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# Histological, ultrastructural, and biochemical study on the possible role of *Panax ginseng* in ameliorating liver injury induced by Lambda cyhalotherin

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## Abstract

**Background:** Lambda-cyhalotherin (LCT) is a pyrethroid type 2 pesticide that is broadly utilized in pest control in public health, animal health, and agriculture. Although claiming that LCT has a low mammalian toxicity, several investigations reported its mammalian hepatotoxicity by mediating oxidative stress causes severe hepatotoxicity and liver damage.

**Results:** LCT significantly decreased catalase (CAT), superoxide dismutase (SOD), and total thiol (T. thiol) and increased lipid peroxidation (LPO). mRNA and protein expression levels of p53 were upregulated, whereas Bcl-2 mRNA and protein expression levels were downregulated in LCT-intoxicated animals. Also, light microscopic and ultrastructure studies for liver tissues of LCT-intoxicated animals showed mononuclear leukocytic infiltration in the parenchyma, congested portal vein with thickened wall, and proliferation of bile duct and hepatocytes with cytoplasmic vacuolations, fatty changes, and collagen fibers. *Panax ginseng* co-treatment attenuated oxidative stress biomarkers. Both tested doses of *Panax ginseng* (100 and 200 mg /kg b. wt./day) significantly decreased p53 and elevate Bcl-2 mRNA and protein expression levels and reveals significant amelioration and restoration of normal histology and ultrastructure of liver, but 200 mg/kg b. wt. of *Panax ginseng* seems to be more potent.

**Conclusion:** *Panax ginseng* exhibited ameliorative effect against hepatic oxidative stress, apoptosis, histopathological, and ultrastructural changes induced by LCT.

**Keywords:** Lambda-cyhalothrin, *Panax ginseng*, Antioxidants enzyme activities, p53, Bcl-2, Histopathology and ultrastructure of liver

## 1 Background

Pesticides are among the most environmental pollutant chemicals and their indiscriminate usage is growing [1]. Pyrethroids are a group of greater than 1000 strong wide range pesticides [2] presenting approximately 25% of the worldwide insecticide market [3]. They are synthesized analogues of natural pyrethrins which is the active ingredient in *Chrysanthemum*, *Cineraria folium*, flowers [4].

According to presence of  $\alpha$ -cyano group, they can be divided into 2 big groups that differ in their action [5].

Lambda-cyhalotherin (LCT), type 2 pyrethroid, utilized for controlling a wide spectrum of ectoparasites mainly on sheep and cattle and to a smaller range in goats and pigs. It is also used for controlling aphids and other pests in cereals, cotton various vegetables, and fruits agriculture. Moreover, its usage in controlling mosquitoes, ticks, flies, and cockroaches that may be vectors of diseases is very important for public health management. Malaria epidemic-prone areas were sprayed with LCT to kill vectors of malaria [6, 7].

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Despite claiming that LCT has a low mammalian toxicity, several investigations reported the mammalian hepatotoxicity of LCT [8, 9]. Numerous mechanisms were proposed as a possible justification for mammalian toxicity of pyrethroids including generation of excess reactive oxygen species (ROS) leading to oxidative damage [10]. ROS react directly with cellular biomolecules leading to lipid peroxidation (LPO), protein oxidation, and DNA damage [11] that eventually conduce to alterations in function and structure of cell [12]. El-Demerdash [13] and Fetoui et al. [14] observed that ROS are involved in LCT-induced hepatotoxicity and oxidative damages. Moreover, Fetoui et al. [15] reported that LCT induce ROS and reduced activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), GPx, GST, and GSH in liver leading to increase lipid peroxidation so induce destruction in structure and function of liver.

Some synthetic antioxidants that were used to avoid oxidative stress may cause side effects [16]. Thus, there is increasing interest in using natural antioxidants of plant origin to ensure safety and efficacy against oxidative stress via decelerating of ROS generation. In the Asian traditional medicine, *Panax ginseng* (G) is considered to be the most precious plant among medicinal herbs for the treatment of various ailments such as metabolic syndrome, hypertension, immune deficiency, respiratory, and hepatic dysfunction [17]. The most important part of G is the root, containing numerous active ingredients: ginsenosides (saponins), polyacetylenes, polyphenolic compounds, and acidic polysaccharides, and among the components, the most pharmaceutically active are ginsenosides [18].

The mechanisms that provide hepatoprotective effects of G are strongly related to its antioxidant activities. G treatment inhibited oxidative stress damage such as lipid peroxidation [19], alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) [20]. Also, G improved the antioxidant defense mechanism [21] and elevated self-antioxidant enzyme activities of SOD, CAT, GPx, GR, GSH, and heme oxygenase-1 in the aged-rat liver [19] and hepatotoxins-induced liver damages in rats [22]. With this background, G with its antioxidant properties is among possible research targets for its promising advantage to be used in alleviating LCT toxicity. Thus the current study was undertaken to define the possible ameliorative effects of G against LCT-induced hepatic damage via oxidative stress, apoptosis, histopathological, and ultrastructural changes in liver of adult albino rats.

## 2 Methods

### 2.1 Chemicals

Lambda-cyhalothrin (LCT), purchased from Pharmacure for Chemicals and Pharmaceuticals, Egypt with

commercial name Lambda C 5%. Pure ginseng was purchased from PHARCO Pharmaceutical Industries (Cairo, Egypt). All chemicals with analytical quality and were obtained from standard commercial products.

### 2.2 Animals and experimental design

Thirty-six male adult laboratory rats (*Rattus norvegicus domestica*), which weighed around  $135 \pm 10$  g. They were collected from the National Research Center (NRC), Doki, Giza, Egypt and kept under observation to exclude any intercurrent infection for 14 days before the start of the experiment. The animals selected were kept in plastic cages with good aeration at temperature ( $25 \pm 5$  °C), normal dark/light cycle of 12 h, and humidity ( $55 \pm 5\%$ ). The rats were supplied with known composition basal diet and water ad libitum throughout study time. In this study, animal care was carried out following the European Community Directive (86/609/EEC) and national rules, this is in accordance with the NIH Guidelines for care and use of Laboratory Animals, 8<sup>th</sup> editions. This was administrated by the committee of the Zoology Department, Beni-Seuf University, Egypt. At the end of experiment, the rats were sacrificed by anesthesia inhalation under light diethyl ether (5%).

Six groups of rats (6 per group) were separated randomly as follows:

Group 1 (C, control): rats received normal saline through intraperitoneal (i.p.) injection daily for 60 days.

Group 2 (G 100): rats received G at a daily dose of 100 mg/kg b. wt. dissolved in normal saline [23] by i.p. injection for 60 days.

Group 3 (G 200): rats received G at a daily dose of 200 mg/kg b. wt. [24] by i.p. injection for 60 days.

Group 4 (LCT): rats received LCT at a daily dose of 61.2 mg/kg b. wt. (1/10 of LD50) [25] by oral gavage for 60 days.

Group 5 (LCT + 100): rats received the same dose of LCT, as in group 4, followed immediately by G, as in group 2, daily for 60 days.

Group 6 (LCT + 200): rats received the same dose of LCT, as in group 4, followed immediately by G, as in group 3, daily for 60 days.

The doses were adjusted weekly regarding to body weight changes to sustain comparable dose per kg rat's body weight till the end of experiment period.

### 2.3 Samples preparation

Body weight change was determined from the gap between the initial weight at the beginning and the final weight upon completion of study. Before weight recording, all rats were fasted for 10 h (water ad libitum) to exclude feeding error. All groups of rats were sacrificed

**Table 1** Primer pairs used for qPCR

	Primer sequence	Gene bank accessi-on number
P53	Forward primer: 5'-GAGCTGAATGAGGCCTTGG-3' Reverse primer: 5'-CTGAGTCAGGCC CTTCTGTCT T-3'	NM_022112
Bcl-2	Forward primer: 5'-CCT GTG GAT GAC TGA GTA CC-3' Reverse primer: 5'-GAG ACA GCC AGG AGA AAT CA-3'	NM009741
Beta actin	Forward primer: 5'-GGTCGGTGTGAACGGATTTGG-3' Reverse primer: 5'-ATGTAGGCCATGAGGTCCACC-3'	XM_017593963.1

under anesthesia of diethyl ether at the end of the 2 months. Liver were rapidly taken out, washed with ice-cold saline, and splitted into 3 parts. The first was the liver part (10% w/v) which was homogenized using Teflon tissue homogenizer (Omni International Inc., Kennewaw, GA, USA) in phosphate-buffered saline (PBS), then for 10 min using centrifuge at 3000 rpm the clear homogenates were obtained and frozen at  $-80^{\circ}\text{C}$  for subsequent analysis of oxidative stress parameters; the second part was kept frozen at  $-80^{\circ}\text{C}$  for gene and protein expression analysis. The third part was used for electron and light microscopic examination.

## 2.4 Biochemical assays

### 2.4.1 Assay of serum biochemical

For serum, the activities of alanine transaminase (ALT) and aspartate transaminase (AST) were spectrophotometrically determined using the methods of Schumann and Klauke [26]. Also, alkaline phosphatase (ALP) activity was estimated by spectrophotometer using the methods of Wenger et al. [27] and Rosalki et al. [28].

### 2.4.2 Assay of oxidative stress and antioxidant defense system

For liver homogenates, LPO content was calculated by measuring malondialdehyde (MDA) content using the Preuss et al. [29] method. Total thiol (T. thiol) content and the antioxidant enzyme activities, catalase (CAT), and superoxide dismutase (SOD) were estimated using the methods of Koster et al. [30], Cohen et al. [31], and Marklund and Marklund [32], respectively.

## 2.5 RNA isolation and quantitative reverse transcription polymerase chain reaction

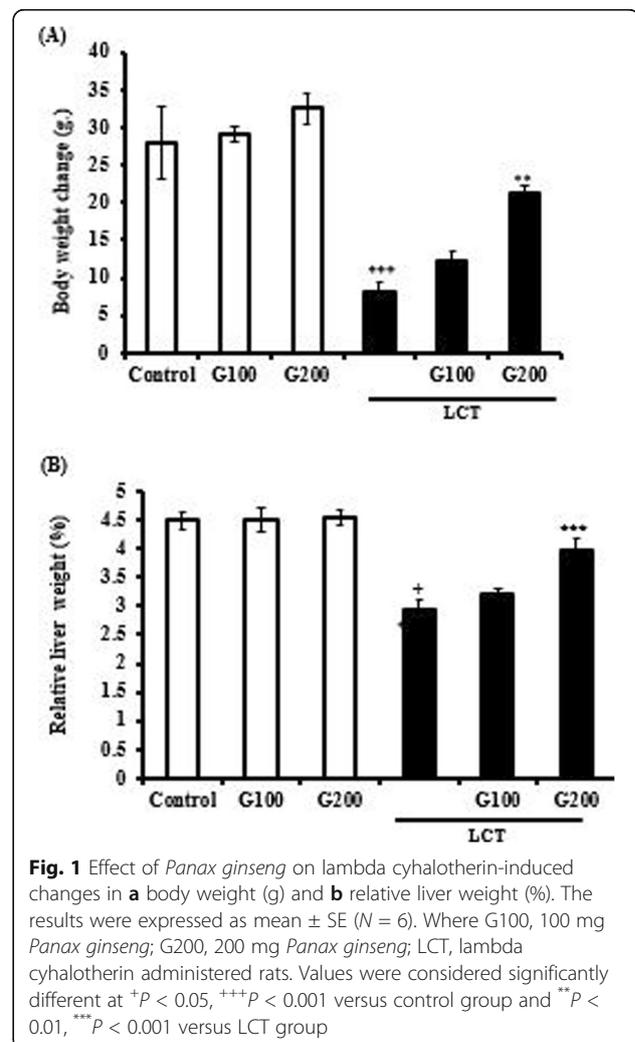
LCT effect on mRNA abundance of p53 and Bcl-2 was studied by qRT-PCR according to Mahmoud et al. [33]. Complementary DNAs were synthesized from 2  $\mu\text{g}$  RNA and were amplified using SYBR Green master mix (Thermo Fisher Scientific, USA) with the primer sets listed in Table 1. Quantitative polymerase chain reaction (qPCR) was conducted and the amplification data was analyzed by the  $2^{-\Delta\Delta\text{Ct}}$  method [34]. The results were normalized to  $\beta$ -actin and shown as percentage of control.

## 2.6 Western blot analysis

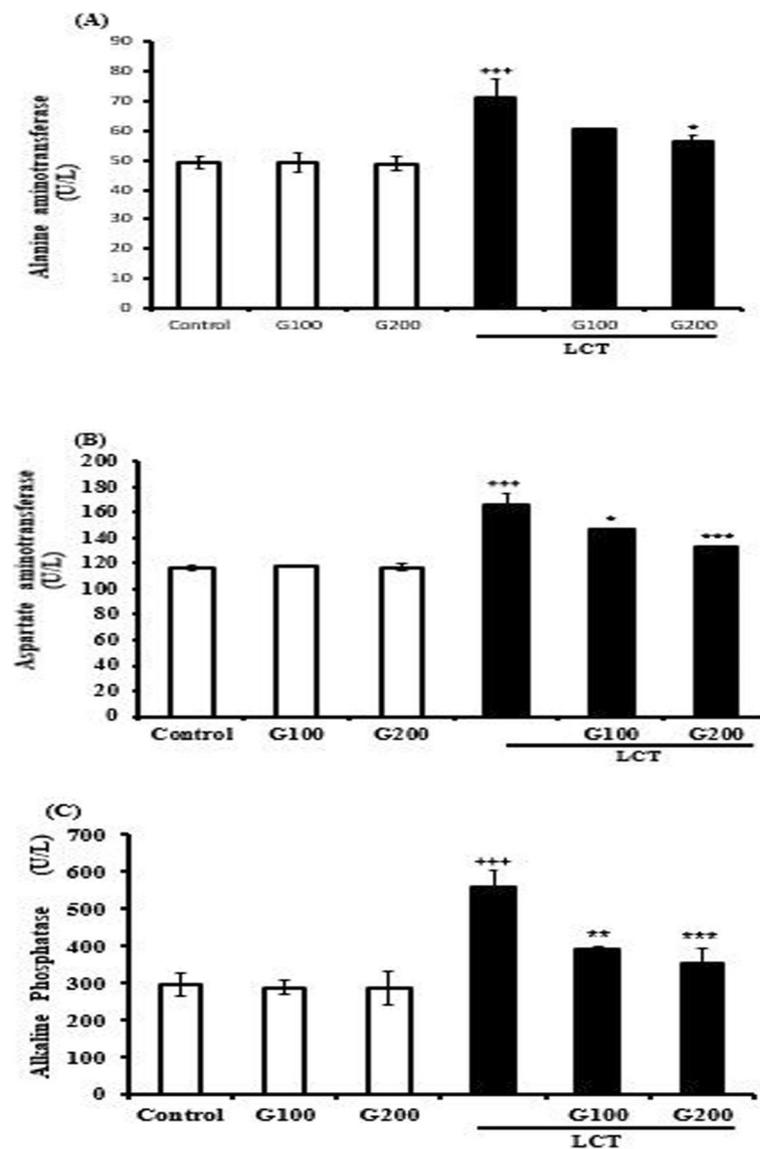
LCT effect on p53 and Bcl-2 expression levels were evaluated from the frozen liver samples by chemiluminescence kit (BIORAD, USA) [35].  $\beta$ -actin was used as a loading control.

## 2.7 Light microscopic study

Small parts of liver tissue were fixed for 24 h by 10% formalin-buffered phosphate. The tissue was processed, sectioned with a microtome at 4–5  $\mu\text{m}$



**Fig. 1** Effect of *Panax ginseng* on lambda cyhalothrin-induced changes in **a** body weight (g) and **b** relative liver weight (%). The results were expressed as mean  $\pm$  SE ( $N = 6$ ). Where G100, 100 mg *Panax ginseng*; G200, 200 mg *Panax ginseng*; LCT, lambda cyhalothrin administered rats. Values were considered significantly different at \* $P < 0.05$ , \*\*\* $P < 0.001$  versus control group and \*\* $P < 0.01$ , \*\*\* $P < 0.001$  versus LCT group



**Fig. 2** Effect of *Panax ginseng* on lambda cyhalothrin-induced changes in **a** alanine aminotransferase (U/L), **b** aspartate aminotransferase (U/L), and **c** alkaline phosphatase (U/L). The results were expressed as mean  $\pm$  SE ( $N = 6$ ). Where G100, 100 mg *Panax ginseng*; G200, 200 mg *Panax ginseng*; LCT, lambda cyhalothrin administered rats. Values were considered significantly different at  $^{+++}P < 0.001$  versus control group and  $^{*}P < 0.05$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$  versus LCT group

thickness, and then treated with hematoxylin and eosin stain (H&E) [36].

## 2.8 Ultrastructural study

Small liver portions of all groups were immediately fixed at 4 °C for 18–24 h in 3% glutaraldehyde-formaldehyde, washed in phosphate buffer, followed by 1% post-fixation in osmium tetroxide. In a sequence of alcohols, the specimens were then dehydrated, washed in propylene oxide, and eventually embedded in Epon epoxy resin. After that, an ultra-microtome trimmed the blocks, sectioning them with glass knives. Semi-thin sections (1 mm) have been

treated with toluidine blue stain and examined on light microscope in order to choose the correct region for the ultrathin parts. The same ultra-microtome was used for cutting sections of ultrathin (70–90 nm) and then treated with uranyl acetate and lead citrate stain [37]. Joel CX 100 transmission electron microscope, operated at an accelerating voltage of 60 kV, performed an examination of the stained sections.

## 2.9 Statistical analysis

Version 20 of the Social Sciences Statistical System (SPSS) for the Windows software system (SPSS Inc,

Chicago, IL, USA) was used for analyzing data. All statistical comparisons were created according to one-way ANOVA method accompanied by post-hoc analysis, and the results were shown as mean  $\pm$  standard error (SE). A value of  $P < 0.05$  was rated significant.

### 3 Results

#### 3.1 Effect of *Panax ginseng* on body weight change of LCT-intoxicated rats

Experimental animals orally administered LCT (61.2 mg/kg/day) for 60 exhibited a significant ( $P < 0.001$ ) decrease in body weight change in comparison with normal control group. However, intraperitoneal injection of G (200 mg/kg b. wt./day) caused a significant ( $P < 0.01$ ) elevation in body weight change in comparison with LCT-intoxicated rats. While intraperitoneal injection with 100 mg G /kg b. wt. daily for 60 days exhibited a non-significant ( $P > 0.05$ ) elevation in body weight change in comparison with LCT-intoxicated rats. Body weight of control groups treated with either tested doses of G did not show any difference from normal control group (Fig. 1a).

#### 3.2 Effect of *Panax ginseng* on relative liver weight of LCT-intoxicated rats

Relative liver weight exhibited a significant ( $P < 0.05$ ) decrease in LCT-treated rats in comparison with normal control group. Intraperitoneal injection of G (200 mg/kg b. wt./day) caused a significant ( $P < 0.001$ ) elevation in relative liver weight in comparison with LCT-treated rats. While intraperitoneal injection with 100 mg G/kg b. wt. daily for 60 days exhibited a non-significant ( $P > 0.05$ ) increase in relative liver body weight (Fig. 1b).

#### 3.3 Effect of *Panax ginseng* on liver function of LCT-intoxicated rats

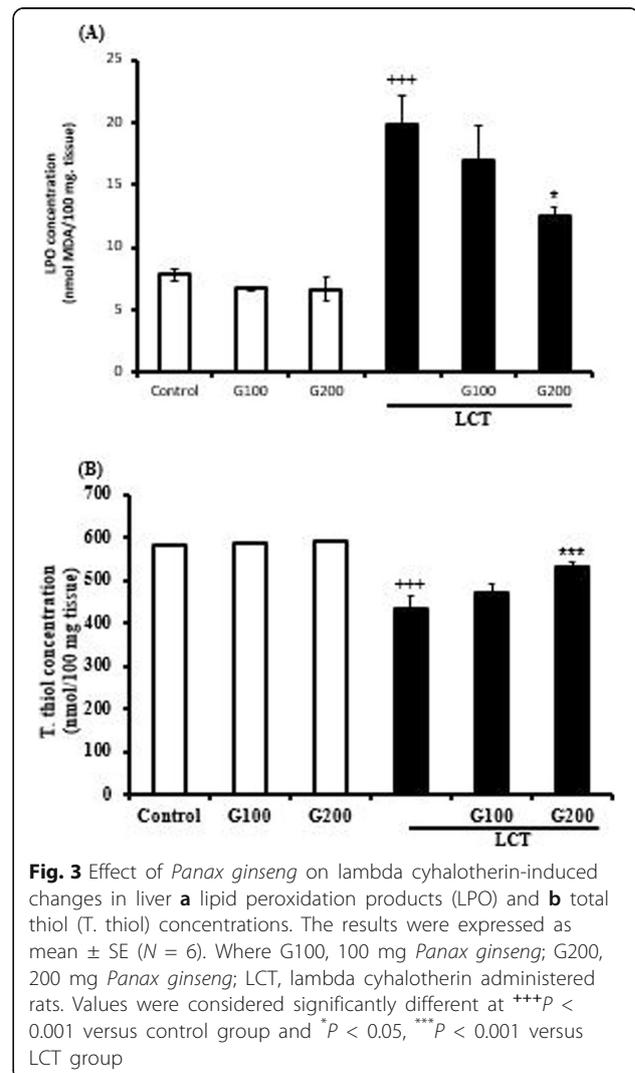
Both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) exhibited a significant ( $P < 0.001$ ) increased activity in serum of LCT-treated rats in comparison with control group. Intraperitoneal injection of G (200 mg/kg b. wt./day) caused a significant ( $P < 0.05$ ) decrease in ALT activity and a significant reduction in AST activity when compared to LCT-intoxicated rats. While intraperitoneal injection with 100 mg G/kg b. wt. daily for 60 days showed a non-significant ( $P > 0.05$ ) decrease of ALT activity and a significant ( $P < 0.05$ ) decrease in AST activity (Fig. 2a, b).

Alkaline phosphatase (ALP) showed the same behavioral pattern as ALT and AST. It was significantly ( $P < 0.001$ ) elevated in serum of LCT-administered rats and decreased as a result of G administration. However, 200 mg G seemed to be more effective ( $P < 0.001$ ) than 100 mg G ( $P < 0.01$ ) in decreasing serum ALP activity (Fig. 2c).

#### 3.4 Effect of *Panax ginseng* on liver LPO and T. thiol content of LCT intoxicated rats

As elucidated in Fig. 3a, liver lipid peroxidation (LPO), estimated as nmol malondialdehyde (MDA)/100 mg tissue, exhibited a significant ( $P < 0.001$ ) elevation in LCT-treated rats in comparison with normal control group. According to the administered dose of G, There is a varying significance in MDA level decrease. Intraperitoneal injection of G (200 mg/kg b. wt./day) showed a significant ( $P < 0.05$ ) reduction in MDA levels. While intraperitoneal injection with 100 mg G/kg b. wt. daily for 60 days showed a non-significant ( $P > 0.05$ ) decrease in MDA level (Fig. 3a).

LCT-intoxicated rats showed a significant ( $P < 0.001$ ) reduction of liver T. thiol contents in comparison with normal control group. However, intraperitoneal injection of 100 mg G/kg b. wt. for 60 days produced a non-significant ( $P > 0.05$ ) elevation in T. thiol content in comparison with LCT-intoxicated group. While



treatment with 200 mg G /kg/day significantly ( $P < 0.001$ ) increased hepatic total thiol level when compared to LCT-intoxicated rats as shown in (Fig. 3b).

### 3.5 Effect of *Panax ginseng* on liver superoxide dismutase (SOD) and catalase (CAT) activities of LCT-intoxicated rats

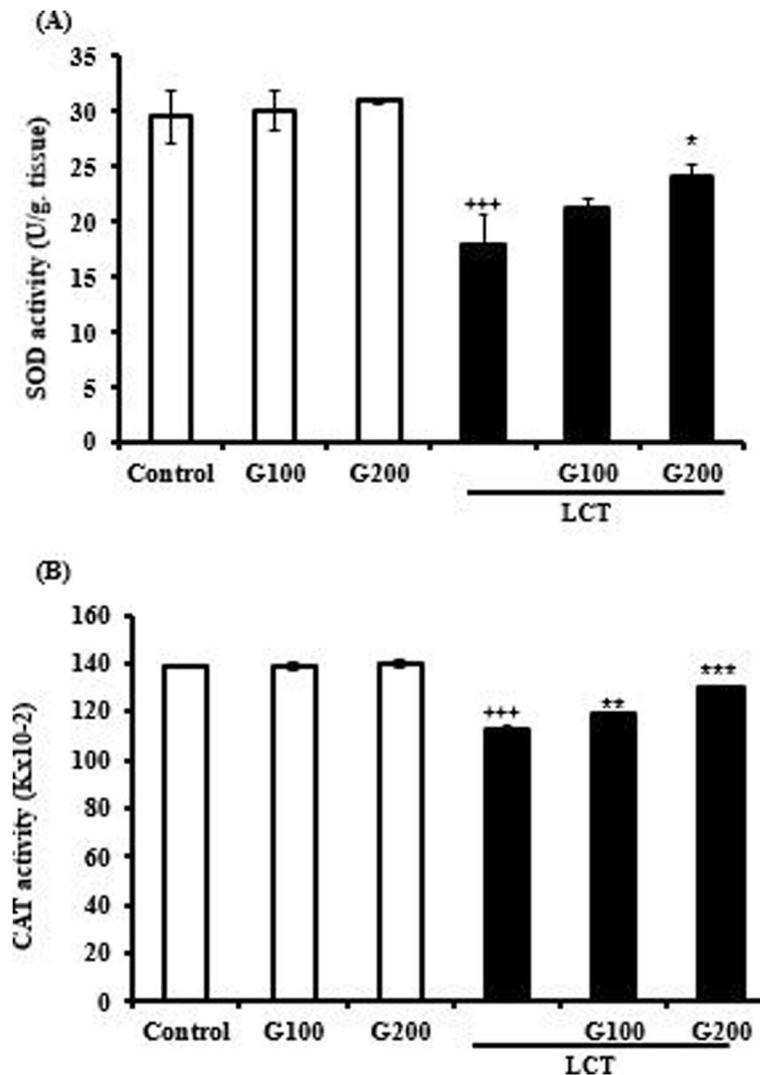
Figure 4 shows the effect of LCT administration on liver SOD and CAT activities. The data recorded obviously showed a significant ( $P < 0.001$ ) reduction in both CAT and SOD activities in LCT-administered group in comparison with control rats. Along concurrent injection with LCT, G elevated the activities of CAT with varying significance. However, 200 mg G seemed to be more effective ( $P < 0.001$ ) than 100 mg G ( $P < 0.01$ ) in increasing the activities of CAT (Fig. 4b). Regarding SOD (Fig.

4a), only 200 mg produced a significant ( $P < 0.05$ ) increase in liver SOD activity while 100 mg produced a non-significant ( $P > 0.05$ ) increase.

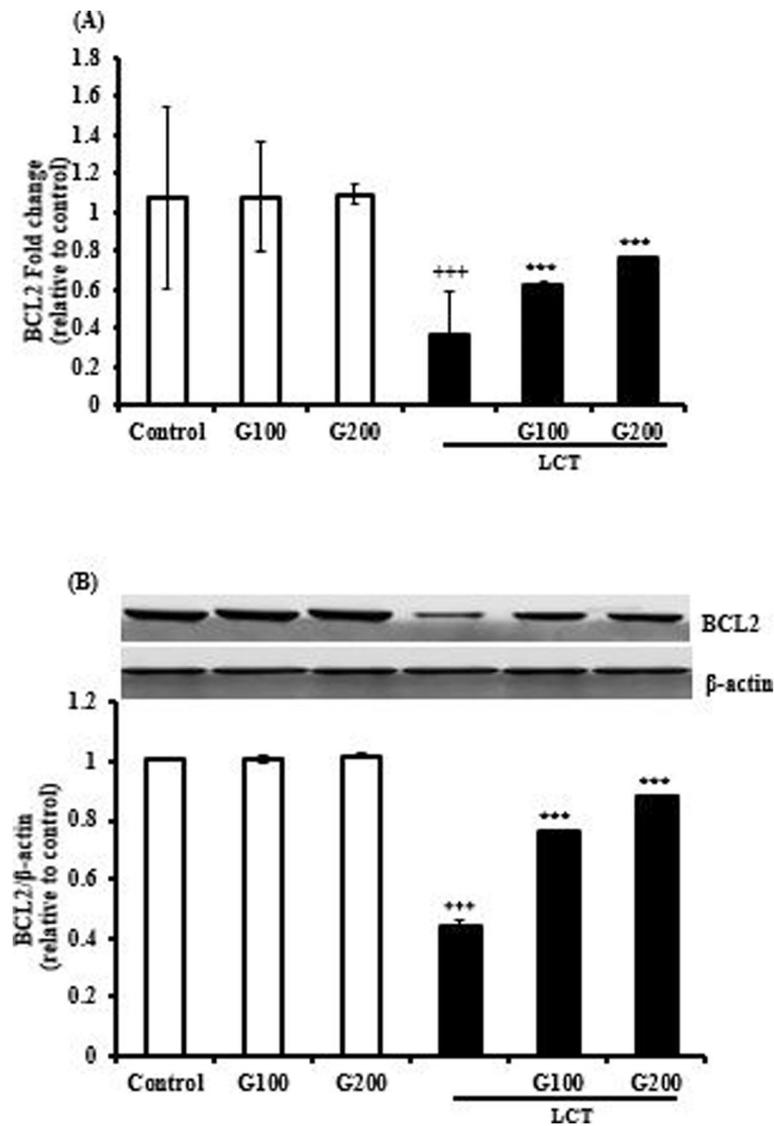
### 3.6 Effect of *Panax ginseng* on liver apoptotic P53 gene and protein expression levels of LCT-intoxicated rats

As shown in Fig. 5 a, mRNA expression of P53 in liver tissue of LCT-intoxicated rats exhibited a significant ( $P < 0.001$ ) upregulation as compared to normal control group. While intraperitoneal co-injection with both tested doses of G induced a significant ( $P < 0.001$ ) downregulation of P53 mRNA expression levels as compared to rats that received LCT only.

Protein expression level of apoptotic protein P53 in liver tissue of LCT-intoxicated rats showed a significant



**Fig. 4** Effect of *Panax ginseng* on lambda cyhalothrin-induced changes in liver enzymatic antioxidant defenses **a** superoxide dismutase (SOD) and **b** catalase (CAT) activities. The results were expressed as mean  $\pm$  SE ( $N = 6$ ). Where G100, 100 mg *Panax ginseng*; G200, 200 mg *Panax ginseng*; LCT, lambda cyhalothrin administered rats. Values were considered significantly different at  $^{+++}P < 0.001$  versus control group and  $^*P < 0.05$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$  versus LCT group



**Fig. 5** Liver **a** BCL2 mRNA and **b** BCL2 protein expression levels in control, G, LCT, and LCT rats co-treated with G. The results were expressed as mean  $\pm$  SE ( $N = 6$ ). Where C, control group; G, *Panax ginseng* injected rats; and LCT, lambda cyhalothen administered rats. Values were considered significantly different  $^{+++}P < 0.001$  versus control group and  $^{***}P < 0.001$  versus LCT group

( $P < 0.001$ ) elevation in comparison with control group. Intraperitoneal injection of both tested doses of G concomitant with LCT produced a significant ( $P < 0.001$ ) reduction in P53 protein expression levels in comparison with LCT-administered rats as illustrated in (Fig. 5b).

### 3.7 Effect of *Panax ginseng* on liver anti-apoptotic B cell lymphoma-2 (Bcl-2) gene and protein expression level of LCT-intoxicated rats

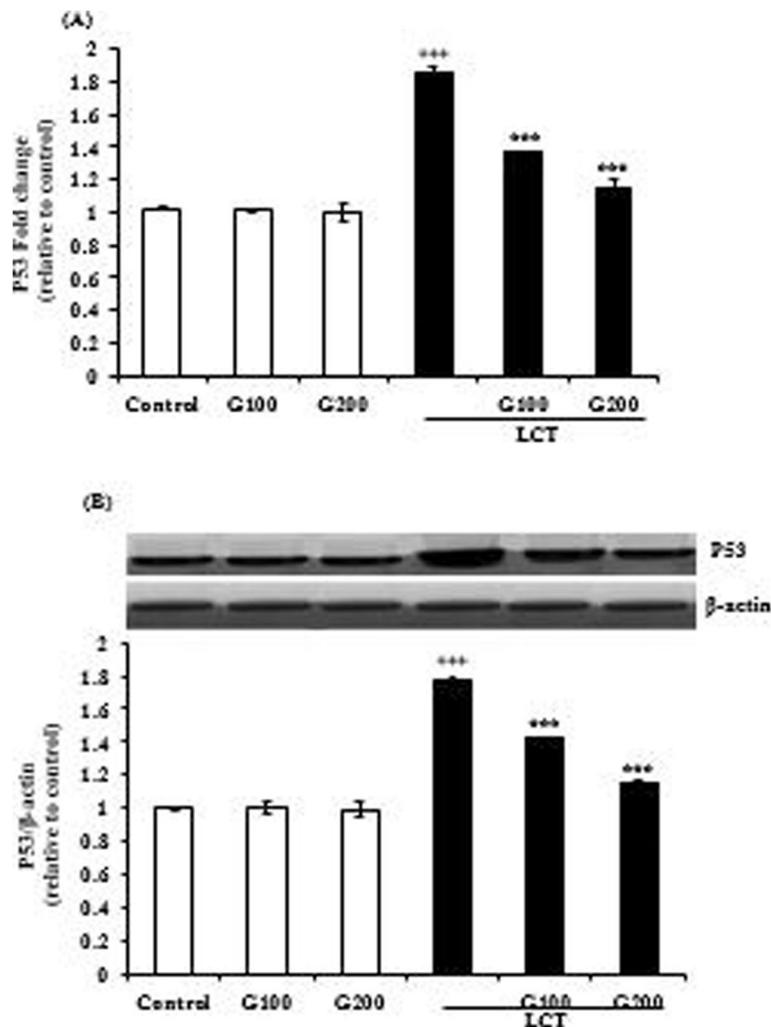
qPCR analysis of Bcl-2 gene expression in liver of LCT-intoxicated rats exhibited a significant ( $P < 0.001$ ) downregulation as compared to normal rats. Simultaneous administration of both tested doses of G with LCT induced a

significant ( $P < 0.001$ ) upregulation of Bcl-2 mRNA expression levels as compared to LCT-intoxicated group (Fig. 6a).

Western blotting data exhibited a significant ( $P < 0.001$ ) decrease in Bcl-2 protein expression level in liver tissues of LCT group as compared to the normal control group. Intraperitoneal co-injection of G with LCT produced a significant ( $P < 0.001$ ) elevation in protein expression levels in comparison with LCT group (Fig. 6b).

### 3.8 Effect of *Panax ginseng* on liver histopathology of LCT-intoxicated rats

The histological examination of the liver of control, G100 mg/kg b. wt./day and G200 mg/kg b. wt./day (Fig. 7 a–c)



**Fig. 6** Liver **a** P53 mRNA and **b** P53 protein expression levels in control, G, LCT, and LCT rats co-treated with G. The results were expressed as mean  $\pm$  SE ( $N = 6$ ). Where C, control group; G, *Panax ginseng* injected rats; and LCT, lambda cyhalothen administered rats. Values were considered significantly different  $***P < 0.001$  versus control group and  $***P < 0.001$  versus LCT group

showed that it consists of numerous hepatic lobules; in the center of each of these hepatic lobules, there is a central vein. Each lobule is consisted of cords of hepatocytes radiating from the central vein. The cell cords are separated by narrow blood sinusoids which lined by Kupffer cells and endothelial cells. The hepatocytes are polygonal in shape with acidophilic cytoplasm and rounded darkly stained nucleus. The portal triads are distributed at the corners of the lobules and representing branches of the portal vein, hepatic artery, and bile duct.

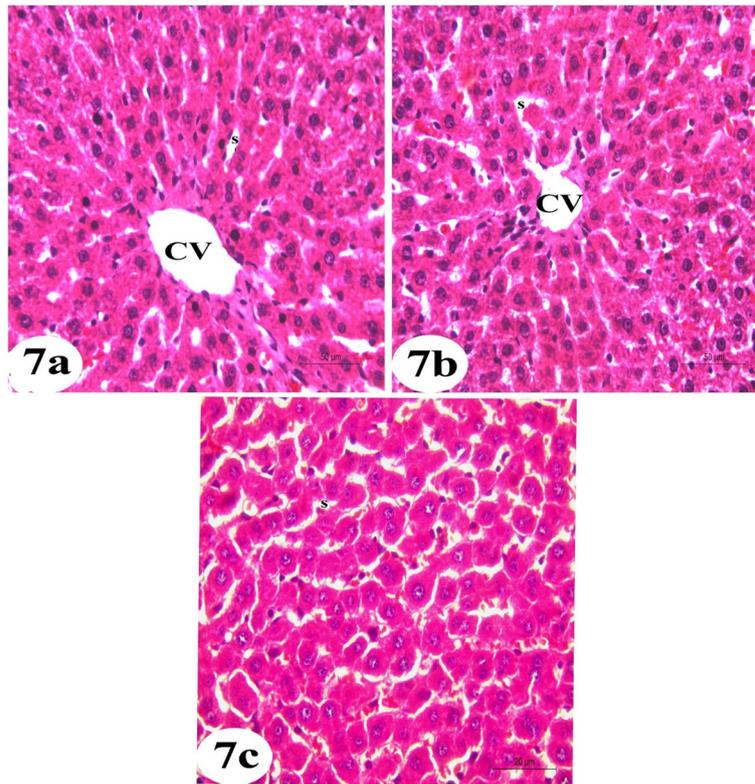
LCT-intoxicated group showed obvious histopathological changes; these include mononuclear leukocytic infiltration in the parenchyma, congested portal vein with thickened wall, proliferation of bile duct, where some of them with hyperplastic wall, edema, and hepatocytes with hyper eosinophilic cytoplasm were also detected (Fig. 8 a, b). Dilated hyperemic sinusoids,

extensive vacuolar degeneration of hepatocytes, and many hepatocytes with pyknotic and karyolytic nuclei were also seen (Fig. 8 c, d). Some hepatocytes that undergo fatty change also detected (Fig. 8 d).

Liver of LCT-intoxicated rat treated with G 100 mg/kg b. wt./day revealed relative recovery except some vacuolar degeneration of hepatocytes and dilated sinusoids (Fig. 9 a). Liver of LCT-intoxicated rat treated with G 200 mg/kg b. wt./day showed marked amelioration and restoration of normal appearance of hepatocyte and central vein (Fig. 9 b).

### 3.9 Effect of *Panax ginseng* on liver ultrastructure of LCT-intoxicated rats

Electron microscopic examination of control rats liver sections showed normal ultrastructure. The hepatocyte cytoplasm includes various mitochondria that appear round in shape. The rough endoplasmic reticulum



**Fig. 7** Photomicrographs of sections of liver of **a** control rats and **b, c** ginseng groups, treated with 100 and 200 mg/kg b. wt./day respectively, stained with H&E: showing normal histological structure of liver; radially arranged polyhedral hepatocytes forming cords around the central vein (CV) and sinusoids (S) (scale bar = 50 µm)

consists of closely packed parallel and flattened cisternae. Several electron-dense glycogen rosettes were obviously observed. The hepatocyte nucleus appears spherical with a distinct nuclear envelope and one or more prominent nucleoli. The nucleoplasm revealed a univacuolar distribution of a little quantity of heterochromatin peripherally and centrally large quantity of euchromatin. Normal bile canaliculi with normal structure of microvilli appeared. Also, Kupffer cell with normal nucleus was observed (Fig. 10 a, b).

LCT-intoxicated rats revealed marked ultrastructural changes. In hepatocytes, the mitochondria appeared vacuolated, cytoplasmic vacuolations, fat droplets, and collagen fibers were also observed (Fig. 11 a–c, e). Damaged bile canaliculi with destructed microvilli (Fig. 11 d) and damaged Kupffer cell with shrunken nucleus (Fig. 11 a) were also seen.

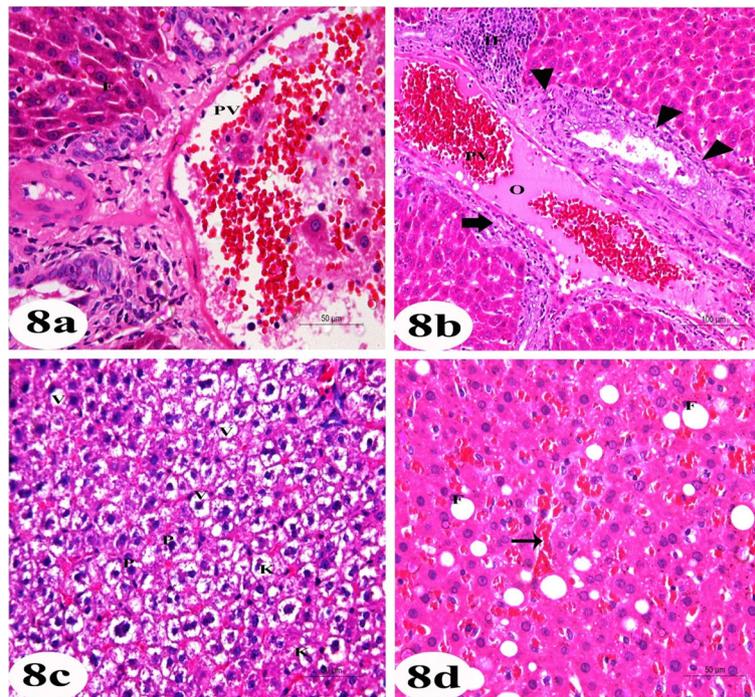
On the other hand, treatment of LCT-intoxicated rats with 100 mg G/kg b. wt./day resulted in relative improvement in the constituents except some cytoplasmic vacuolation and some fat droplets in hepatocyte (Fig. 12 a–c). While treatment of LCT-intoxicated rats with 200 mg G/kg b. wt./day restore normal appearance of hepatocyte, Kupffer cell, and bile canaliculi (Fig. 13 a–c).

#### 4 Discussion

LCT, pyrethroid insecticide type II, is extensively utilized in public health and agriculture controlling pests [38]. Its widespread use consequently leads to the exposure of its possible toxic effects either directly by exposure of field applicators, manufacturing workers, and the ecosystem or indirectly by consumption of vegetables, fruits [39], and meat or milk of animals that exposed to LCT [40, 41].

Liver is the principal site of pyrethroid metabolism where large quantities of their metabolites are accumulated [42]. LCT metabolism occurs rapidly in liver by hydrolytic cleavage of ester and oxidative pathways via CYP450 enzymes leading to ROS [43] causing damage of various components of cell membranes [14] resulting in alterations of the antioxidant status, ROS, the antioxidant enzymes, LPO [44], and apoptosis that represent major contributors in hepatic damage. Thus, our study estimates the potential ameliorative effect of G in rat model of LCT-induced hepatotoxicity.

In the current study, terminal body weight and relative weight of liver of LCT-intoxicated rats were reduced that run in a good agreement with previous studies [14, 45–48].



**Fig. 8** Photomicrographs of sections of liver of LCT-treated rats for 60 days stained with H&E. **a, b** Showing mononuclear leukocytic infiltration (IF), congested portal vein (PV) with thickened wall (thick arrow), edema (O), eosinophilic hepatocytes (E), and proliferation of bile duct, where some of them with hyperplastic wall (arrow head) (scale bar = 50 and 100  $\mu\text{m}$  respectively). **c, d** Showing extensive vacuolar degeneration (V) of hepatocytes, dilated hyperemic sinusoids (thin arrow), and many hepatocytes with pyknotic (P) and karyolytic (K) nuclei, fatty changes (F) (scale bar = 50  $\mu\text{m}$ )

Elhalwagy et al. [1] demonstrated that decreasing body weight and organs relative weight is one of the chronic effects of pyrethroids toxicity. In LCT-intoxicated rats, the observed decrease may be directly linked to hypophagia or emaciation because of LCT's direct cytotoxicity [45].

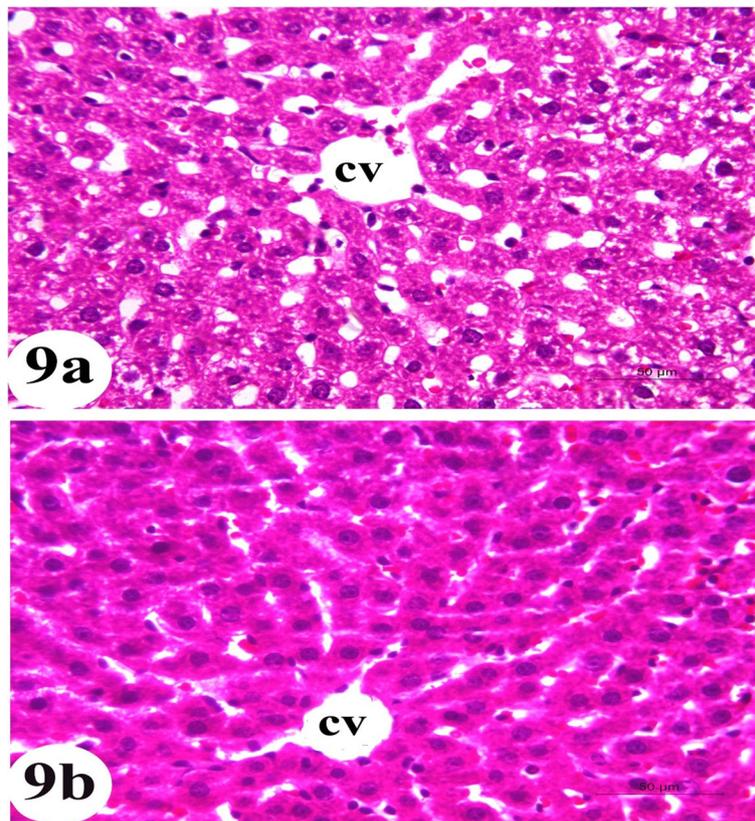
Both *in vivo* and *in vitro* researches reported that G decrease biological, physical, and chemical stress and maintain general vitality [49]. In our study, treatment of rats with LCT in concomitant with G 100 or 200 mg/kg b. wt./day increased terminal body weight, relative weight of liver, as compared to LCT-intoxicated ones matching with previous studies. Qadir et al. [50] demonstrated that G protects mice from body weight loss and improved kidney weight induced by gentamicin toxicity.

The current study indicated that LCT-intoxication caused extensive damage in the liver of rats that was confirmed by the histopathological and ultrastructural alterations and reflected by marked elevations of AST, ALT, and ALP activities, which are the circulating markers of hepatocytes injury.

Increased serum ALT and AST activities reflect LCT-induced hepatotoxicity, causing these enzymes to leak into blood [51, 52]. These observations matched with earlier finding of LCT toxicity in rats [1, 8, 15, 47, 53,

54]. ALT activity reflects general hepatic damage and AST activity indicates mitochondrial damage; elevated levels of ALP in blood are regarded as an indicator of hepatic necrosis and cholestasis [55, 56]. The elevated levels of serum ALT and AST have been related to liver structural integrity damage [57, 58], as they are cytoplasmic enzymes and are released into blood after hepatic injury [59]. Releasing of metabolically toxic intermediates able to cause hepatocellular injury which happen during LCT metabolism in the liver, leading to these enzymes leakage to blood [60]. The increase or decrease of enzyme activity depends on the intensity of cellular damage. In the present study, the increased LPO products (MDA) level in liver of LCT-intoxicated rats and the elevation AST and ALT activities in serum proposed that LCT induced hepatocellular injury and this injury happened likely by ROS. That suggests that an elevation in the activity ALT in parallel with reduction in free radical scavenging enzymes activities [1, 14, 53] could be representative of LCT-induced hepatic pathological alterations.

ALP is excreted through the liver by bile. Cholestasis, due to bile duct obstruction, cause enzymes to regurge that raise its blood level due to its back pressure that lead it to leach into blood. Biliary impairment could be



**Fig. 9** Photomicrographs of sections of liver of treated rats stained with H&E **a** with LCT plus ginseng 100 mg/kg b. wt./day showing relative recovery except vacuolar degeneration of hepatocytes and dilated sinusoids (scale bar = 50 µm). **b** With LCT plus ginseng 200 mg/kg b. wt./day group showing approximate regain of normal appearance of hepatocytes and central vein (CV) (scale bar = 50 µm)

explained similarly. Elevated ALT, AST, and LDH activities concomitant with an increase in level of bilirubin in serum indicate cholestasis that lead to elevated serum ALP level [61]. Destruction of hepatic tissue (necrosis) also may have accounted for elevation in ALP level in serum. Because of pesticide toxicity, parenchymal cells of liver are deteriorated and necrosed, thus ALT, AST, and ALP release into the circulating blood stream, resulting in increased levels [62, 63]. Bhushan et al. [56] reported that an increase of serum ALP could be linked initially to some hepatic patho-physiological alterations as a result of pesticide toxicity, likely because of hepatocytes membrane permeability damage, so enzymes leak to blood stream.

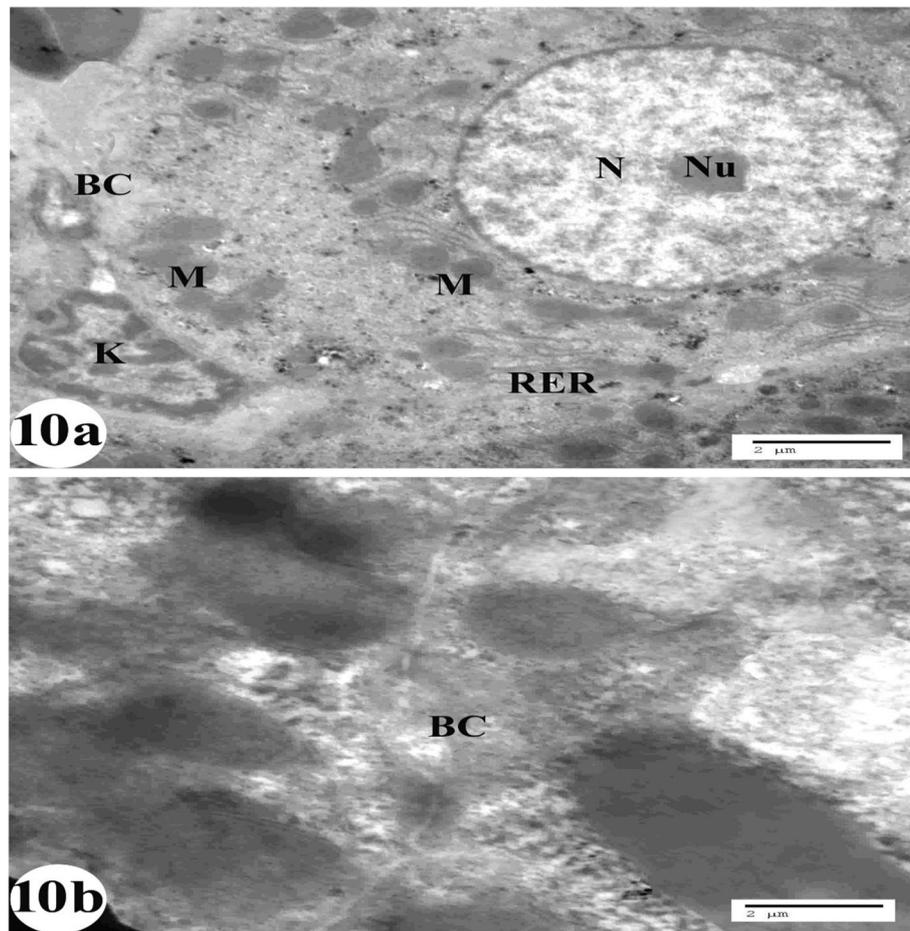
In the present study, treatment of LCT-intoxicated rats with either 100 or 200 mg G/kg b. wt./day produced a profound improvement of the altered ALT, AST, and ALP activities in serum. G reduced the elevated of AST, ALT, and ALP activities in serum of fipronil intoxicated rats and 100 mg G/kg b. wt./day [64] or chlorpyrifos and profenofos and 200 mg G/kg b. wt./day [65], conforming the protective effect of G against liver damage.

Oxidative stress has been described as losing balance between antioxidants and oxidants due to general elevation in ROS cellular levels [66]. It is known as a risk factors for the development of various diseases [67].

ROS are compounds that contain oxygen generated via general metabolic pathways, which are reported to cause oxidative damage to lipids, proteins, and nucleic acid [68].

Pesticides cause oxidative stress that leads to free radicals formation, changes in the levels of antioxidants, and LPO [69]. LPO is widely used as an oxidative stress marker. MDA is a main oxidation product of peroxidized polyunsaturated fatty acids and elevated MDA level is a LPO's accurate determinant [70]. In the current study, LCT produced a significant increase in the level of MDA in liver tissues. The increase in extent of LPO likely because of free radicals production as a result of LCT exposure. These observations are matched with earlier finding of LCT-hepatotoxicity [1, 8, 14, 53, 54, 71].

LPO results from lipid reaction with free radicals and this is regarded as a significant aspect of cellular damage caused by attacks of free radical [72]. This indicates that



**Fig. 10** Electron micrographs of a sections of liver of a control rats **a** showing hepatocytes contains numerous mitochondria (M), rough endoplasmic reticulum (RER) consists of closely pack parallel and flattened cisternae, spherical nucleus (N) appears with a distinct nuclear envelope and prominent nucleoli (Nu), Kupffer cell (K) with normal nucleus and normal bile canaliculi (BC) (scale bar = 2  $\mu\text{m}$ ). **b** Showing high magnification of normal bile canaliculi (BC) with normal structure of microvilli (scale bar = 2  $\mu\text{m}$ )

elevated LPO could be among the molecular mechanisms implicated in the toxicity caused by LCT in rats.

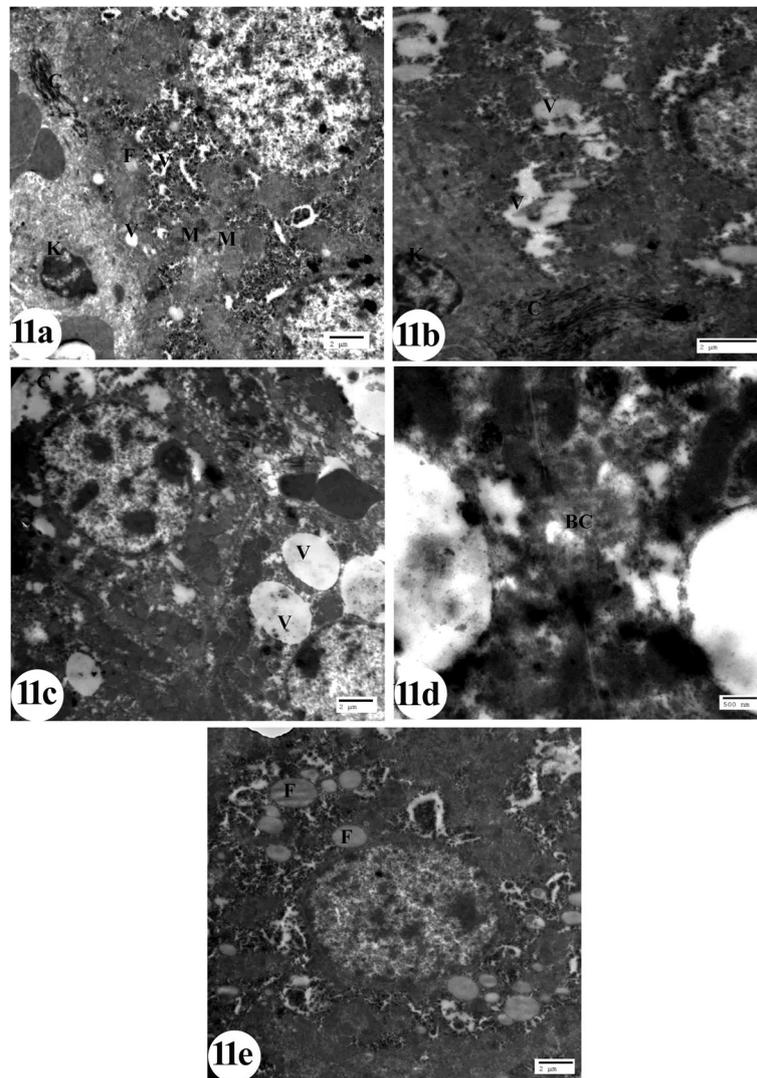
Even so, in case of heavily established oxidative stress, the defense capacities against ROS becomes insufficient; in turn, ROS also affect the antioxidant defense mechanisms, decreases the intracellular thiol level, the antioxidant enzymes activities is altered, and MDA is increased [71]. Indirectly, these indicate an elevated generation of oxygen free radicals. Highly reactive oxygen metabolites, especially  $\cdot\text{OH}$ , produce MDA by acting on unsaturated fatty acids of phospholipid components of membranes [73].

CAT and SOD play a major role in quenching of ROS. SOD act as catalyst for breaking down of  $\text{O}_2\cdot^-$  to  $\text{H}_2\text{O}_2$ , while CAT act as catalyst for decomposition of  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and  $\text{O}_2$  to inhibit oxidative stress and to maintain cell homeostasis. In the current study, CAT and SOD activities were significantly reduced in liver of LCT-treated rats that run in parallel with the results of

[8, 15, 25, 74, 75]. Both CAT and SOD act with each other for ROS elimination, and minor deviations in physiological levels could have a dramatic effect on cellular proteins, nucleic acid, and lipids resistance to oxidative stress [76]. In the LCT group, low CAT and SOD levels may be attributed to these enzymes consumption due to elevated oxidative stress in liver.

Thiols are a sulphhydryl group containing organic compounds. Among all the body's antioxidants, thiols represent the main part of the total body antioxidants and they have an important role in defense against ROS. Thiols comprised of both extracellular and intracellular thiols either in the free form as reduced or oxidized glutathione, and protein-bound thiols [77]. In the current study, T. thiol is decreased in liver of LCT-intoxicated rats matching those of previous study [71].

Antioxidants are the compounds which react with ROS for slowing their action and for neutralizing them, thus reducing oxidative stress and protecting us from



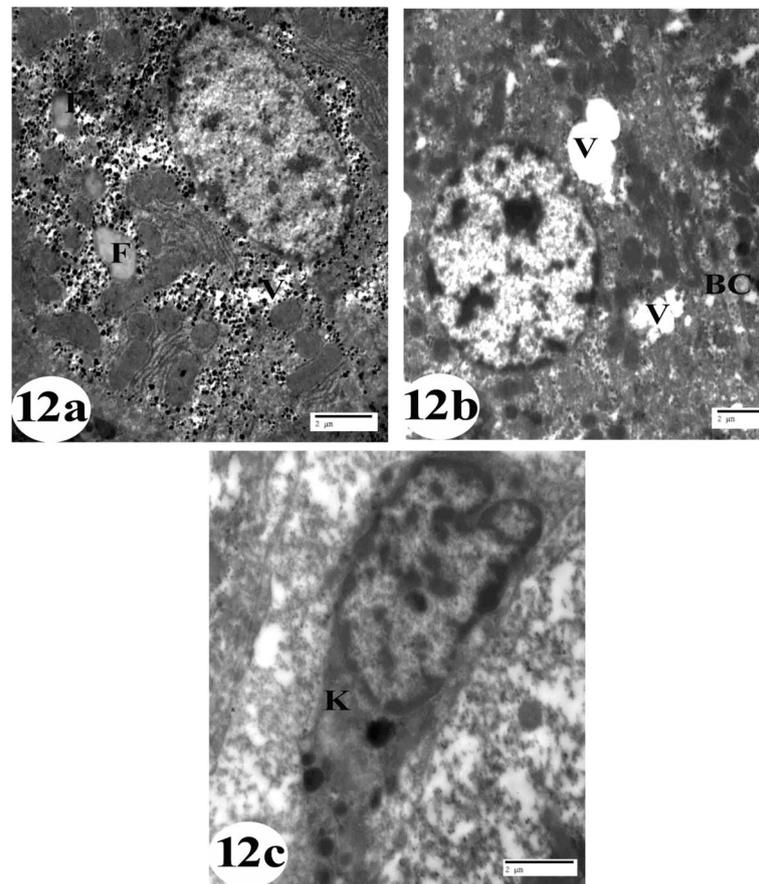
**Fig. 11** Electron micrographs of a sections of liver of rats treated with LCT **a-c, e** showing hepatocytes with cytoplasmic vacuolations (V), vacuolated mitochondria (M), fat droplets (F), collagen fibers (C), and damaged Kupffer cell (K) (scale bar = 2  $\mu$ m). **d** Bile canaliculi with damaged microvilli (BC) (scale bar = 500 nm)

ailments [78]. Normally, our cells are able to inhibit ailments caused by free radicals by producing its endogenous antioxidants or via taking them from food [79]. Some synthetic antioxidants were used for preventing oxidative stress may cause side effects [16]. Thus, the regular consumption of natural antioxidant in diets is regarded very important to ban a broad spectrum of ailments, such as allergies, some types of cancer, hepatic and cardiovascular ailments, and inflammation that include free radical-mediated damage in pathologically generating processes [80].

The antioxidant properties of G are well documented. G exhibits antioxidant activity by improving the expressions of antioxidant enzyme genes that help to scavenge ROS. G intake stimulates both antioxidant enzyme

activity and scavenging of free radicals [81]. G improved the antioxidant defense mechanism by increasing self-antioxidant enzyme activities (SOD, CAT, GPx, GR, GSH) and heme oxygenase-1 in the aged-rat liver [19, 21] and inhibition of lipid peroxidation [23, 28]. However, its effect on inhibition of hepatotoxins-induced liver damages in rats cannot be neglected [22, 82].

In the present study, co-administration of G with LCT caused a significant decrease in the mean value of MDA and a significant increase in the mean values of antioxidant enzymes (SOD and CAT) activities and T. thiol in liver as compared with LCT-treated group. Similarly, Al-Harbi et al. [64] demonstrated that G, due to its antioxidant properties, reduced MDA level, and elevated CAT and SOD activities in fipronil treated rats. Also, Diab

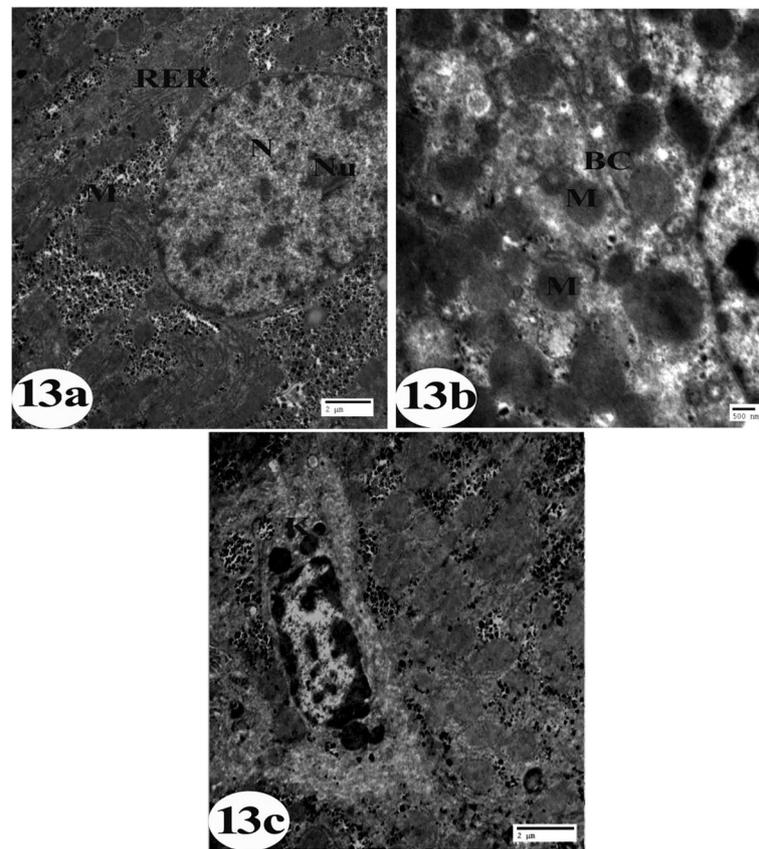


**Fig. 12** Electron micrographs of a sections of liver of LCT + 100 mg ginseng/kg b. wt./day treated rats **a, b** showing hepatocyte except some cytoplasmic vacuolations (V), some fat droplets (F), and normal bile canaliculi (BC) (scale bar = 2  $\mu$ m). **c** Showing Kupffer cell (K) (scale bar = 2  $\mu$ m)

et al. [65] reported that G administration decreased MDA levels, and increased SOD, CAT activities in chlorpyrifos and profenofos-treated animals.

In conjunction with impaired antioxidant defense system, the present study exhibited significant elevation in mRNA and protein expression levels of hepatic p53 concomitant with decrease in mRNA and protein expression levels of Bcl2 in LCT-treated rats in comparison with control group. Apoptosis, the process of programmed cell death, is necessary for management of numbers of cells by elimination of damaged cells for better functioning of the body. But excessive tissue damage is caused by an uncontrolled and unconditioned apoptosis. Exposure of rats to LCT results in the generation of ROS, DNA fragmentation, and apoptosis [53, 66]. A possible mechanism of LCT-caused apoptosis was demonstrated to be related with activation of a transcription factor, nuclear factor-kappa B (NF- $\kappa$ B) which is a critical activator of genes involved in immunity, inflammation, and apoptosis [66]. However our study did not estimate NF- $\kappa$ B; Martínez et al. [66] reported an elevation of mRNA

expression level of NF- $\kappa$ B and interleukin (IL-1 $\beta$ ) in hepatic tissues secondary to LCT-induced oxidative stress. Activation of NF- $\kappa$ B causes reduction in proliferation of cells and elevation in apoptosis by ROS-caused DNA damage and p53 activation. Activated p53 due to LCT oral exposure also activates the intrinsic mitochondrial apoptotic pathways responding to DNA damage by stimulating the expression of pro-apoptotic proteins (Bax, Casp-3), downregulates Bcl-2 expression, and shifts the balance toward pro-apoptotic effects [66]. Co-treatments with G significantly decrease mRNA and protein expression of p53 and increase mRNA and protein expression of Bcl-2 in hepatic tissue in comparison with LCT group. Thus, it is logical to believe that an enhanced apoptotic rate in co-treated groups can be ascribed to antioxidant properties of G and the subsequent ROS scavenging, suppression of NF- $\kappa$ B activation, and cytokines release. G decreased the gene expression of the pro-apoptotic proteins p53 and caspase-3, while elevated expression of the anti-apoptotic Bcl2 in neuroblastoma cells suggesting the protective effects of G



**Fig. 13** Electron micrographs of a sections of liver of LCT + 200 mg ginseng/kg b. wt./day treated rats **a, b** showing normal hepatocyte with spherical nucleus (N) appears with a distinct nuclear envelope and prominent nucleoli (Nu), numerous mitochondria (M), rough endoplasmic reticulum (RER), and normal bile canaliculi (BC) (scale bar = 2  $\mu$ m and 500 nm respectively). **c** Showing normal ultrastructure of Kupffer cell (K) (scale bar = 2  $\mu$ m)

against the cell death in oxidative stressed brain cells [83]. Also, G suppresses TNF- $\alpha$ /IFN-c-induced thymus- and activation-regulated chemokine (TARC) expression through NF- $\kappa$ B-dependent signaling in HaCaT cells. G improved 2,4-dinitrochlorobenzene (DNCB)-induced dermatitis severity, serum levels of IgE and TARC, and mRNA expression of TARC, TNF- $\alpha$ , IFN-c, IL-4, IL-5, and IL-13 in mice. G inhibited TNF- $\alpha$  /IFN-c-induced NF- $\kappa$ B activation [84].

Another molecular mechanism study found that G suppresses nuclear factor-kappa B (NF- $\kappa$ B) [85, 86].

The present histopathological observations of liver sections of LCT-intoxicated rats showed mononuclear leukocytic infiltration, portal vein congestion with thickening in wall, and bile duct proliferation was also observed. In addition to hyperemic sinusoids, fatty changes, and edema. Degenerative changes were seen in the hepatocytes in the form of vacuolar degeneration, hyper eosinophilic cytoplasm, pyknosis, and karyolysis, in agreement with previous studies [15, 87, 88]. Also, Abdul Basir et al. [89] reported that LCT-intoxicated

rabbits exhibited hemorrhages in sinusoidal spaces hyperplasia of bile duct, karyolysis, and vacuolation. LCT-intoxicated mice also showed hepatocyte degeneration, vascular degeneration, mononuclear leukocytic infiltration, and portal vein appeared dilated and congested [90, 91].

In addition to similar histopathological observations, it has been reported by Mossa et al. [92] and Abdulhamid et al. [93] on the effect of cypermethrin (CYP), type II pyrethroids, on liver tissue. These histopathological changes in the hepatocytes due to CYP's inhibitory action on total adenine triphosphate (ATP) activity in the liver, that can disturb active transport of Ca<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup> ions, inducing hepatocytes damage [94].

Matching with histopathological findings, our ultrastructural study showed that LCT-intoxicated rats exhibited damaged bile canaliculi with destructed microvilli, Kupffer cell showed shrunk nucleus, hepatocytes with vacuolated mitochondria, cytoplasmic vacuolations, fat droplets, and collagen fibers in parallel line with similar

previous studies. Marked ultrastructural alterations were exhibited in previous studies of CYP intoxicated rats. In hepatocytes, mitochondria looked swollen, vacuolated with destructed cristae, cytoplasmic vacuolations, complete lysis of nucleus and dilated bile duct with destruction in microvilli, fat droplets, and cytoplasmic vacuoles were also observed. Moreover, Kupffer cell appeared with elongated nucleus, endocytic vesicles, and noticeable elevation in number of lysosomes [93, 95, 96].

Concerning the hepatocytes, fatty changes in the form of fat droplets in the cytoplasm can be rationalized by the high levels of LPO that resulted from LCT-induced oxidative damage, and its effect on membrane phospholipids.

After several types of hepatocellular damage, healing normally occur by regeneration of parenchymal cells and substitution of stromal elements coordinately that preserves accurately the lobular architecture of hepatic tissue. While, in some cases for unknown causes, abnormal quantities of collagen accumulate impairing the normal relationships between the parenchymal cells and its blood supply. That is known as liver fibrosis or cirrhosis, which can impair hepatic function or even death [97] that matching with the current ultrastructural observation of collagen accumulation in hepatic tissue of LCT-intoxicated rats.

In the current study, treatment of LCT-intoxicated rats with G 100 mg/kg b.wt./day and 200 mg/kg b. wt./day plus LCT protected liver of LCT hepatic toxicity and maintain approximately the normal histological structure matching with similar previous studies. El-Bialy et al. [9] demonstrated that G 200 mg/kg. b. wt./day ameliorates LCT and acetamiprid pesticides mixture for hepatotoxicity in rats and restore normal hepatic architecture.

## 5 Conclusion

The current study reported that G has shown therapeutic efficacy against LCT-induced histopathological and ultrastructural hepatic damage in rats. Moreover, the mechanisms sharing in its therapeutic efficacy involved free radicals scavenging, enhancing the antioxidants status, and anti-apoptotic properties were shown. Further researches are needed to illustrate the molecular mechanism of G against LCT-induced hepatotoxicity.

### Abbreviations

LCT: Lambda-cyhalothrin; LPO: Lipid peroxidation; MDA: Malonaldehyde; ROS: Reactive oxygen species; CAT: Catalase; SOD: Superoxide dismutase; T. thiol: Total thiol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; qPCR: Quantitative polymerase chain reaction; G: *Panax ginseng*

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### Authors' contributions

All authors have contributed significantly, Prof. Dr. MA, Dr. Prof. HMA, and Prof. Dr. SA have contributed in suggesting design of the work, preparation and analysis of the results, interpretation of data and discussion. In addition, Mr. KZ has performed the practical part. All authors are in agreement with

the contents of the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Ethics approval and consent to participate

In this study, animal care was carried out following the European Community Directive (86/609/EEC) and national rules, this is in accordance with the NIH Guidelines for care and use of Laboratory Animals, 8<sup>th</sup> editions. This was administrated by the committee of the Zoology Department, Beni-Suef University, Egypt.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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