their normal appearance. The hepatocytes appeared more or less

normal except for few hepatocytes with vacuolated cytoplasm and dilated congested blood vessels.

A disturbance in the pro-oxidant/antioxidant system has been defined oxidative stress which plays a major role in diclofenac-induced hepatotoxicity. The normal balance between radical formations and antioxidants system protects the cells against oxidative stress [48]. Previous studies using ginger had reported the significant antioxidant activities [49&50]. It is likely that the preventive effects of ginger extract in diclofenac toxicity are based on its antioxidant activity through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals and inhibiting lipid peroxidation, leading to regeneration of damaged tissues and cells [51].

This is coincided with other investigators [52&53] who reported that the ginger extract or its constituents have protective effects on hepatic damage which induced by hepatotoxicants, CCl4 and acetaminophen through their antioxidant properties. Results of the previous study of Attyah and Ismail [14] demonstrated that ginger extract improve the elevated levels of the serum of liver enzymes due to cisplatin treatment. They reported that ginger components stabilize hepatocytes plasma membrane and prevent delivery of liver enzymes to the extracellular fluid [52].

In addition to antioxidant effects of ginger extract, previous studies demonstrated that ginger components may exert its hepatoprotective effect by inhibition the activity of pro-inflammatory signaling compound prostaglandin-E2(PG-E2) from COX-II in lipopolysaccharide-activated macrophages [54] and also by blocking the enzyme cytochrome P450-2E1[55].

In the present study, the hepatocyte's mitochondria of ginger extract and diclofenac treated group appeared normal with intact cristae. Mitochondria are natural targets of phytochemical antioxidant protection [56]. The results of study of Dugasani et al**.,** [57] indicated that the ginger extract had protective effects against mitochondrial damage caused by ethanol administration and the subsequent organ damage by a number of mitochondrial actions including protection of the electron transport chain up-regulation of specific anti-reactive oxygen species proteins. Furthermore, other researchers [58] detected that antioxidants was able to prevent caspase cascade activation when incubated with diclofenac which could decrease generation of reactive oxygen species and oxidant stress to hepatocytes.

In conclusion, the results of the present study showed that ginger extract partially ameliorate hepatotoxicity induced by high dose of diclofenac.

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