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Hepato-morpholoy and biochemical studies on the liver of albino rats after exposure to glyphosate-Roundup®

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Abstract

Background: The object of this work was to evaluate of the hepatic effects of the herbicides glyphosate-Roundup® by different doses in both sexes of albino rats.

Methods: Forty animals divided into four groups with ten animals for each (both sexes) were treated orally with vehicle (controls) and 25, 50, and 100 mg/kg bwt of glyphosate-Roundup® (treated groups) for 15 days daily.

Results: The most conspicuous changes occurred on the liver treated groups due to glyphosate toxicity were the increase of enzymes activities of ALT and AST, cellular infiltration, many signs of nucleus degeneration, focal necrosis, rarified cytoplasm, disorganization of cellular organelles, and deposition of lipid droplets. The increase in the amount of collagenous fibers and the number of the mast cell were also observed.

Conclusion: Our results indicated that the administration of glyphosate-Roundup® in different doses may cause adverse effects on the histopathological, ultrastructure, and biochemical alternations on the liver of the albino rats.

Keywords: Glyphosate-Roundup®, ALT and AST, Liver, Histopathology, Ultrastructure

Background

Glyphosate [N-(phosphonomethyl) glycine] (GLP) is an organophosphorated non-selective agrochemical used widely in a lot of countries, such as Turkey, and after the sprout, it acts in a systemic way (WHO (World Health Organization) 1994). In 1974, the agricultural company, Monsanto, was developed and commercialized GLP in a formulation marketed as Roundup® (Guyton et al. 2015). GLP shows lower level of toxicity for animals and a lower mobility in comparison to other pesticides (Gasnier et al. 2009) and environmental friend lines (Franz et al. 1997). It is perhaps the most important herbicide ever developed (WHO (World Health Organization) 1994).

GLP is used in commercial formulations, which include other chemical additives (including surfactant) that enhance its efficiency as a weed killer, by promoting toxicity and improving the plant's ability to take up the herbicide (Vincent and Davidson 2015 and Defarge et al. 2016). It has a low permanence and because of employment of this herbicide for prevailing of weeds in agricultural fields, large quantities find their way into water bodies. The random use of the herbicide therefore makes it a potential source of danger to animals, not only in grazing fields but also in the water bodies (Ayoola 2008).

Glyphosate metabolites into aminomethyl phosphoric acid (AMPA) and formaldehyde are found as a contaminant in environments such as soil and rivers (WHO (World Health Organization) 1994). Two possible metabolic pathways for GLP in the human body lead eventually to formaldehyde. Six different enzymes that have the capability to catalyze the conversion of formaldehyde to formic acid is present in animal tissue (Swanson et al. 2016). It is known that formic acid has the ability to cause the malfunction of metabolic acidosis and mitochondria (Kruse 1992). EFSA (European Food Safety Authority) (2015) reported that about 20% of ingested glyphosate is absorbed from the gastrointestinal tract, and according to US EPA

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(U.S. Environmental Protection Agency) (2006), the figure is 30–36%. The absorbed portion is spread widely in the bone, kidneys, and liver with highest concentrations.

Roundup causes inhibition of enzymes implicated in the detoxification of chemicals in the body (Acquavella et al. 2004). GLP have negative effects on some number of enzymes in the CYP (cytochrome P450) superfamily (McLaughlin et al. 2008) located in cell mitochondria and microsomes which play a main function in the liver (Gasnier et al. 2009).

There was a controversy about safety and toxicity of glyphosate. On the one hand, it is considered as a safe compound (Vereecken 2005) since it has not been bioaccumulated, biomagnified, or preserved in a biologically available form in the environment (Helander et al. 2012). Recent work has shown that both glyphosate and AMPA were eliminated slowly from the plasma. On the other hand, although the bioavailability of glyphosate and AMPA was only 23.21%, it is likely that GLP is distributed through the body by the blood's circulation and there may be considerable diffusion of it into tissues to exert systemic effects (Anadón et al. 2009). Myers et al. (2016) reported that metabolism studies strongly point to bioaccumulation in the kidney and liver.

Gasnier et al. 2009reported that GLP was used for a period of time as the minimal harmful herbicides, while recent studies display that GLP can get many risks and not be as safe as conceptualized before (Franz et al. 1997). The present study represents an effort to identify the effects of different doses of GLP for a short period of time on the liver of adult albino rats.

Methods

Experimental design

This study was performed to investigate the effects of different doses of glyphosate on rats. Forty adult male and female albino rats were organized into four groups of ten individuals each (both sexes). The first group (M1 and F1) served as a control received vehicle. The second group (M2 and F2) of animals was treated with 25 mg/kg bwt of glyphosate. The third group (M3 and F3) of animals was treated with 50 mg/kg bwt of glyphosate. The fourth group (M4 and F4) of animals was treated with 100 mg/kg bwt of glyphosate. The herbicide in all treated groups diluted in distilled water and was administered orally, by gavage, on a daily basis for a period of 15 days. Collections of blood and hepatic tissue were made at the end of this period of time.

Materials

Chemicals

Roundup (glyphosate 48% WSC Monsanto Co.) was obtained from the Central Agricultural Pesticide Laboratory (CAPL) in Dokki, Giza, Egypt.

Animals

Male and virgin female albino rats (90-day-old, 129–230 g) were bought from the Assiut University Joint Animal Breeding, Assiut, Egypt. All rats kept under the same laboratory conditions of temperature (25 ± 2) lighting (12:12 light-dark cycle) and were given free access to standard food and tap water. They were allowed to acclimatize for 3 weeks before experiments. The experiment was approved by the Ethics Committee of Assiut University, Egypt.

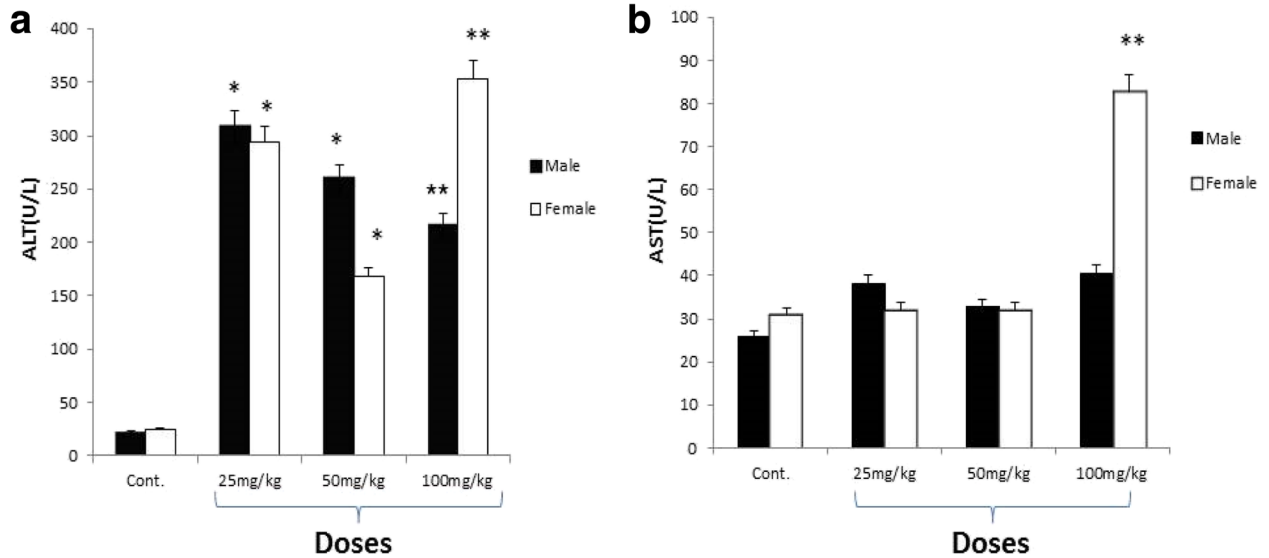

Fig. 1 **a** and **b** Values of treated groups with respect to control are significantly differing at: *$P < 0.05$, **$P < 0.01$

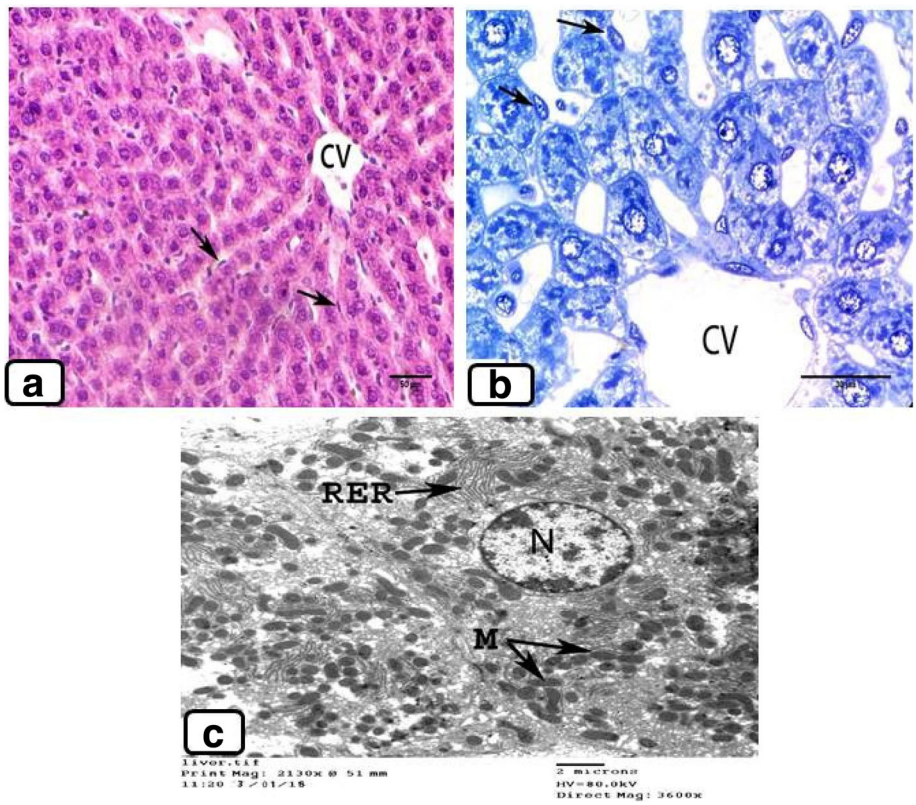

Fig. 2 **a** Liver section of control rat showing normal polyhedral hepatocytes radiating from the central vein (CV) with eosinophilic cytoplasm and centrally located nuclei. The blood sinusoids are often seen with phagocytic Kupffer cells (arrows) between the cords of hepatocytes (H&E). **b** Semithin section of control rat liver showing the normal structure of hepatocytes and central vein (CV). Note: Kupffer cells (arrows) (toluidine blue stain). **c** Electron micrograph of the hepatocyte of control rat showing centrally located rounded nucleus (N), normal distribution of hetero and euchromatin, well-developed rough endoplasmic reticulum (RER), and mitochondria with dense matrices and vary in shape (M)

Experimental procedures

Measurements of liver enzymes in serum

Blood samples were collected from orbital sinus of all animals immediately before animals were killed. Serum was separated by centrifugations at 3000 rpm for 10 min. The serum was used for liver function assessment employing measurements of the enzymes alanine aminotransferase (ALT) and alanine aspartate aminotransferase (AST) by following the instructions of enzymatic colorimetric assay kits (Diamond Diagnostic).

Histopathological experiments

Liver samples were taken from all animals by surgical processing after the animal was killed. All samples were kept in formal alcohol for 24 h. Blocks were made following standard methods. Sections were cut 5–7 μm thick, stained with hematoxylin and eosin according to Drury and Wallington (1980). These sections were investigated under light microscopy.

Transmission electron microscopy study

Immediately after sacrificing the animals, small specimens were taken from the liver and fixed in 5% cold glutaraldehyde for 24 h; the specimens were then washed in three to four changes of cacodylate buffer (pH 7.2) for 20 min in each change and postfixed in cold osmium tetraoxide for 2 h. Thereafter, the specimens were washed in four changes of cacodylate buffer for 20 min each. Dehydration was carried out using ascending grades of ethyl alcohol of 30, 50, and 70%, each for 2 h, and two changes of 90 and 100% for 30 min each. Embedding was carried out in Epon 812. The embedded samples were kept in an incubator at 35 °C for 1 day, at 45 °C for another day, and at 60 °C for 3 days (Woods and Stirling, 2008).

Semithin sections of 0.5–1 μm were prepared using an LKB ultramicrotome, Germany. The sections were stained with toluidine blue, examined with a light microscope, and photographed. Ultrathin sections (50–80 nm) from selected areas of the trimmed blocks were taken

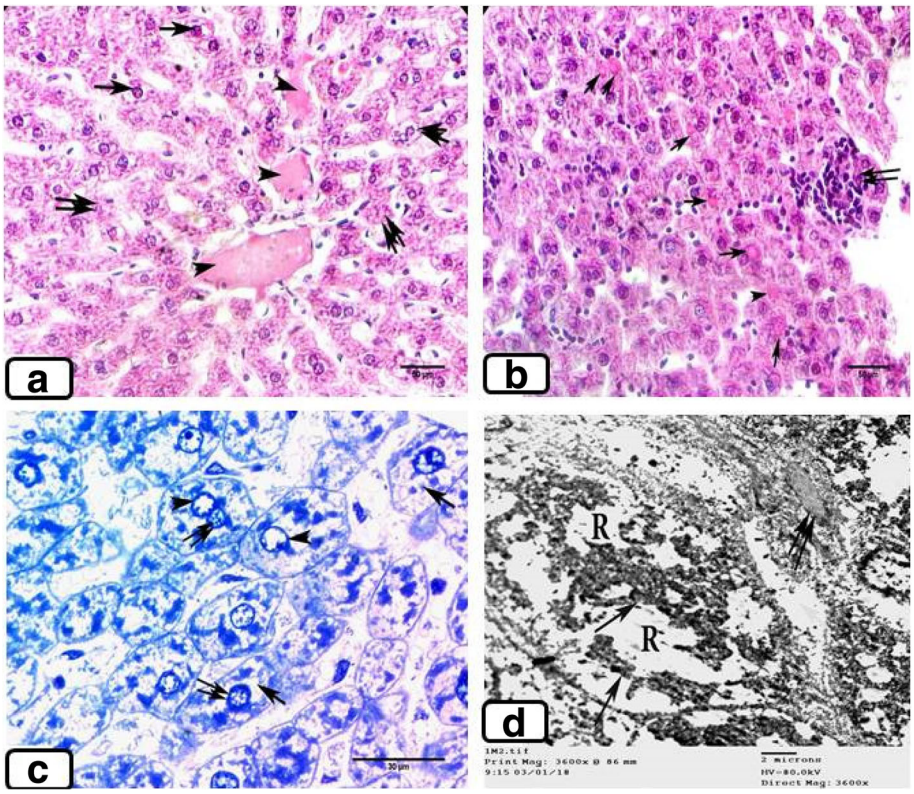

Fig. 3 M2 group liver showing **a** vacuolated cytoplasm with deeply stained nuclei (↑), degeneration of some hepatocytes (↑↑), and congestion of blood vessels and central vein (arrowhead) (H&E). Note: the Kupffer cell puched into the sinusoids lumen. **b** Eosinophilic stain (red color) of nuclei (↑), leucocytic aggregation between hepatocytes (↑↑), degeneration of many hepatocytes (arrowhead), and other hepatocytes with vacuolated cytoplasm and pyknotic nuclei (H&E). **c** Vacuolated cytoplasm of many hepatocytes (↑) with deeply stained nuclei (↑↑) and the nucleoplasm of some nuclei devoid chromatin (arrowhead) (toluidine blue). **d** Ultrastructure of hepatocytes showing many rarified areas in the cytoplasm (R) and disorganization and dissociation of cellular organelles (necrosis) (↑). Note: collagenous fibers between hepatocytes (↑↑)

and collected on a copper grid. The ultrathin sections were contrasted with uranyl acetate for 10 min and with lead citrate for 5 min, examined by transmission electron microscopy (JEOL 100 CX; JEOL, Japan), and photographed at 80 kV in the Assiut University Electron Microscopy Unit.

Statistical analysis

The quantitative results of the present work were expressed as means ± SEM. Differences between means were tested by one-way analysis of variance (ANOVA) followed by the student–Newman–Keul t test and were considered different with respect to control data at two levels of significance: $^{*}P < 0.05$ and $^{**}P < 0.01$.

Results

Biochemical study

Hepatotoxicity was detected by quantitative analysis of the serum ALT and AST activities, which were used as the biochemical markers of liver damage as showed in Fig. 1. The result showed that the higher dose results in higher level of enzyme activities exposed to glyphosate. There was a significant difference in ALT enzymes activities between glyphosate-treated groups and controls (Fig. 1a). While AST activity in treated groups showed an increase but insignificant difference between glyphosate-treated groups and controls. However, F3 group showed high significantly difference ($p < 0.01$) compared to treated groups and controls (Fig. 1b).

Morphology

A normal liver structure was observed in the control animals (M1 and F1) as shown in Fig. 2a, b. Electron microscope examination revealed that the liver showed normal appearance of hepatocytes with centrally placed prominent nuclei and cytoplasm that contained a large number of mitochondria and rough endoplasmic reticulum (RER) (Fig. 2c).

In the present study, many cellular changes on the hepatic tissue of all treated groups compared to controls were evident. In male rats group that received 25 mg/kg bwt of glyphosate (M2), it showed obvious histopathological changes in the form of hepatocyte cordon plan disarrangement in parenchymal cells due to dilatation of blood sinusoids. Damage of the wall of the blood

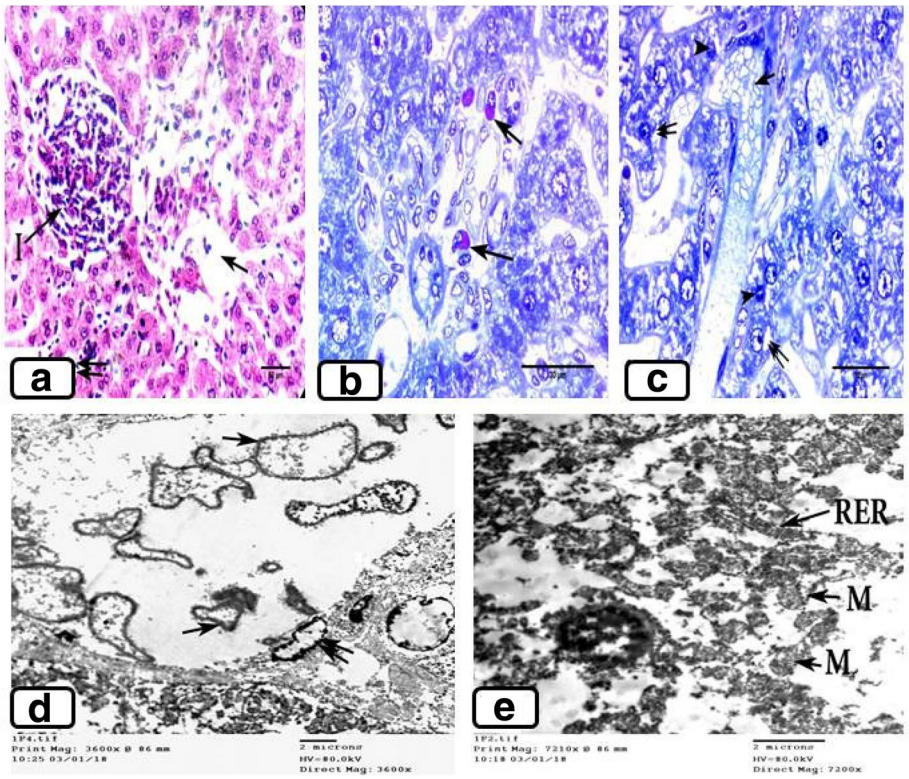

Fig. 4 F2 group liver showing **a** focal necrotic area (↑), foci of cellular infiltration around the portal area (I), leucocytic aggregation between liver cells (↑↑), and vacuolation and degeneration of many hepatocytes (H&E). **b** Many of mast cells between portal areas (↑) (toluidine blue stain). **c** Large dilatation of blood sinusoids filled with deformed RBCs (↑), highly vacuolated cytoplasm of many hepatocytes (↑↑), and pyknotic nuclei (arrowhead). Note: deposition of RBCs debris in the wall of blood sinusoids (toluidine blue stain). **d** Ultrastructure of hepatocytes showing deformed RBCs with thickened their plasma membrane (↑) and the nuclei of endothelial cell appeared irregular in shape with condensed chromatin (↑↑). **e** Ultrastructure of hepatocytes showing destructed mitochondria (M) and fragmented rough endoplasmic reticulum (RER). Note: degenerated nuclei

vessel and blood sinusoid was observed (Fig. 3a). Other signs of degeneration were reflected by small leucocytic aggregation among the liver cells and unusual red color stain (eosinophilic stain) of the nuclei was prominent (Fig. 3b). Semithin sections revealed highly vacuolated cytoplasm of some hepatocytes. In the binucleated hepatocytes, one appeared deeply stained while other nuclei chromatin may be aggregated in their inner membrane or the chromatin were disintegrated; the nucleoplasm devoid from chromatin and appeared empty (Fig. 3c). Ultrastructural investigation in male rats received 25 mg/kg bwt revealed significant changes compared to control group. The cellular organelles of the liver cell showed disorganization and dissociation (necrosis). Moreover, the amount of collagenous fibers in-between hepatocyte was detected (Fig. 3d).

Examination of the liver sections of female rats after administration of 25 mg/kg of glyphosate (F2) showed some of the similar changes to those observed in male rats administered with 25 mg/kg (M2). However, additional remarkable histopathological changes were represented by large foci of necrotic area and also increase the amount of cellular infiltration around the portal area (large foci) compared to M2 group (Fig. 4a). Toluidine blue examinations revealed few of mast cells between portal areas (Fig. 4b). Also, vacuolation and aggregation of cell organoids in many hepatocytic cytoplasms were observed (Fig. 4b, c). The blood sinusoids appeared more widening and congested with deformed RBCs (Fig. 4c, d). The RBCs's plasma membrane was thick and the nuclei of endothelial cell is irregular in its shape and with condensed chromatin (Fig. 4d). Moreover, the damage and destruction of mitochondria and the remaining of fragmented RER were obvious (Fig. 4e).

The male rats that received 50 mg/kg bwt of glyphosate (M3) showed bilirubin pigments (yellow materials) in blood vessels, blood sinusoids, and between liver cells (Fig. 5a). Marked extensive necrosis of large area of hepatocytes with dispersion of all their contents and areas devoid of hepatocytes associated with hemorrhage was detected (Fig. 5b). Moreover, polyploidy nuclei (red and blue color) of many hepatocytes were prominent in many liver sections. Also, most of the liver cells degenerated and vacuolated. Sometimes, lipofuscin pigment (yellow-brown color) filled the macrophage cells (Kupffer cells) of the liver tissue (Fig. 5c).

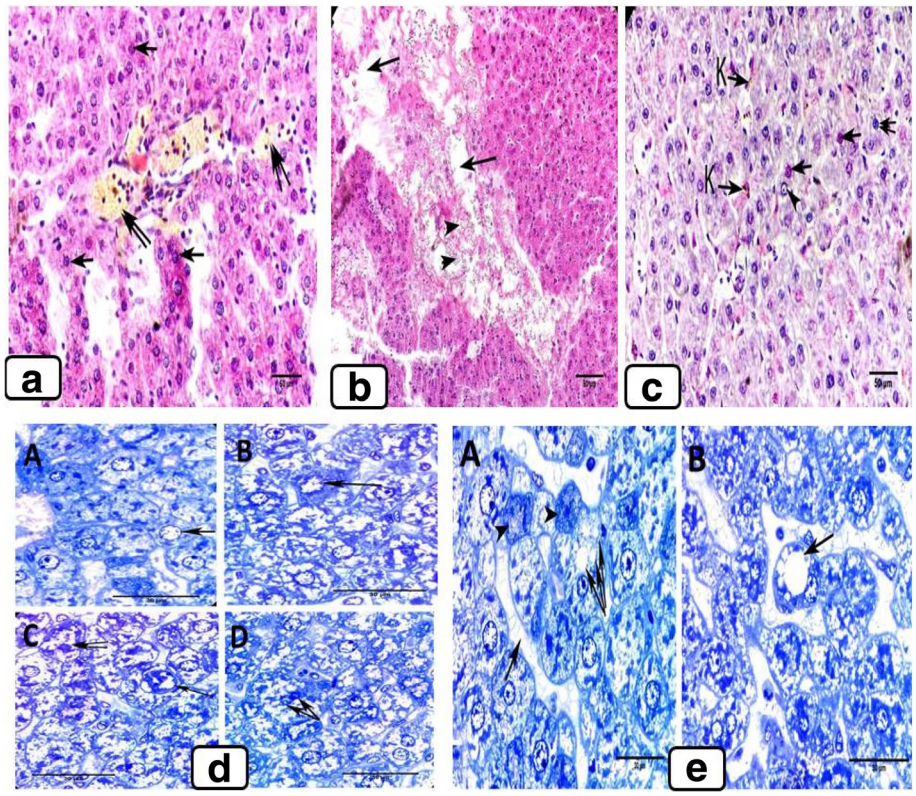

Fig. 5 M3 group liver showing **a** many necrotic areas (n), bilirubin filled the blood sinusoid and between hepatocytes (↑↑), degeneration of most of the hepatocytes and small dark stain nuclei was observed (↑) (H&E). **b** Marked extensive necrosis of large area of hepatocytes with dispersion of their contents and areas devoid of hepatocytes (↑). Note: RBCs in the destructed area (hemorrhage) (arrowhead) (H&E). **c** Polyploid nuclei (red and blue color) (↑), chromatin of few nuclei segregation in their inner membrane and appeared empty (arrowhead) and other hepatocytes with small dark stained nuclei (↑↑). Notice lipofuscin pigments (yellow-brown color) in few Kupffer cells (K) (H&E). **d** *A* nuclei showed chromatolysis, *B* Irregular shape of nuclei, *C* nuclei enlarged in size with clumped of their chromatin and *D* hypertrophic of binucleated hepatocytes with pyknotic nuclei (toluidine blue stain). **e** A Hepatocyte with many of vacuoles differed in size (↑↑), apoptotic cells (arrowhead), and dilated and congested blood sinusoids with a thickened wall (↑). B Hepatocyte contains large vacuole (toluidine blue stain)

Different liver sections in toluidine blue-stain semithin sections of the M3 group revealed many pathological changes in the nuclei of hepatocytes. The nuclei sometimes showed segregation of euchromatin in their inner membrane or chromatolysis (nucleoplasm depleted from chromatin) and appeared empty (Fig. 5d (A)), while in other hepatocytes, the nuclei appeared irregular in shape (Fig. 5d (B)), enlarged in size, and clumped of their chromatin (Fig. 5d (C)) and pyknosis in hypertrophic binucleated hepatocyte (Fig. 5d (D)). In other liver sections, many hepatocytes showed vacuolation and others with many vacuoles different in size (Fig. 5e (A, B)), and many of the apoptotic cells were detected (Fig. 5e (A)). Dilatation and congestion of blood sinusoids were filled by abnormally shaped RBCs; their plasma membrane appeared thick. Moreover, apoptotic cell was also observed (Fig. 6a). Other affected hepatocytes with pyknotic nuclei and large parts of cytoplasm depleted from cellular organelles (Fig. 6b).

Histological investigation of female rats taking 50 mg/kg bwt of glyphosate (F3) revealed lesion of most hepatocytes and huge focal leucocytic aggregation with deposition of amount of fibers was prominent (Fig. 6c). Other changes appeared more or less similar to the M3 group. Electron microscopic investigation of this group revealed hepatocyte with large lipid droplet between the fragmented RER and other organelles (Fig. 6d). In other severe hepatocytes, activated mast cell was detected and its nuclei has condensed chromatin. Many of the collagenous fibers between liver cells were observed (Fig. 6e).

In male rats administered with 100 mg/kg bwt, many histopathological changes were revealed. In binucleated hepatocytes, the nuclei were dissimilar in color stain and disintegration in most cytoplasmic contents (Fig. 7a). An increase in the number of Kupffer cells was noticed. Many of them were filled with lipofuscin pigments (Fig. 7b). Fusions of the lumen of both congested blood vessels and blood sinusoid were detected due to the disruption of their walls, and most of the hepatocytes had extensive vacuolar degeneration (Fig. 7c). In severe hepatocytes, the fragmentation of cell organelles forming

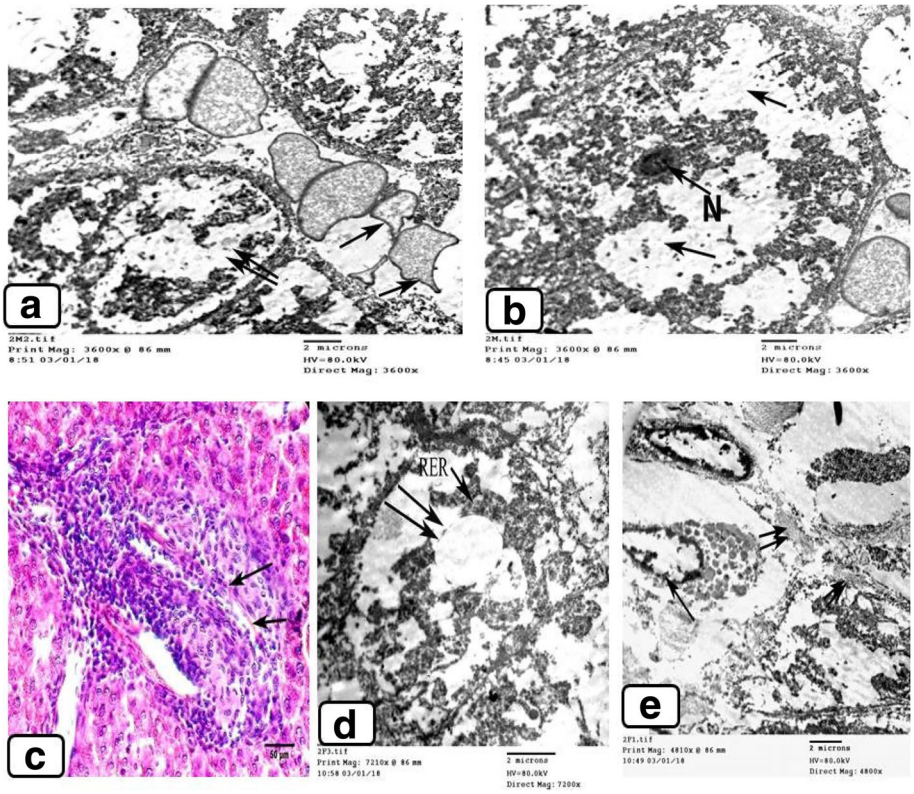

Fig. 6 a Ultrastructure of the M3 group hepatocytes showing congested blood sinusoid with abnormal shaped RBCs and thickened of their plasma membrane (↑). Note: apoptotic cell (↑↑). **b** Ultrastructure of the M3 group hepatocytes showing large parts of cytoplasm depleted completely from cellular organelles (↑) and with pyknotic nuclei (N). **c** F3 group liver showing large foci of leucocytic aggregation with deposition of the amount of fibers (↑) (H&E). **d** Ultrastructure of F3 group hepatocytes showing large lipid droplet (↑↑) between fragmented rough endoplasmic reticulum (RER) and other organelles (↑). **e** Ultrastructure of F3 group hepatocytes showing activated mast cell and their nuclei with condensed chromatin (↑) and many amounts of collagenous fibers between liver cells (↑↑)

electron dense clusters and the remaining of the fragments of RER were observed (Fig. 7d).

In female rats groups that were administered with 100 mg/kg glyphosate (F4), a large necrotic area with hemorrhage and cellular infiltration was obvious (Fig. 8a). Toluidine blue stained sections revealed an increase in pathological changes compared to previously treated groups and appear more or less similar to the M4 group (treated with 100 mg/kg). These changes represented by an increase in the widening of blood sinusoids and damage of their walls led to the fusion of their lumens which filled with RBCs (Fig. 8b (A, B)), and the nuclei of some hepatocytes showed karyolysis and many hepatocytes depleted from the cytoplasm (Fig. 8b (B)). Hepatic ultrastructural alternation revealed the abnormal appearance of three nuclei in one cell, one of them showed their nuclear membrane was damaged, the other one showed a separation between their inner and outer membrane, while the last one appears degenerated (Fig. 8c, d).

Discussion

Based on the results of the present study, glyphosate-Roundup® causes biochemical and histopathological changes in the liver of both sexes of treated groups. These changes were more intensified in the case of high dose (100 mg/kg bwt) used in our study duration.

Statistically, the levels of AST and ALT enzymes showed an increase in the serum of treated groups compared to those of controls in the present study after 15 days of exposure to GLP. This observation is in line with Benedetti et al. (2004) who demonstrated an increase in the levels of the enzymes ALT and AST that causes cellular alterations. These alternations were represented by an increase of connective tissue and deposition of collagen in hepatocytes of Wistar rats exposed to glyphosate-Biocarb®.

Large deposition of collagenous fibers may cause modifications in the diffusions of solutes, like proteins, between the hepatocytes and plasma. This may induce liver cells dysfunction (Pratt and Kaplan 2001). Jyothi and Narayan (1999)

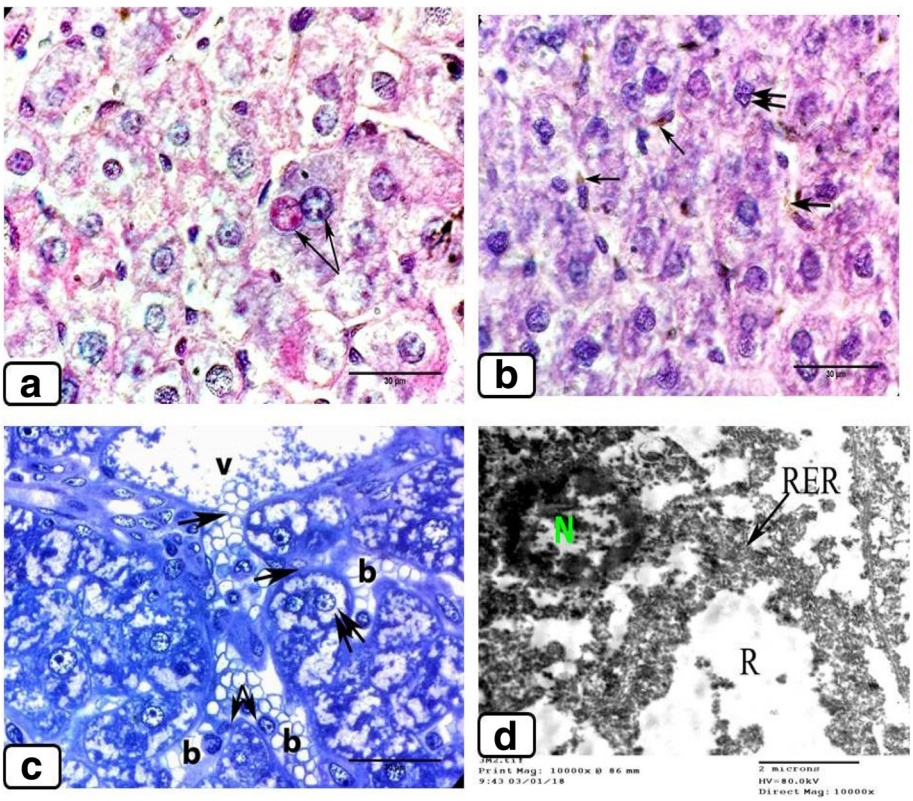

Fig. 7 M4 group liver showing **a** binucleated hepatocytes were dissimilar in their color stain (↑↑) and most of the hepatocytes devoid completely from the cytoplasm (H&E). **b** Yellow-brown color of lipofuscin pigments in many of Kupffer cells (↑) and the nuclei of some hepatocytes appeared irregular in its shape (↑↑) (H&E). **c** Damage of the wall of both blood vessels (V) and blood sinusoids (b) and fusion of their congested lumen (↑) and most hepatocytes with extensive vacuolar degeneration (↑↑) (toluidine blue stain). **d** Ultrastructurally of hepatocyte showing remaining of fragment RER (↑↑) rarified cytoplasmic space (R) and degenerated nucleus (N)

suggested that damages of the liver may cause the production of mitochondrial enzymes like alkaline phosphatase which was thinly released into the blood. The toxic effects could be lipolytic in nature, as a result of which the cell membrane, lysosomal membrane, and other organelles underwent dissolution, releasing the enzymes through blood. Hence, the increased plasma enzyme activities in the present investigation may be a result of cell necrosis in the liver.

Histological investigations revealed many alternations. These alternations were in the form of disarrangement in parenchymal cells, mononuclear cell infiltration, many apoptotic hepatocytes, and many focal necrotic areas. Also, an increase in the number of Kupffer cells with deposition of lipofuscin pigments and the amount of collagenous fibers was noticeable.

In the present investigation, a remarkable collection of inflammatory cells adjacent to some blood vessels and invading the hepatic tissue were observed after administration of glyphosate. A similar result was reported by Caglar and Kolankaya (2008) and EFSA (European Food Safety Authority) (2015) in the rat liver. Wunderlich et al. (2005) suggested that the abundance of leucocytes in general and lymphocyte in particular is a prominent response of body tissues facing injurious impact. Also, Trasher et al. (1988) reported that formaldehyde (which is one of the metabolites of glyphosate) might be conjugated with human serum albumin and yield a new antigen. This antigen encouraged the body to manufacture anti-formaldehyde human serum antibodies and raise the antigen memory cells. This led to sustained stimulation of the immune system.

Our study indicates cellular degeneration followed by necrosis, especially after 100 mg/kg bwt dose of glyphosate. Similar findings were obtained in juvenile African catfish (*Clarias gariepinus*) exposed to glyphosate (Ayoola 2008). Also, Cox (1995) and Jashni et al. (2013) found that glyphosate causes damage and overgrowth in the renal cells in the female albino rat. Moreover, Caglar and Kolankaya (2008) reported focal necrosis and some of the apoptotic hepatocytes on rats after exposure to glyphosate-based herbicide Roundup.

John et al. (2001) suggested that organophosphorus including glyphosate produce free radicals in the cells that cause structural changes in cell proteins and unsaturated lipid peroxidation in the cells. These complications can cause cell necrosis and neoplastic changes. Biagiant-

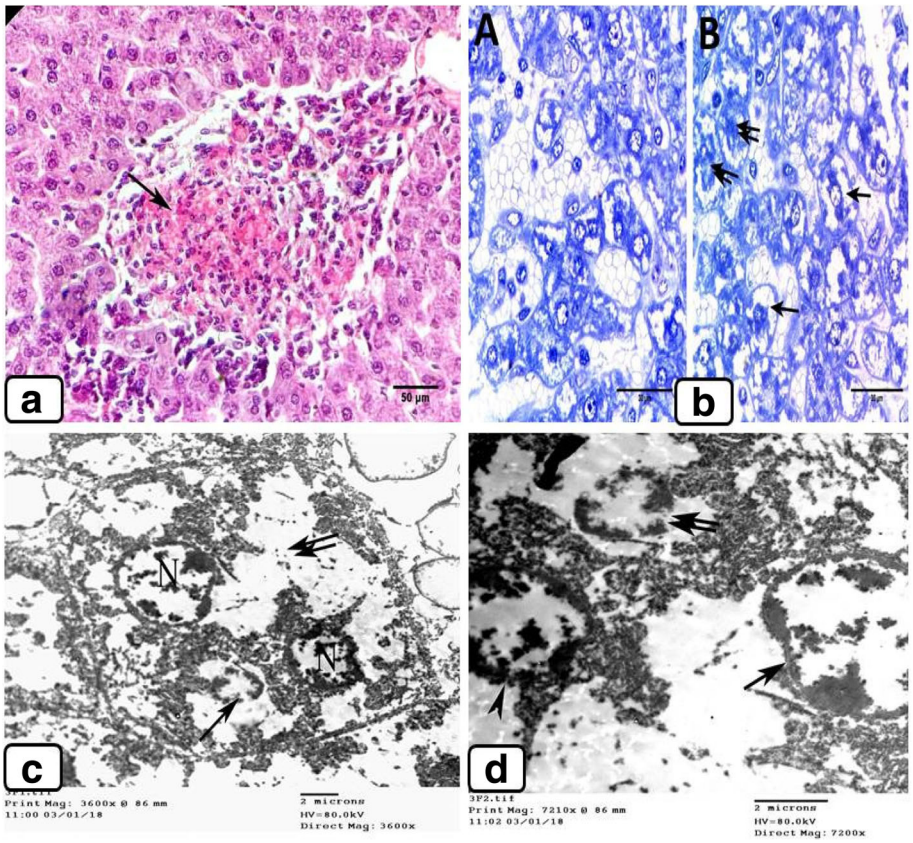

Fig. 8 F4 group liver showing **a** large necrotic area with leucocytic aggregation and hemorrhage (H&E). **b** F4 group liver showing A congested blood sinusoids and damage of their walls led to the fusion of their lumens (toluidine blue stain). B Most cells depleted from the cytoplasm (↑) and degeneration of most of the nuclei (↑↑) (toluidine blue stain). **c** Ultrastructure of hepatocytes showing three degenerated nuclei (N); one of them their nuclear membrane was damaged (↑). Large empty spaces due to the destruction of most cell organoids (↑↑). **d** Ultrastructure of hepatocytes showing three nuclei, one of them their nuclear membrane was damaged (↑↑), the other one showed separation between their inner and outer membrane (↑) while the last one appears degenerated (arrowhead)

Risbourg and Bastide (1995) explain the necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work and needs to dispose of the toxicant from its body during the process of detoxification by the liver.

The present study revealed that Kupffer cells increased in number and sometimes filled with lipofuscin pigments after glyphosate administration. These results agree with EFSA (European Food Safety Authority) 2015who found diffuse acute inflammation in the liver with pigment deposits in rats. Also, Benedetti et al. (2004) and Caglar and Kolankaya (2008) indicated that after higher doses of glyphosate, the Kupffer cells in hepatic sinusoids were increased.

Benedetti et al. (2004) illustrated that the infiltration of Kupffer cells was followed by large deposition of reticular fibers, mononuclear cell infiltration, and congestion of the liver tissues. They also reported that these could have contributed to the weakness of regular liver function due to xenobiotic modification in detoxification processes.

Curran (1987) explained that lipofuscin granules and residual bodies, which represent the indigestible remnants of cell organelles and cytoplasmic materials, increased in cases of increasing age or the presence of a wasting disease, the condition of brown atrophy results. In this case, death followed prolonged cachexia (weakness and wasting of the body due to severe chronic illness) caused by malignant disease.

It is evident from the present study that the administration of glyphosate resulted in distinctive subcellular alterations in hepatocytes. Glyphosate administration induced rarified cytoplasm, disorganization of cellular organelles, and deposition of lipid droplets. In addition, the presence of activated mast cells between the portal areas and the increase of collagenous fibers also were observed.

The deposition of lipid droplets and vacuolization on hepatocytes of rats exposed to GLP were observed in the present study and were reported by Biagiant- Risbourg and Bastide (1995), who found that fish atrazine liver display an increased in the lipid droplets size and

vacuolization after exposure to herbicide. Also, they were reported by Samanta et al. (2016) who found excess of fat deposition in liver fish (*Heteropneustes fossilis*). The presence of sufficient number of lipid droplets suggested a decay in the synthesis of protein in the cytoplasm (which involved in transport triglycerides and serve as lipid metabolizing enzymes) which ultimately blocks the utilization of lipid–protein conjugation (Cheville 1994). In this case, the lipids are continuously produced at a normal rate, which leads to progressive accumulation of lipid globules (Holm et al. 1993). Liver cells vacuolization perhaps illustrate an imbalance between the average synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation (Gingerich 1982).

In the present investigation, the nucleus showed a depletion from chromatin and the nucleoplasm appeared empty and sometimes, a very few amount of chromatin aggregate in the inner nuclear membrane. So, this may illustrate the changes of the nucleus from blue to red color in the present study and this is maybe due to that chromatin is known to give the nucleus its basophilic stain. Gasnier et al. 2009) and Mañas et al. (2009) reported injury of DNA in human hepatocytes with 50% DNA strand breaks after exposure to GLP and Roundup. Also, Cutler (2010) added that the damage of DNA in the liver cells can foster that cell death and possibly create a cancerous cell. These previous reports may illustrate that the chromatins became decays and the nucleoplasm appeared empty in some cells and appeared red in others.

The mast cell can be recognized by its content of metachromatic granules when appropriately fixed and stained with metachromatic dyes such as toluidine blue. It is implicated in neoplastic, immunological, inflammatory, and other conditions (Yong 1997). Based on the ultrastructure appearance of mast cells positive granules, morphological sings of activation of many of the mast cells were distinguished in our present study. Those positive granules of mast cell when appeared and invigorate in liver play a critical role in the adjustment of the fibrogenesis and inflammation. This inflammation and fibrogenesis were known to cause dangerous damage to the liver as nonspecific reactive hepatitis (Gulubova et al. 2005).

Conclusion

From the present study, it may be concluded that glyphosate induced biochemical, histopathological, and subcellular alterations in hepatocytes of liver structure in male and female rats. Results from this investigation may contribute to the understanding of the effect(s) of different doses and simultaneous exposition to glyphosate on cell survival, and they may be of clinical interest in the evaluation of the degree of damage to which humans are involuntarily exposed due to environmental pollution.

Our findings suggest that we should be very careful during use of glyphosate as an herbicide in our fields and that we should use it in a specific dose according to WHO to reduce as much as possible its passive effects. We should also overcome the problems resulting from eating food may contain glyphosate. Taking a good antioxidant to strengthen our immune system can provide one of the possible remedies.

Abbreviations

ALT: Alanine aminotransferase; AST: Alanine aspartate aminotransferase; GLP: Glyphosate; RER: Rough endoplasmic reticulum; US EPA: US Environmental Protection Agency; WHO: World Health Organization

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Availability of data and materials

Rats purchased from Assiut University Joint Animal Breeding, Assiut, Egypt, and the kits purchased from the specific producing company. Glyphosate-Roundup® was obtained from Central Agricultural Pesticide Laboratory (CAPL) in Dokki, Giza, Egypt. All data are available from the corresponding author upon request.

Authors' contributions

Dr. SSHMM designed the experiment, practical work, histological analysis, carried out the biochemical data analysis, and wrote the manuscript. ELTA contributed to the practical work. Dr. IMA contributed his idea of work. Dr. MIA and Prof. EHA contributed by providing the chemicals. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was carried out in accordance with the ethical rules for handling the experimental animals, Zoology Department, Faculty of Science, Assiut University, Egypt.

Consent for publication

No human subjects are included. No individual person's data are included.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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