Ameliorative Effects of Panax Ginseng on Lung of Lambada Cyhalotherin-intoxicated Rats

Original Article

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ABSTRACT

Introduction: Lambada-cyhalotherin (LCT) caused severe oxidative damage in liver, lung and testis.

Aim of the Study: The objective of this research was to evaluate the ameliorative activity and underlying techniques of pure Panax ginseng (G) using rat model of LCT-induced lung damage.

Materials and Methods: 36 male adult laboratory rats Rattus norvegicus domestica in weights around 135±10 g were separated into 6 experimental groups: 1st control group. 2nd and 3th groups were G groups (100 and 200 mg G/kg b. wt.) LCT group was given by oral gavage LCT (61.2 mg /kg b. wt.) that is equivalent to 1/10 of LD50. The 5th and 6th groups were co-treated with two doses of G.

Results: LCT significantly decreased superoxide dismutase (SOD), catalase (CAT) and total thiol (T. thiol) and increased lipid peroxidation (LPO). mRNA and protein expression levels of p53 gene (apoptotic gene) were increased, whereas, Bcl-2 gene (anti-apoptotic gene) mRNA and protein expression levels were decreased in LCT-treated animals. Also, light microscopic and ultrastructure studies for lung tissues of LCT-treated animals showed marked hyperplasia of dilated bronchioles wall, RBCs extravasation in bronchiolium lumen and mononuclear leukocytic infiltration in parenchyma. Additionally, blood vessels congested with thickened walls, alveoli appeared collapsed with compensatory expansion of adjacent alveoli divided by thickened inter-alveolar septa, besides to damage in type 1 and type 2 pneumocytes. G co-treatment attenuated oxidative stress biomarkers. Both tested doses of G significantly decreased p53 and elevate Bcl-2 mRNA and protein expression levels and revealed significant amelioration and restoration of normal histology and ultrastructure of lung.

Conclusion: In summary, G has exhibited ameliorative activity against oxidative stress induced by LCT in lung, apoptosis, histopathological and ultrastructural changes in albino rats.

INTRODUCTION

Pesticides are directly applied to the environment, agricultural land, vegetation, animal housing areas and animals[1]. Controlling flies, mosquitoes, ticks and cockroaches, which can act as disease vectors for both human and animals, is the most important application of it[2]. Pyrethroids are artificial analogs for natural pyrethrum but more toxic and longer lasting[3,4]. Because of their high effectiveness, low toxicity to birds and mammals and fast biodegradability, they are chosen over organophosphorus, organochlorine and carbamate pesticides[5]. Despite claiming that they have a low mammalian toxicity, several investigations reported the toxicological evidence of pyrethroid among various species of animals; in rats[6], in mice[7], in fishes[8] and in rabbit[9].

Lambda cyhalothrin (LCT), type II pyrethroid pesticide, used in public and animal health applications[10,11]. Residues of LCT was observed in dairy cows' blood and milk[13], vegetables, fruits[12] and in meat of cattle[14]. Placental transference of LCT in goats was observed[15]. LCT has been found to accumulate in biological membranes of tissue initiating reactive oxygen species (ROS) which alter the antioxidant systems and increase lipids peroxidation (LPO) in mammals[16]. LCT metabolism occur rapidly in liver by hydrolytic cleavage of ester and oxidative pathways via CYP450 enzymes leading to ROS[17]. Large quantities of ROS able to oxidize lipids, proteins and nucleic acids, thus lead to the formation of a wide spectrum of diseases as diabetes, obesity, neurodegenerative diseases, atherosclerosis, cardiovascular disease, and cancer[18] and associated with accelerated levels of inflammation[19].

Lung damage is one of pesticides' side effects on mammals[20]. Intraperitoneal injection of LCT to albino rats showed some clinical symptoms include nasal discharge, severe coughing, anorexia, fatigue, emaciation and depression[21]. In addition some macroscopic (lung consolidation and congestion) and microscopic (bronchiolitis, abscessation, pulmonary oedema and hemorrhage findings were previously reported[9,21,22]. Arafa *et al.*[23] reported that ultrastructurally, lung of α -cypermethrin- treated rats exhibited thickening of

inter-alveolar septa with multiple cellular infiltration. Pneumocytes type II showed multiple vacuoles in the cytoplasm. The interstitium exhibited severe hemorrhage. Alveolar spaces appeared narrow.

Panax ginseng (G) is a worldwide famous medicinal herb. It is utilized for treatment of various diseases like gastrointestinal diseases, respiratory diseases and as a general tonic[24]. Ginsenosides is the most important active ingredient in G. It protected isolated cells from oxidative damage[25], suppress LPO[26], regulate lipids metabolism, Foster anti-oxidation activity and improve immune activity[27]. Small limited clinical trials have shown potential therapeutic efficacy of G extract in chronic obstructive pulmonary disease COPD patients[28,29]. Korean Red Ginseng (KRG) extract has was observed to prevent acute respiratory disease in a clinical trial[30]. In preliminary study of Lee *et al.*[31], KRG hot water extract had significant inhibitory activity against an acute lung injury model for the mouse. Mohi El-Din *et al.*[21] found that G showed a reduction in lung sensitivity against LCT toxicity; showing moderate hyperplasia in bronchioles wall with inflammatory cell clearance and mild interstitial alveolitis with little neutrophils number observed in the rats compared with LCT- treated rats.

With this background, G was found to have the most hopeful proof for regulating the immune response, especially cell-mediated immunity and ability of clinical application for chronic pulmonary diseases. Thus our research was conducted to determine the possible therapeutic potential of G toward pulmonary damage caused by LCT via oxidative stress, apoptosis, histopathological and ultrastructural changes in lung of adult albino rats.

MATERIALS AND METHODS

Chemicals

Lambda-cyhalothrin (LCT), with commercial name Lambada C 5%, was purchased from Pharmacure for Chemicals and Pharmaceuticals, Egypt. Pure ginseng was purchased from PHARCO Pharmaceutical Industries (Cairo, Egypt). All chemical with analytical quality and were obtained from standard commercial products.

Animals and experimental design

The present study used male adult laboratory rats (Rattus norvegicus domestica), which weighed around 135±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 ± 5 °C), normal dark / light cycle of 12 hours and humidity (55 ± 5 %). According to the 1993 Canadian Council for Animal Care (CCAC) principles and guidelines, the rats were supplied with known composition basal diet and water ad libitum throughout study time.

Six groups of Rats (6 per group) were separated randomly (6 per group) 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[32] by i.p. injection for 60 days.

Group 3 (G 200): rats received G at a daily dose of 200 mg/kg b. wt[21] by i.p. injection for 60 days.

Group 4 (LCT): rats received LCT at a daily dose of 61.2 mg/kg b. wt (10% of LD50)[33] by oral gavage for 60 days.

Group 5 (LCT+G100): 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+G200): 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.

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 weights recoding all rats were fasted for ten hours (water ad libitum) to exclude feeding error. All groups of rats are sacrificed under anesthesia of diethyl ether at the end of the two months. Two lungs were rapidly taken out, washed with ice-cold saline and splitted into 3 parts. 1st lung part (10% w/v) was homogenized using Teflon tissue homogenizer (Omni International Inc., Kennesaw, 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° C for subsequent analysis of oxidative stress parameters, the 2nd part was kept frozen at -80° C for gene and protein expression analysis. The 3rd part was used for electron and light microscopic examination.

Biochemical assays

Assay of oxidative stress and antioxidant defense system

For lung homogenates, LPO content was calculated by measuring malondialdehyde (MDA) content using the Preuss *et al.*[34] method. Total thiol (T. thiol) content and the antioxidant enzymes activities; catalase (CAT) and superoxide dismutase (SOD) were estimated using the methods of Koster *et al.*[35], Cohen *et al.*[37] and Marklund and Marklund[36], respectively.

RNA isolation and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

LCT effect on on mRNA abundance of p53 and Bcl-2 by qRT-PCR according to Mahmoud *et al.*[38]. Complementary

DNAs were synthesized from 2 µg RNA and were amplified using SYBR Green master mix (Thermo Fisher Scientific, USA) with the primer sets listed in (Table 1). qPCR was conducted and the amplification data was analyzed by the 2-ΔΔCt method[39]. The results were normalized to β-actin and shown as percentage of control.

Table 1: Primer pairs used for qPCR

	Primer sequence	Gene bank accessi-on number
P53	Forward primer :5'- GAGCTGAATGAGGCCTTGGA -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

Western blot analysis

LCT effect on p53 and Bcl-2 expression levels were evaluated from the frozen lung samples by chemiluminescence kit (BIORAD, USA)[40]. β-actin was used as a loading control.

Light microscopic study

Small parts of Lung tissue were fixed for 24 hours by 10 percent formalin buffered phosphate.The tissue was processed, sectioned with a microtome at 4–5 µm thickness then treated with Hematoxylin and Eosin stain (H&E)[41].

Ultrastructural study

Small lung portions of all groups were immediately fixed at 4 ° C for 18-24h in 3% glutraldehyde-formaldehyde, phosphate buffer rinsing, followed by 1 percent osmium tetroxide post-fixation. 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[42]. Joel CX 100 transmission electron microscope, operated at an accelerating voltage of 60 kV, performed an examination of the stained sections.

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 ± standard error (SE). A value of $P < 0.05$ was rated significant.

RESULTS

Effect of Panax ginseng on body weight change of LCT- treated rats

Oral gavage of LCT (61.2 mg/kg/day) for two months showed a very highly significant ($P < 0.001$) decrease in body weight change in comparison with normal control group. On the other hand, injection, intraperitoneally, with G (200 mg/kg b. wt. /day) caused a highly significant ($P < 0.01$) increase in body weight change when as compared to LCT- treated rats. While intra-peritoneal injection with 100 mg G /kg b. wt. daily for 60 days showed a non-significant ($P > 0.05$) elevation in body weight change when in comparison with LCT- treated group. Body weight change of control groups treated with either tested doses of G did not show any difference from normal control group (Figure 1A).

Effect of Panax ginseng on relative lung weight of LCT-treated rats Relative lung weight exhibited a very highly significant ($P < 0.001$) reduction in LCT-treated rats in comparison with control group. Intra-peritoneal injection of both tested doses of G showed a highly significant ($P < 0.01$) elevation in relative lung in comparison with control group LCT-treated rats (Figure 1B).

Effect of Panax ginseng on lung LPO and total thiol contents of LCT-treated rats

As illustrated in (Figure 2A); lung LPO, calculated in nmol (MDA)/100 mg tissue, exhibited a very highly significant ($P < 0.001$) elevation in LCT-treated rats in comparison with control group. As a result of G administration, MDA level decrease with varying significance. However, 200 mg G seemed to be more effective ($P < 0.01$) than 100 mg G ($P < 0.05$) in decreasing MDA Level (Figure 2B).

LCT-treated rats exhibited a very highly significant ($P < 0.001$) reduction of lung T. thiol contents in comparison with control group. Otherwise, intra-peritoneal injection of both tested doses of G produced a significant ($P < 0.05$) elevation in T. thiol contents when in comparison with LCT-treated rats as illustrated in (Figure 2B).

Effect of Panax ginseng on lung catalase (CAT) and superoxide dismutase (SOD) activities of LCT-treated rats

The effect of LCT administration on lung SOD and CAT activities are shown on (Figure 3). Its data recorded obviously showed a very highly significant ($P < 0.001$) reduction in activities of both SOD and CAT in LCT-administered group in comparison with

control group. Concurrent with LCT administration, G elevated CAT activity with a very highly significance ($P < 0.001$) in both tested doses (Figure 3B). Regarding SOD (Figure 3A), 200 mg G appeared to be more potent ($P < 0.001$) than 100 mg G ($P < 0.05$).

Effect of Panax ginseng on lung apoptotic p53 gene and protein expression levels of LCT-treated rats

As illustrated in (Figure 4A), mRNA expression of p53 in lung tissue of LCT-treated rats exhibited a very highly significant ($P < 0.001$) up regulation in comparison with control group. On the other side, intra-peritoneal co-injection with both of G tested doses induced a very highly significant ($P < 0.001$) down regulation of p53 mRNA expression levels as compared to rats received LCT only.

Protein expression level of apoptotic protein p53 in lung tissue of LCT-treated rats exhibited a very highly significant ($P < 0.001$) increase in comparison with control group. Intra-peritoneal injection of both tested doses of G concomitant with LCT produced very highly significant ($P < 0.001$) decrease in p53 protein expression level in comparison with LCT-administered rats as illustrated in (Figure 4B).

Effect of Panax ginseng on lung anti-apoptotic B-cell lymphoma-2 (Bcl-2) gene and protein expression level of LCT-treated rats

qPCR analysis of Bcl-2 gene expression in lung of LCT-treated rats exhibited a very highly significant ($P < 0.001$) down-regulation in comparison with control group. Simultaneous administration of both tested doses of G with LCT induced a very highly significant ($P < 0.001$) up-regulation of Bcl-2 mRNA expression levels in comparison with LCT-treated group (Figure 5A).

The result of Western blotting exhibited a very highly significant ($P < 0.001$) decrease in Bcl-2 protein expression levels in lung tissues of LCT group in comparison with control group. Intra-peritoneal co-injection of G with LCT produced very highly significant ($P < 0.001$) elevation in protein expression levels in comparison with LCT group (Figure 5B).

Effect of Panax ginseng on lung histolopathology of LCT-treated rats

Microscopic examination of the lung of control, ginseng 100 mg/kg b. wt. and ginseng 200 mg/kg b. wt. /day respectively (Figures 6a, b, c) showed normal histological structure including blood vessels and clear alveoli with thin inter-alveolar septa, squamous type I pneumocytes, cuboidal type II pneumocytes and bronchioles.

LCT-treated group lung revealed obvious histopathological changes. Alveoli appeared collapsed with compensatory expansion of adjacent alveoli divided by thickened inter-alveolar septa (Figure 7a), remarkable hyperplasia of dilated bronchioles wall (Figures 7b, c), RBCs extravasation in bronchiolium lumen (Figure 7c), and mononuclear leukocytic infiltration in parenchyma additionally, blood vessels congested with thickened walls (Figures 7a, b).

Lung of LCT-treated rat treated with ginseng 100 mg/kg b. wt. /day revealed relative recovery except some collapsed alveoli (Figure 8a). Lung of LCT- treated rat treated with ginseng 200 mg/kg b. wt. /day showed great recovery and restoration of normal pulmonary tissue configuration (Figure 8b).

Effect of Panax ginseng on lung ultrastructure of LCT-treated rats

Electron microscopic observations of control group exhibited type 1 pneumocyte which appeared as a ell with a fsquamous clattened nucleus which occupied most of the volume of the cell and thin cytoplasm with few organelles (Figure 9a) and type 2 pneumocyte appeared cuboidal in shape with big spherical nuclei and chromatin condensed at the periphery. Membrane bounded lamellar bodies containing electron-dense secretions and several electron-dense mitochondria appeared in its cytoplasm. Short microvilli were noticed projecting from the borders of type II penumocytes (Figure 9b).

The present study showed that the alveolar tissue of LCT-treated group revealed ultrastructural alterations including, cytoplasmic vacuolation, pyknotic nuclei, empty lamellar bodies and absence of microvilli of type 2 pneumocytes (Figures 10a, b). Type 1 pneumocytes revealed shrinkage in nucleus and vacuolated cytoplasm (Figure 10c). Inter-alveolar septum exhibited thickened (Figure 10d).

Injection of LCT-treated rats with 100 mg G /kg.b.wt/ day resulted in relative improvement in the constituents of the alveolar tissue. Partially empty lamellar bodies, short microvilli and normal nucleus of type 2 pneumocyte (Figure 11a). Type 1 pneumocyte appeared with normal flattened nucleus except some cytoplasmic vacuolation (Figure 11b). Inter-alveolar septum with normal thickness (Figure 11c).

While injection LCT-treated rats with 200 mg G /kg b. wt /day restore normal shape of nuclei, lamellar bodies, mitochondria, border thickness and short microvilli (Figures 12 a,b,c).
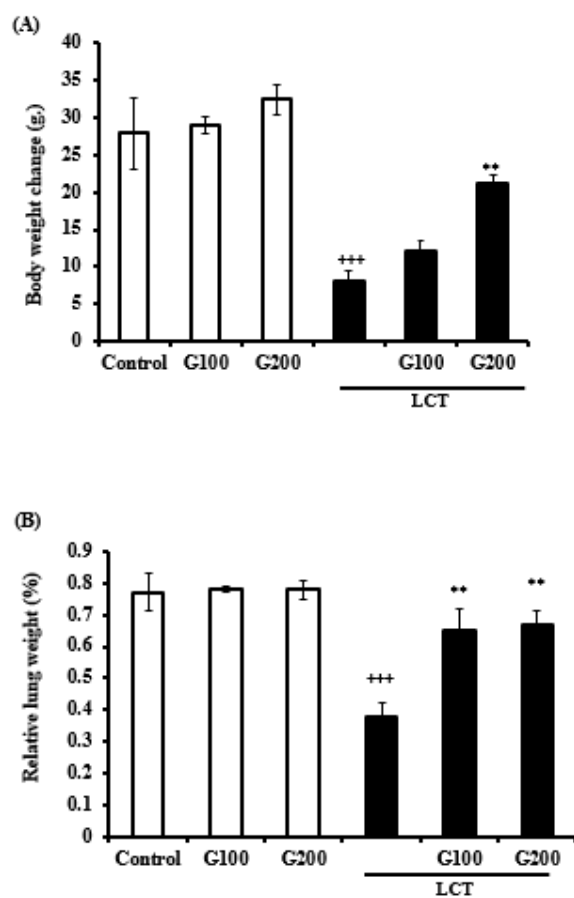
Fig. 1: Effect of Panax ginseng on lambada cyhalotherin-induced changes in (A) body weight change (g) and (B) relative lung weight (%). The results were expressed as Mean ± SE (N=6). Where, G100, 100 mg Panax ginseng; G200, 200 mg Panax ginseng; LCT, lambada cyhalotherin administered rats. Values were considered significantly different at +++$P < 0.001$ versus control group and **$P < 0.01$ versus LCT group.
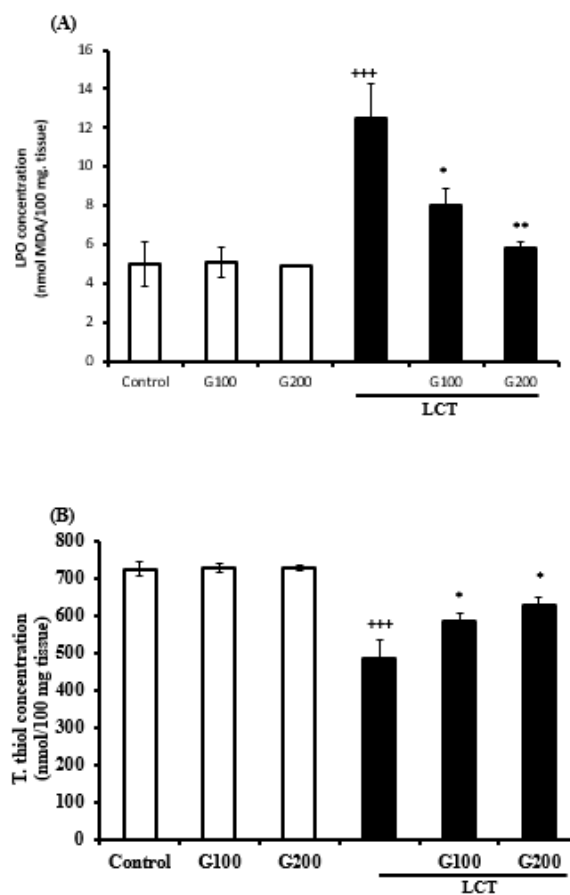
Fig. 2: Effect of Panax ginseng on lambada cyhalotherin-induced changes in lung (A) Lipid peroxidation products (LPO) and (B) Total thiol (T. thiol) concentrations. The results were expressed as Mean ± SE (N=6). Where, G100, 100 mg Panax ginseng; G200, 200 mg Panax ginseng; LCT, lambada cyhalotherin administered rats. Values were considered significantly different at +++$P < 0.001$ versus control group and *$P < 0.05$, **$P < 0.01$ versus LCT group
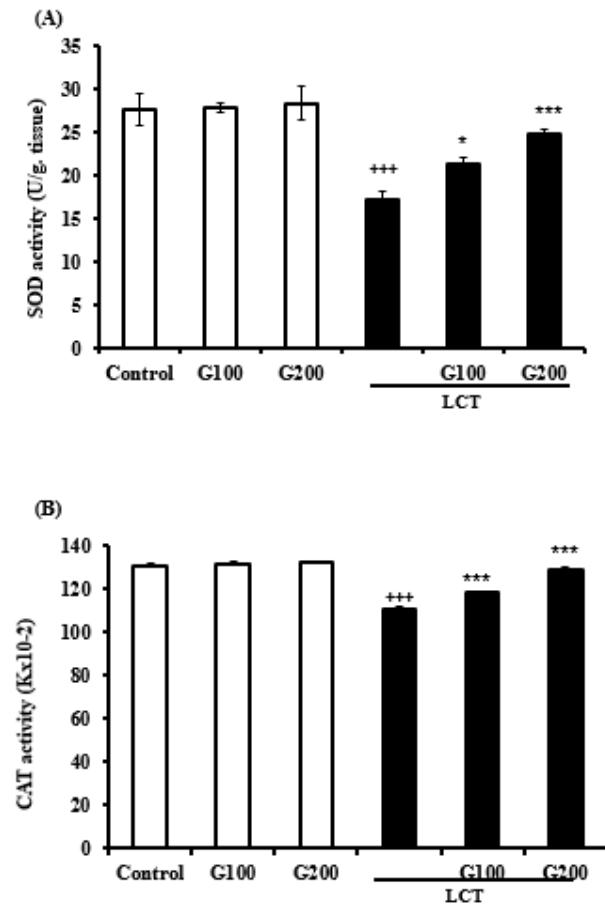
Fig. 3: Effect of Panax ginseng on lambada cyhalotherin-induced changes in lung enzymatic antioxidant defenses (A) Superoxide dismutase (SOD) and (B) Catalase (CAT) activities. The results were expressed as Mean ± SE (N=6). Where, G100, 100 mg Panax ginseng; G200, 200 mg Panax ginseng; LCT, lambada cyhalotherin administered rats. Values were considered significantly different at $+++P < 0.001$ versus control group and $*P < 0.05$, $***P < 0.001$ versus LCT group.
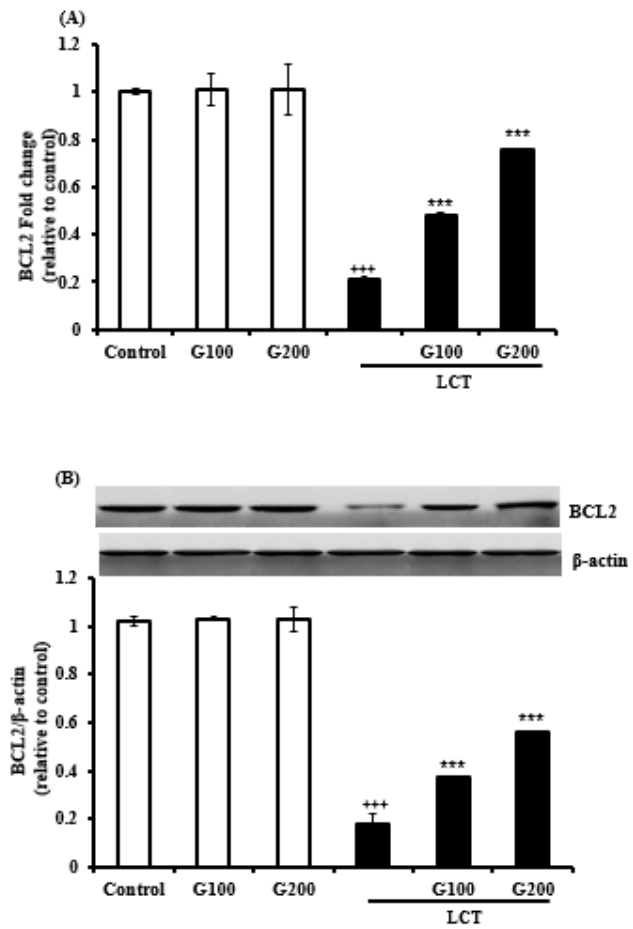
Fig. 4: lung (A) BCL_2 mRNA and (B) BCL_2 protein expression levels in control, G, LCT and LCT rats co-treated with G. The results were expressed as Mean ± SE (N=6). Where, C, control group; G, Panax ginseng injected rats and LCT, lambada cyhalotherin administered rats. Values were considered significantly different $+++P < 0.001$ versus control group and $***P < 0.001$ versus LCT group.
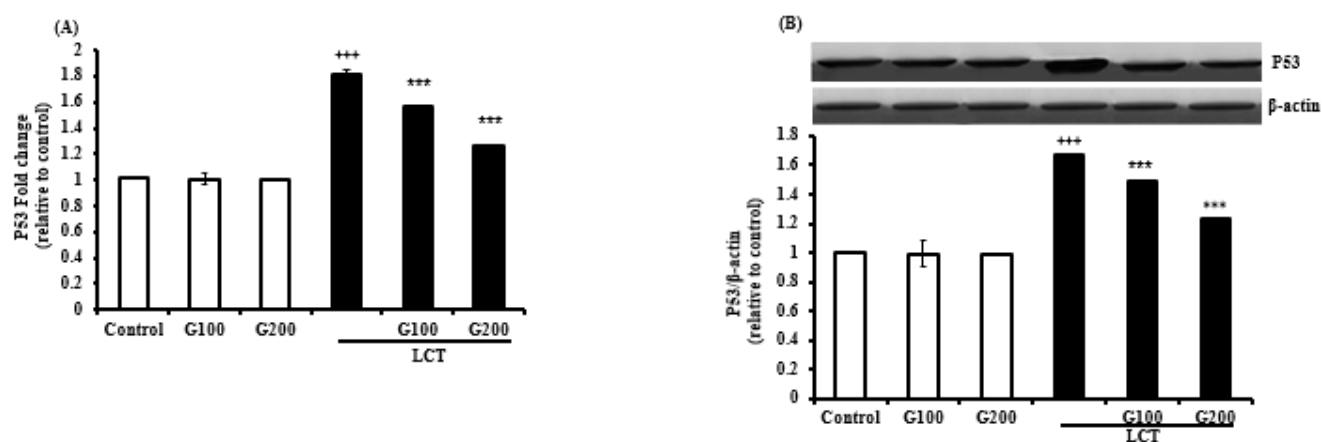
Fig. 5: lung (A) P53 mRNA and (B) P53 protein expression levels in control, G, LCT and LCT rats co-treated with G. The results were expressed as Mean ± SE (N=6). Where, C, control group; G, Panax ginseng injected rats and LCT, lambada cyhalotherin administered rats. Values were considered significantly different +++P < 0.001 versus control group and ***P < 0.001versus LCT group.
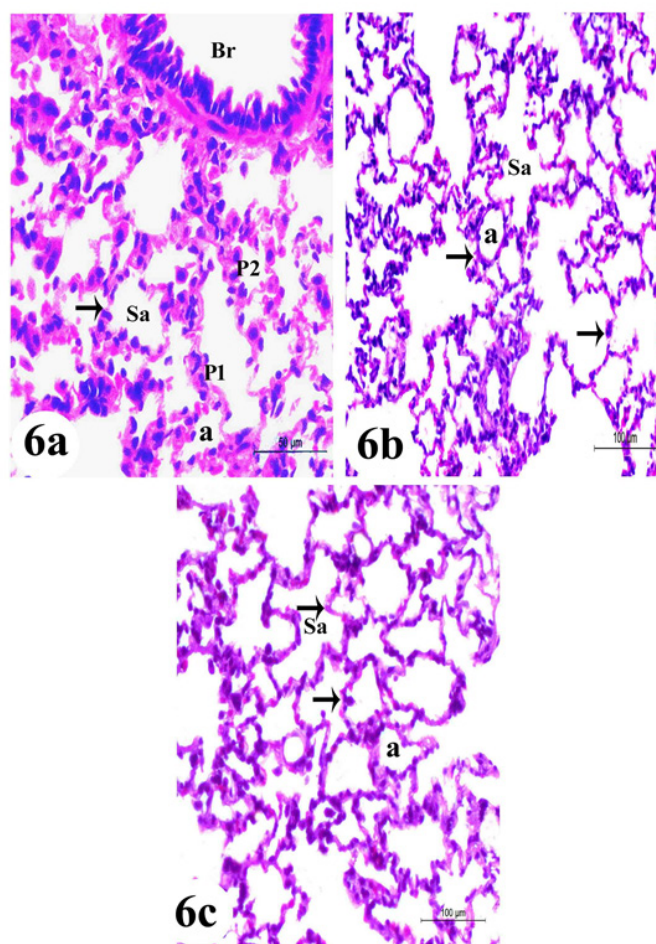

Fig. 6: Photomicrographs of sections of lung of control (a) and control ginseng groups (b & c) 100 and 200 mg /kg b. wt. /day respectively stained with H&E.: showing normal histological architecture of lung including alveoli (a) which are lined with type1 pneumocyte (P1) and type 2 pneumocyte (P2), thin inter-alveolar septum (arrows), bronchiole (Br), Saccule (Sa) (Scale bar =50, 100 and100 μm respectively).
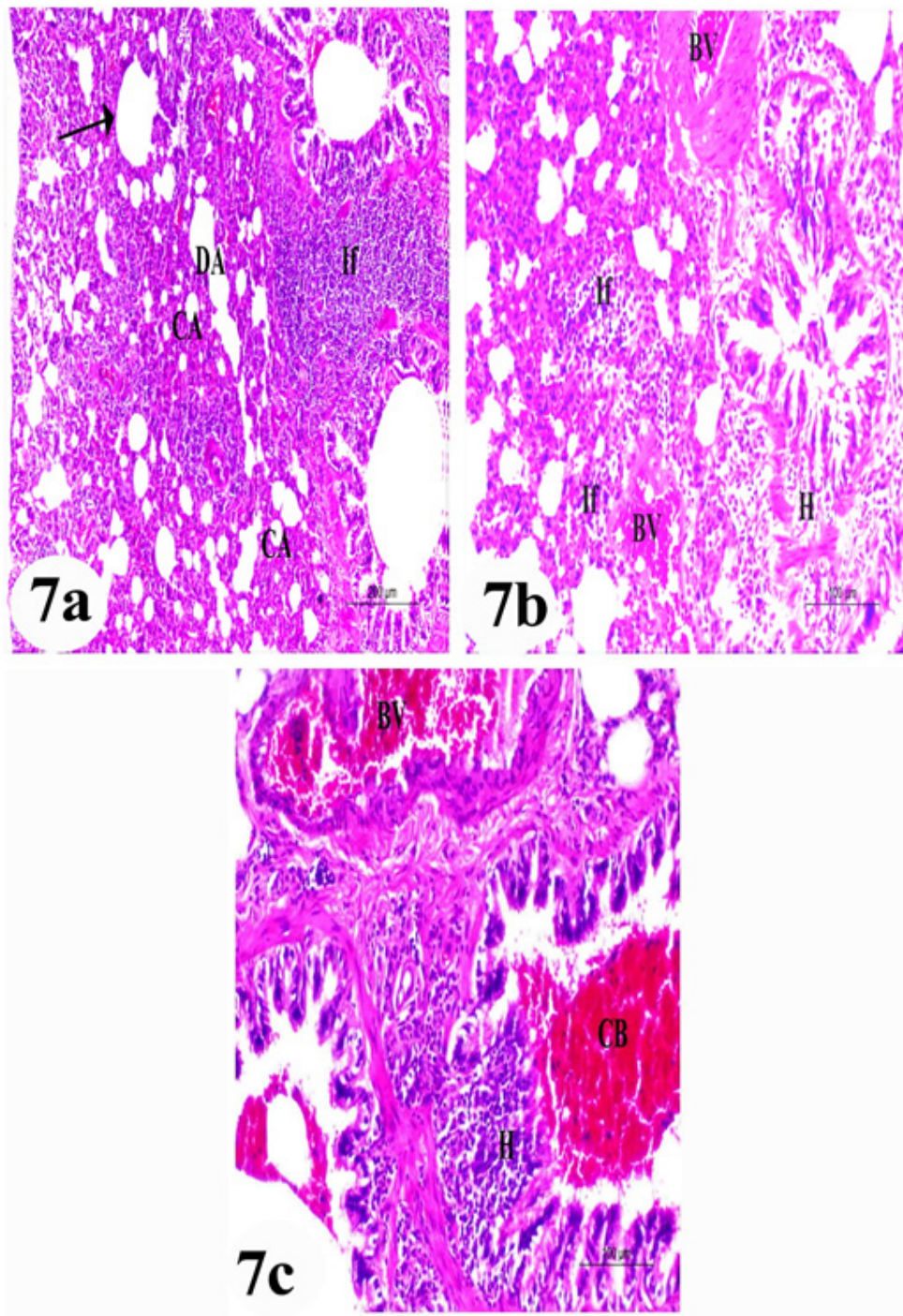
Fig. 7: Photomicrographs of sections of lung of LCT- intoxicated rats for 60 days stained with H&E. (a): showing collapsed alveoli (CA) with compensatory expansion of adjacent alveoli (DA) separated by thickened inter-alveolar septum (arrow) and mononuclear leukocytic infiltration (If) in parenchyma (Scale bar =200 µm). (b): showing congested blood vessels (BV) with thickened wall, marked hyperplasia (H) of dilated bronchioles wall and mononuclear leukocytic infiltration (If) in parenchyma (Scale bar =100 µm). (c): showing extravasation of red blood cells in the bronchiole lumen (CB), congested blood vessels (BV) with thickened wall and marked hyperplasia (H) of dilated bronchioles wall (Scale bar =100 µm).
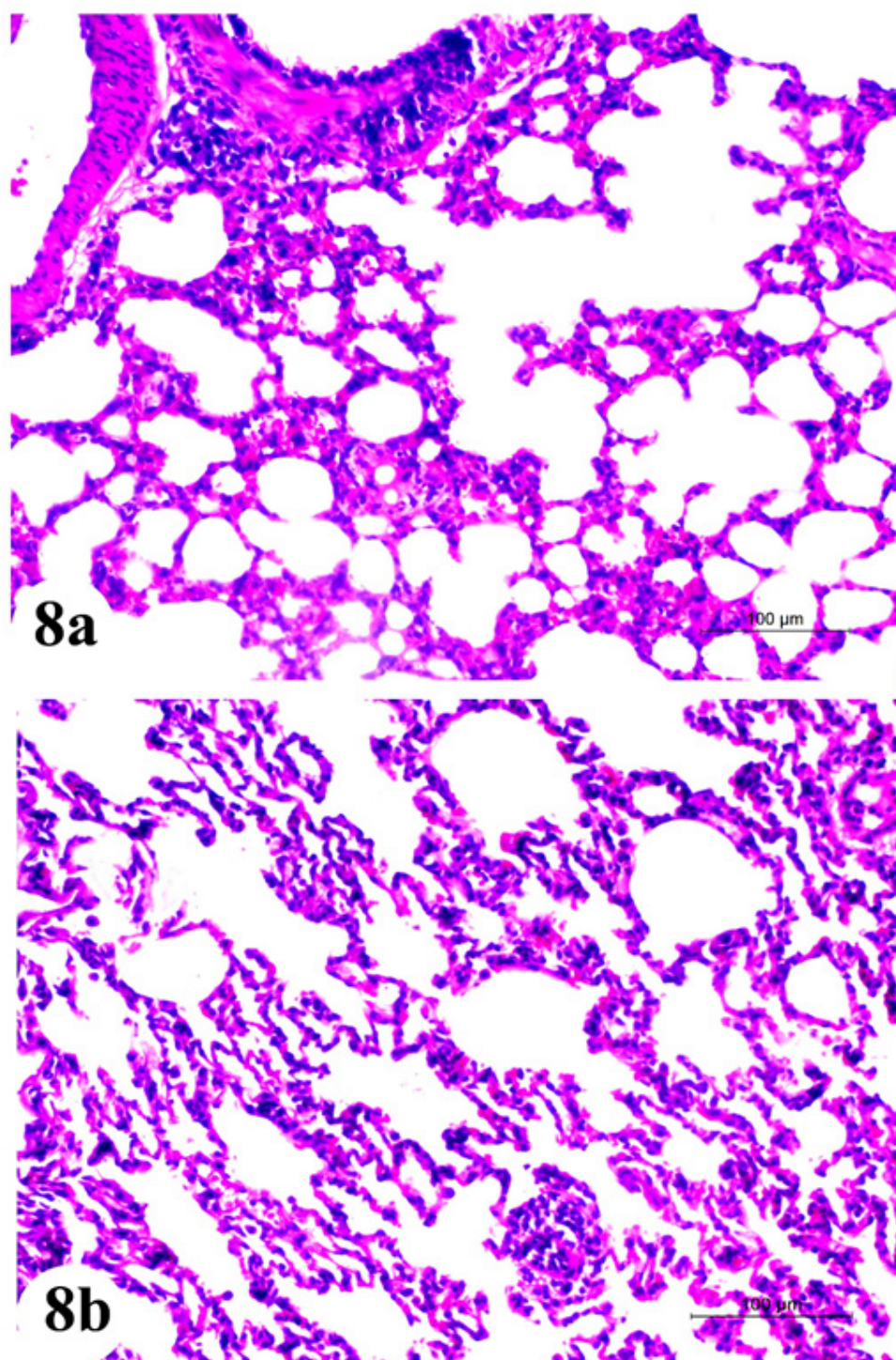
Fig. 8: Photomicrographs of sections of lung of treated rats stained with H&E. (a): LCT plus ginseng 100 mg/kg b. wt. showing relative recovery except some collapsed alveoli (Scale bar = 100 μm). (b): LCT plus ginseng 200 mg/kg b. wt. group showing approximate regain of normal appearance of pulmonary tissue (Scale bar = 100 μm).
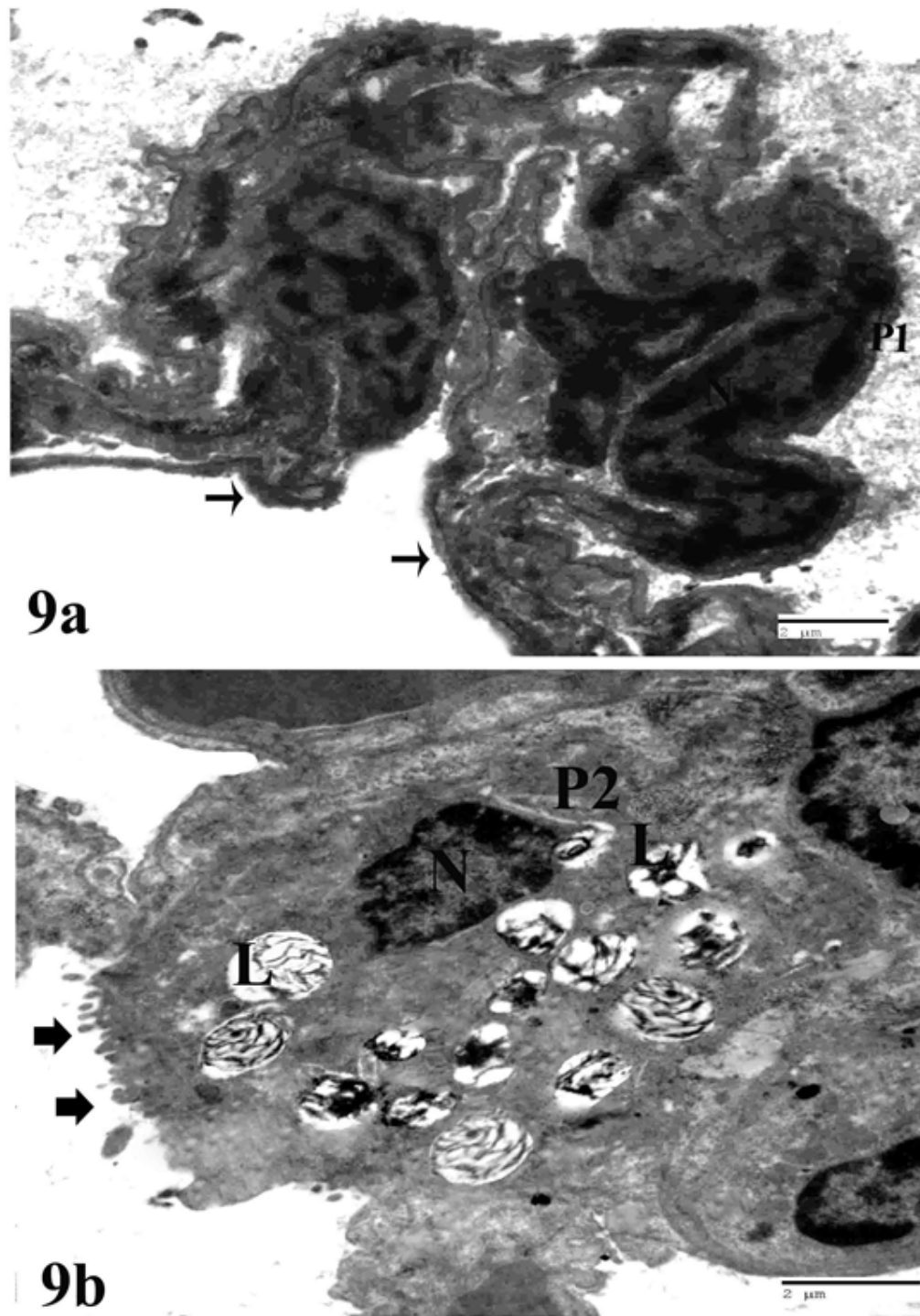
Fig. 9: Electron micrographs of a sections of lung of a control rats (a) Showing normal type 1 pneumocyte (P1) with flattened nucleus (N) and normal inter-alveolar septum (thin arrow) (Scale bar = 2 μm). (b) Showing normal type2 pneumocyte (P2) which is cuboidal in shape with normal lamellar bodies (L), short intact microvilli (thick arrow) and large nucleus (N) (Scale bar = 2μm).
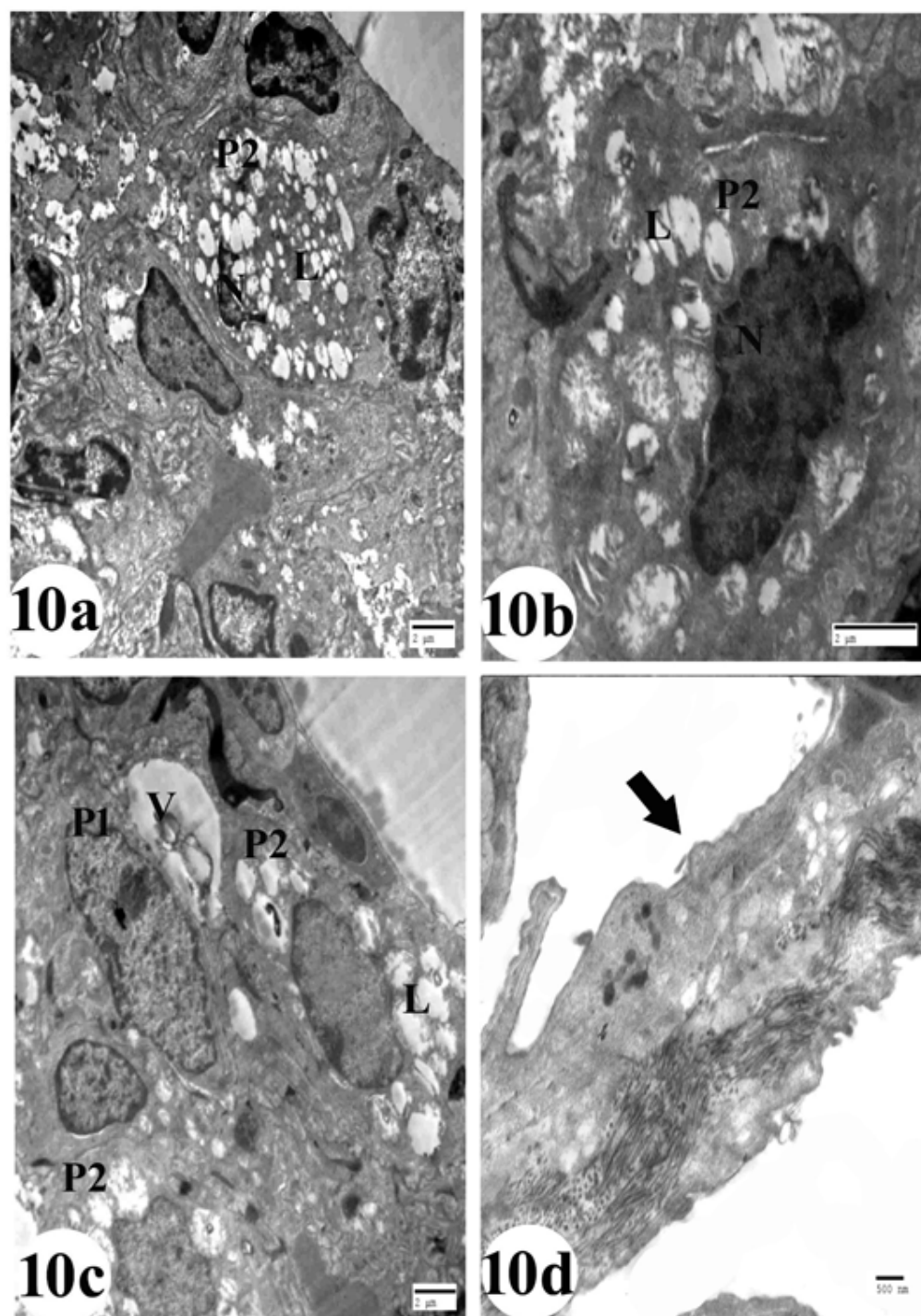
Fig.10: Electromicrographs of sections of lung of rats treated with LCT (a & b) Showing abnormal type 2 pneumocyte (P2) with empty lamellar bodies (L) and shrinked pyknotic nucleus (N) and lack of microvilli (Scale bar = 2μm). (c) Showing abnormal type 1 pneumocyte (P1). Notice vacuolated cytoplasm (v) and damaged type 2 pneumocyte with empty lamellar bodies (L) (Scale bar = 2μm). (d) Showing thickened inter-alveolar septum (thick arrow) (Scale bar = 2 μm).
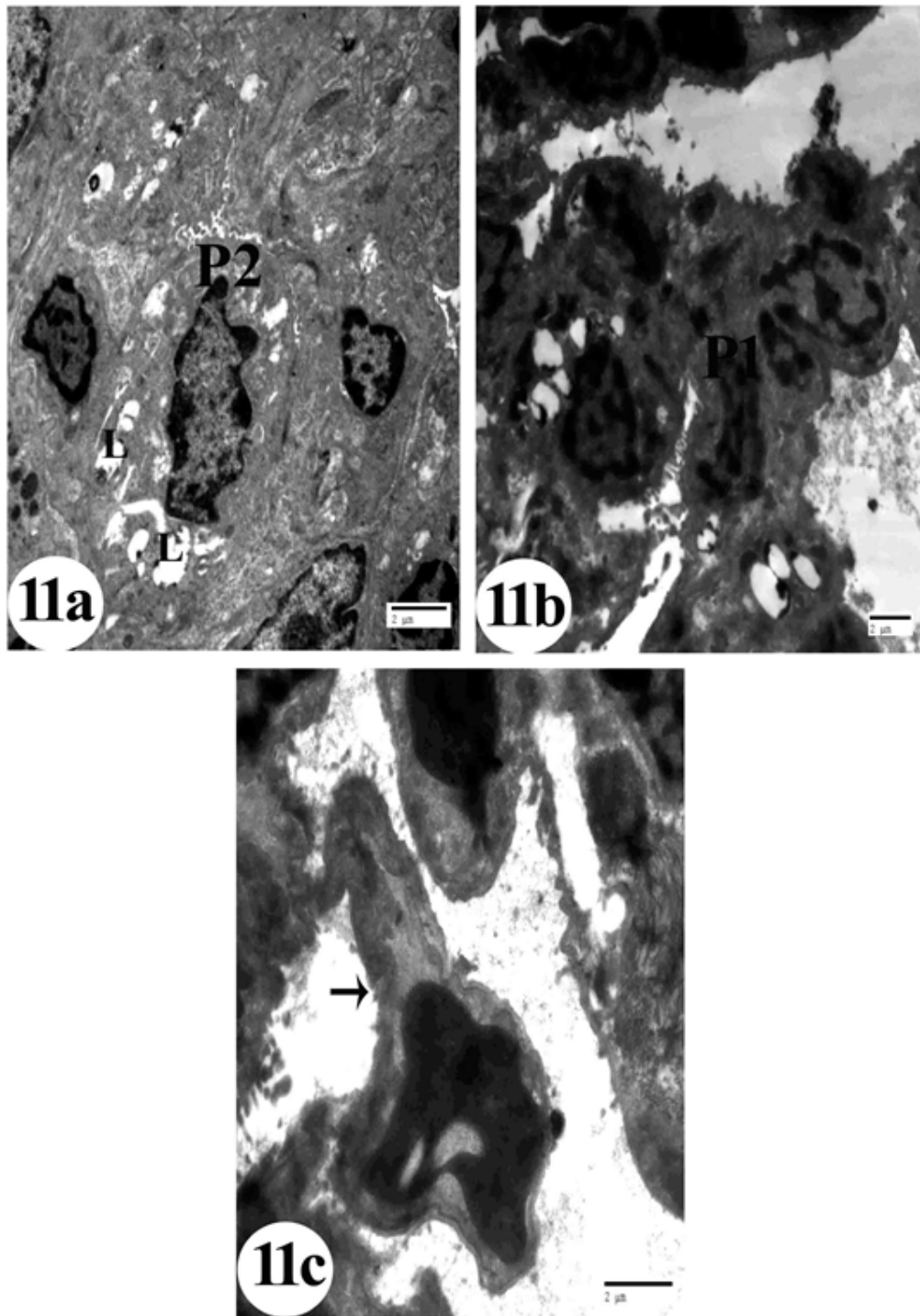
Fig. 11: Electron micrographs of a sections of lung of a LCT+100 mg ginseng/kg b. wt. /day treated rats (a) Showing type 2 pneumocyte (P2) except some empty lamellar bodies (L) and few microvilli (Scale bar = 2µm). (b) Showing type 1 pneumocyte (P1) except some cytoplasmic vacuolation. (Scale bar = 2µm). (c) Showing normal inter-alveolar septum (thin arrow) (Scale bar = 2 µm).

Fig. 12: Electron micrographs of a sections of lung of LCT+200 mg ginseng/kg b. wt. /day treated rats (a) Showing normal type 2 pneumocyte (P2) and normal type 1 pneumocyte (Scale bar = 2μm). (b) Showing normal type 2 pneumocyte (P2) with normal lamellar bodies (L), intact microvilli (thick arrow) and large nucleus (Scale bar = 2μm). (c) Showing normal inter-alveolar septum (thin arrow) (Scale bar = 2 μm).
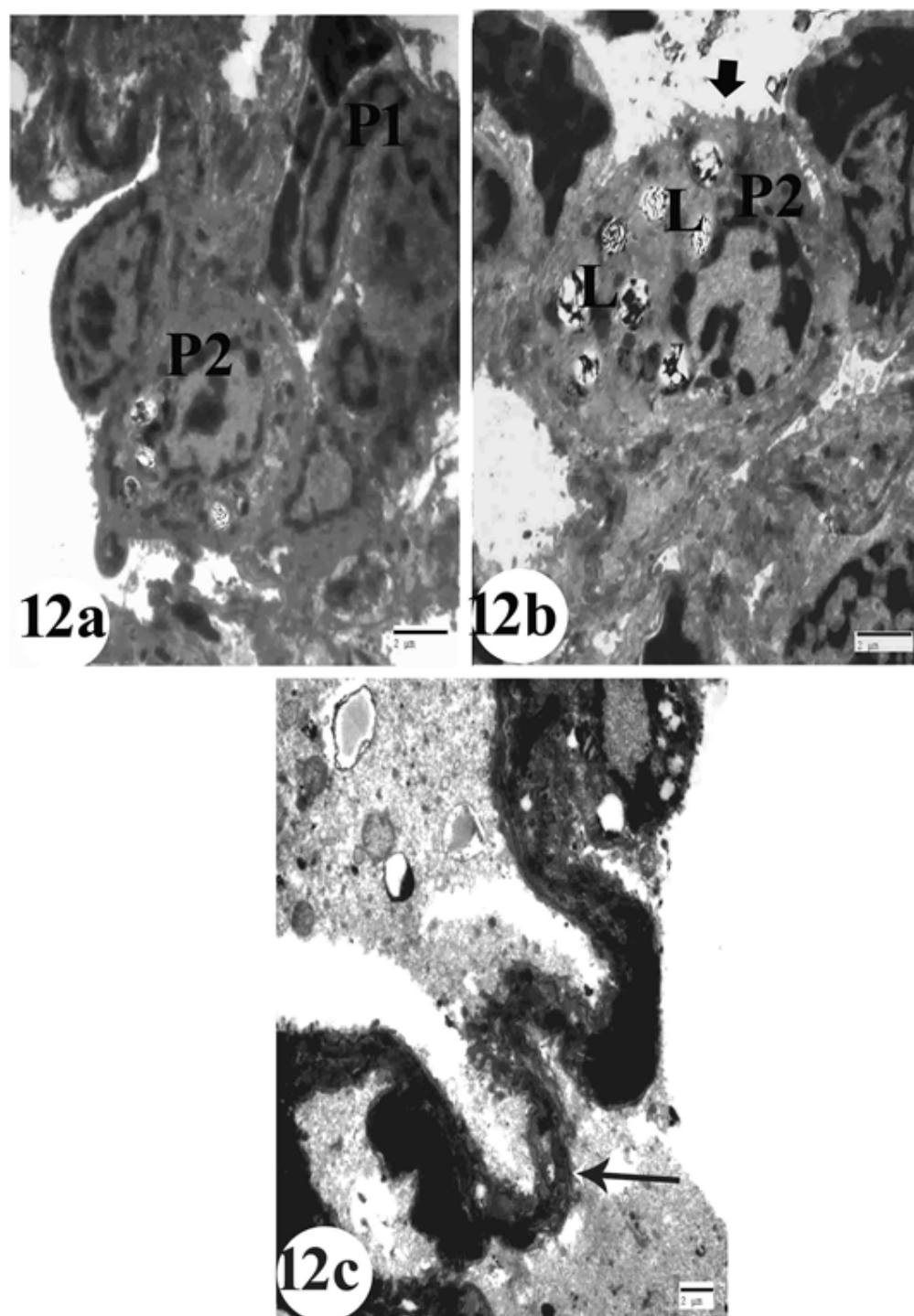
DISCUSSION

Despite the great benefits of pesticides in protecting agricultural crops, animals and humans from harmful pests and diseases vectors, they have extremely harmful effect on non-target creatures including human and other mammalian animals.Nevertheless, the public health effects of pesticide residues are yet to be fully understood. By increasing its use a precise evaluation of their hazards is required.

It is well documented that free radicals have a significant role in pesticide induced toxicity[17]. Pesticides may induce oxidative stress due to alteration in the status of the scavenging antioxidant enzymes, propagation of lipid peroxidation products[43] and apoptosis that represent major contributors in lung damage. Thus, the present study determine the possible therapeutic potential of G toward pulmonary damage caused by LCT in rat model.

In our current study, body weight, relative weight of lung of LCT-treated rats were reduced that run in a good agreement with previous studies[44-49]. Kohner *et al*.[44] observed that decreasing body weight and organs relative weight is one of the chronic effects of pyrithroids toxicity. In LCT-treated rats, the observed decrease can be related to hypophagia or loss of weight because of LCT's direct cytotoxicity[46].

Many studies either In *vitro* or in *vivo* demonstrated that G decreases chemical, physical, and biological stress and maintain general vitality[50]. In our study treatment of LCT-treated rats with 100 or 200 mg G /kg b. wt. /day increased body weight, relative weight of lungs. Qadir *et al*.[51] demonstrated that ginseng protect mice from body weight loss and improved kidney weight induced by gentamicin toxicity.

Oxidative stress has been described as losing balance between antioxidants and oxidants due to general elevation in ROS cellular levels[52]. It is known as risk factors for developing of diverse diseases[53]. ROS are known to induce oxidative damage to proteins, lipids, and DNA[54,55]. Consistent with its lipophilic nature[56]. In biological membranes LCT was found accumulated making oxidative damage and LPO which has been used widely as an oxidative stress marker. MDA is the main oxidation component of polyunsaturated peroxidized fatty acids thus elevated level of MDA is a LPO's powerful marker[57]. In the current study, LCT produced marked increase in lung tissues' MDA level. This suggests that increased LPO may be one of the valuable mechanisms associated with toxicity induced by LCT in rats.

Interestingly, G co-administration significantly protected lung tissues against LCT-induced LPO, suggesting its free radical scavenging action and the mechanisms of chain- breaking. Zhang *et al*.[26] stated that LPO induced by iron- and hydrogen peroxide, via decomposition of unsaturated fatty acid, prevented by long-term administration of G extract. G -extract's antioxidant

effect could happen in both initiation stage and also at the propagation stage.

Furthermore, when an oxidative stress disorder strongly develops, the protection capacities against ROS becomes inadequate, in turn ROS often alter the antioxidant defense mechanisms, decrease T. thiol intracellular concentration of, disrupt antioxidant enzymes activity e.g., CAT and SOD and elevate MDA levels[58,59]. Indirectly, these indicate increased development of oxygen-free radicals.

Highly reactive oxygen metabolites, particularly hydroxyl radicals (•OH), react with unsaturated fatty acids of membrane phospholipid components producing MDA[60].

The role of CAT and SOD in the scavenging of ROS is well known. SOD act as a catalyst in the degradation of the superoxide radicals ($O2\bullet^-$) to hydrogen peroxide (H_2O_2), while CAT makes reduction to H_2O_2 into water (H_2O) and oxygen molecule (O_2) to inhibit oxidative stress and in Preserving homeostasis in cell. In the current study, SOD and CAT activities were decreased markedly in lung of LCT-treated rats.Both CAT and SOD function together to remove ROS, and minor physiological concentration differences may have a significant impact on cellular lipid, protein and DNA resistance to oxidative damage[61]. In the LCT group, low SOD levels may attributed to this enzyme consumption because of elevated oxidative stress in lung tissue.

Thiols are the organic compounds that contain a sulphydryl group. Of all the antioxidants present in the body, thiols constitute the main part of the total antioxidants in the body, and they play a major role in defending against ROS.

Thiols consisting of both intracellular and extracellular thiols in free form as oxidized or reduced glutathione, or protein-bound thiols[62]. In the current study, low levels of total thiol is lung of LCT-treated rats matching with other endogenous oxidative stress-related markers (SOD, CAT) produced clear indications that the pathogenesis of LCT- lung toxicity involves oxidative stress.

The antioxidant properties of G are well-documented. G possesses antioxidant activity through improving the expression of antioxidant enzyme gene that related to ROS scavenging[63]. In the present study, co-administration of G with LCT induced a significant reduction in the mean value of MDA and a marked elevation in the mean values of antioxidant enzymes (SOD and CAT) activities and total thiols in lung as compared with LCT-treated group. Similarly, Al-Harbi *et al*.[64] stated that G, as an antioxidant, reduced level of MDA and elevated activities of CAT and SOD induced by fipronil. Also, Diab *et al*.[65] reported that G administration reduced level of MDA and elevated CAT and SOD activities in chlorpyrifos and profenofos- treated animals.

Matching with reduced endogenous antioxidants, the current study showed marked up-regulation in mRNA

and protein expression levels of lung p53 (Proapoptotic gen) accompanied with down-regulation in mRNA and protein expression levels of Bcl2 (anti-apoptotic gen) in LCT-treated rats in comparison with control group. These genes play a major role in apoptosis regulation[66,67]. In line with the current results, Gupta et al.[68] demonstrated that Bcl2 protein was decreased in corneal epithelial cells of human exposed to Allethrin (type 1 pyrithroid). Also Bcl2 protein expression was decreased in brain of deltamethrin (type 2 pyrithroid) treated rats[69,70] in splenocytes[71]. These obtained results proposed activation of the apoptotic pathway via upregulation of p53 gene and protein as well as Bcl2 gene and protein down regulation could be involved in lung damage induced by LCT.

In normal condition, apoptosis is responsible for tissue remodeling during the development and turnover of normal tissue for better functioning of the body but an uncontrolled apoptosis causes excessive damage of tissue. LCT-treated rats showed ROS generation, DNA fragmentation and apoptosis[52,72,73]. The mechanism of apoptosis induced by LCT has been proposed to be linked with oxidative stress and mitochondrial pathway activation of a transcription factor, nuclear factor-kappa B (NF-κB) is a crucial activator of inflammatory, immune and apoptosis genes[52]. Activation of NFκB induces cell proliferation to decrease and apoptosis to increase by DNA damage induced by ROS and activation of p53. Activated p53 due to LCT oral exposure additionally stimulates the intrinsic mitochondrial apoptotic pathways responding to DNA damage by stimulating pro-apoptotic proteins (Bax, caspase- 3) expression, down regulates Bcl-2 expression and switches the balance to pro apoptotic effects[52]. Injection of G concomitant with LCT produced down regulation of p53 mRNA and protein expression in lung tissue in comparison with LCT group. SO, we can relate the improved rate of apoptosis in co-treated groups to antioxidant activity of G and the ROS scavenging, suppression of NF-κB activation, interleukin (IL-1β) and release of cytokines. Matching with Cheng et al.[74] who reported that G inhibits interleukin (IL-1β)-induced apoptosis in human chondrocytes. Previous research has shown that ginsenosides Rg3 inhibit apoptosis of endothelial cell by elevation the expression of BAX and reducing Bcl-2 in cells of prostate cancer[75,76]. G decreased gene expression of pro-apoptotic proteins p53 and caspase-3, while elevated anti-apoptotic Bcl2 expression in neuroblastoma cells suggested protective effects of G against cell death in oxidatively stressed brain cells[77]. Also, G suppresses TNF-α/IFN-c-induced thymus and activation-regulated chemokine (TARC) expression through NF-κB 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-α, IFN-c, IL-4, IL-5, and IL-13 in mice. G suppressed TNF-α /IFN-c induced NF-κB activation[78].

The current histopathological investigations demonstrated that the administration of the LCT to adult male rats for 60 days induced variable degenerative changes in the pulmonary tissue in comparison with normal control group. These histopathological observations represented by severe damage in alveoli as collapsed alveoli divided by remarkably thickened inter-alveolar septa with compensatory expansion of adjacent alveoli. Bronchiolitis manifested by remarkable hyperplasia of dilated bronchioles wall and extravasation of RBCs in the bronchiole lumen. Blood vessels showed congestion and thickening in wall with mononuclear leukocytic infiltration in the surrounding tissue beside to numerous hyperemia in some tissues. These findings are in line with Mohi-Eldin et al.[21] in rat model and Abdul Basir et al.[9] in rabbit model. Also, similar histopathological observations have been reported by Sheikh et al.[79] on the effect of cypermitherin on lung tissue of mice model and by Arafa et al.[23] in rat model.

In our study LCT-treated rats exhibited pulmonary ultrastructural alterations characterized by cytoplasmic vacuolation and shrinkage nucleus in both type I and type II pneumocytes, empty lamellar bodies, absence of microvilli of type II pneumocytes and thickening of inter alveolar septum.

Type I pneumocytes which cover about 95 percent of the lung's inner surface and responsible for gas exchange[80]. Like neurons, type I pneumonocytes are considered to have lost the capacity for mitosis. Cells of destroyed pneumocytes type I are replaced by cells of pneumocytes type II [81]. According to our ultrastructural observation Type I pneumocytes revealed shrinked nucleus and vacuolated cytoplasm that impair its structure and function in gas exchange Type II includes characteristic lamellar inclusions, which are surfactant's source, the material in charge of alveolar surface tension modifications[82]. Degenerative alterations in pneumocytes type II were observed as vacuolation and reduced surfactant material in their lamellar bodies[23,83]. There is also evidence that type II pneumocytes produce a diverse range of materials participating in alveolar structure and defense as α1 antitrypsin and fibronectin that can inhibit the proliferation of lymphocytes and improve macrophage functioning in the alveolar septa[84,85].

The ultrastructural alterations in pneumocytes type II, Possibly associated with LPO effect on phospholipids of membrane, were associated with surfactant secretion impairment and turnover. This proposition was also confirmed by lamellar body residues existence, that may participate to the functional effect on O_2[86]. These alterations in pneumocytes type II are identical to apoptosis and necrosis picture presented in several studies[87,88] because of an enhanced production of TNF-α and its resultant activation of the FASL/FAS signaling pathway[89].

Thickened inter alveolar septum was similar to earlier finding of arafa et al.[23] reporting that α-CYP-treated lung exhibited elevated alveolar wall thickness which can be rationalized by increased deposition of extracellular matrix

proteins and endothelial hyperproliferation[90,91] and related with low elasticity and respiratory gases exchange[92]. This may be linked to the ROS accumulation in lung due to elevated LPO.

In the current study, treatment of LCT-treated rats with G 100 mg/kg b. wt. /day and 200 mg/kg b. wt. /day plus LCT protected lung of LCT pulmonary toxicity and maintain approximately the normal histological structure matching with similar previous studies. According to Mohi El-Din *et al.*[21] G showed a reduction lung sensitivity against LCT-induced toxicity, in comparison with rats treated with LCT only, represented by bronchial wall with moderate hyperplasia and inflammatory cells clearance from the lumen.

CONCLUSION

The current study reported that G has shown therapeutic efficacy against LCT-induced histopathological and ultrastructural lung 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 investigations are needed to illustrate the accurate molecular mechanism of G against LCT-induced lung toxicity.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائي للجنسنج على السميه الناتجه من لامبادا سيهالوثرين على رئه الجرذان البيضاء

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قسم علم الحيوان، كليه العلوم، جامعه بنى سويف، مصر

المقدمه: يعتبر لامبادا سيهالوثيرين مبيد حشري من النوع الثانى من المبيدات الحشريه البيريثرويديه له تطبيقات عديده في الزراعه والصحه العامه وصحه الحيوان. على الرغم من الادعاءات أن تاثيره السمى على الثديات منخفض فقد أثبتت العديد من الدراسات أن له تاثير سمى على الثدييات عن طريق تحفيز جهد الاكسده مما يسبب تلفًا حادًا في الرئة ومن ثم يشكل خطرا على صحه الانسان والحيوان.

الهدف: تهدف هذه الدراسه الي التحقق من الدور الوقائى والمحسن لجرعتين من مستخلص نبات البانكس جينسنج (١٠٠و٢٠٠ملجم/كيلوجرام من وزن الجسم) وهو نبات طبي له خصائص مضاده للاكسده مثبته وموثقه علميا ضد الآثار السمية الناتجة عن التعرض لمبيد لامبادا سيهالوثيرين في رئه الجرذان البيضاء لمده ٦٠ يوماً.

المواد والطرق: لتحقيق هذا الهدف قمنا بتقسيم ٣٦ من الجرذان البيضاء بأوزان تتراوح بين ١٢٠-١٥٠ جرام إلى ٦مجموعات بواقع ٦ فى كل مجموعة. المجموعة الاولى (المجموعة الضابطة): والتى أعطيت محلول ملحى عن طريق الحقن البريتونى يومياً. المجموعة الثانية (جينسنج ١٠٠): أعطيت جرعه يوميا من الجينسنج قدرها ١٠٠ملجم/ كيلوجرام من وزن الجسم (مذابة فى محلول ملحى) عن طريق الحقن البريتونى. المجموعة الثالثة (جينسنج ٢٠٠): أعطيت جرعة يوميا من الجينسنج قدرها ٢٠٠ملجم/كيلوجرام من وزن الجسم (مذابه فى محلول ملحى) عن طريق الحقن البريتونى . المجموعة الرابعة (لامبادا سيهالوثيرين): باستخدام انبوبة فموية تم تجريعها عن طريق الفم بمبيد لامبادا سيهالوثيرين بجرعة ١/١٠ من LD٥٠ (٦١.٢ مجم / كجم من وزن الجسم) يومياً. المجموعة الخامسة (لامبادا سيهالوثيرين+ ١٠٠): اعطيت يومياً نفس جرعه المجموعه الرابعه من لامبادا سيهالثيرين تليها على الفور نفس جرعة المجموعة الثانية من الجينسنج. المجموعة السادسة (لامبادا سيهالوثيرين+ ٢٠٠): اعطيت يومياً نفس جرعة المجموعة الرابعة من لامبادا سيهالثيرين تليها على الفور نفس جرعة المجموعة الثالثة من الجينسنج. تم تعديل الجرعات اسبوعيا وفقا للتغيرات فى وزن الجسم حتى نهاية فترة التجربة.

النتائج: بعد انتهاء فتره التجريبية (بعد ٦٠ يومًا) تم تسجيل الأوزان النهائية للجرذان، وتم حساب نسبه زيادة وزن الجسم. تم ذبح جميع مجموعات الجرذان تحت التخدير من ثنائي إيثيل الأثير. تم استخراج الرئة ، وغسلها بمحلول ملحي مثلج ثم وزنها لحساب الوزن النسبي للأعضاء ثم تقسيمهم إلى ٣ أجزاء. تم وضع الجزء الأول (١٠٪ وزن / حجم) في محلول ملحي معادل بالفوسفات ، لمدة ١٠ دقائق باستخدام جهاز طرد مركزي عند ٣٠٠٠ دورة في الدقيقة

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تم الحصول على محلول متجانس واضح وتجميده عند ـ٨٠ درجة مئوية للتحليل اللاحق لمعلمات الإجهاد التأكسدي ، تم تجميد الجزء الثاني عند ـ٨٠ درجة مئوية لتحليل التعبير الجيني والبروتينى. تم استخدام الجزء الثالث للفحص بواسطه المجهر الضوئى والالكترونى.

قد تسبب التسمم ب لامبادا سيهالوثيرين فى انخفاض فى الاوزان النهائيه للجرذان والاوزان النسبية للرئه. وقد اظهرت نتائج تحاليل دلالات جهد التأكسد فى انسجه الرئه ان التسمم بلامبادا سيهالوثيرين قد تسبب فى زياده ملحوظه في مستوى (MDA) وانخفاض ملحوظ في نشاط الانزيمات المضاده للاكسده (CAT و SOD ومستوى الثيول الكلى (T. thiol). بالاضافه الى ذلك زياده ملحوظه في مستوي mRNA والتعبير البروتيني لجين p٥٣ (المنشط لموت الخلايا المبرمج) مصحوبا بنقص في و مستوي mRNA والتعبير البروتيني لجين Bcl-٢ (المثبط لموت الخلايا المبرمج) فى الرئة.

بالاضافة الى ذلك اظهر الفحص بالمجهر الضوئي لعينات الرئة للجرذان المسممه بلامبادا سيهالوثيرين ان اعطاء لامبادا سيهالوثيرين يسبب تغيرات تنكسية متعدده فى الأنسجة الرئوية بالمقارنة بالمجموعة الضابطة. هذه التغيرات تتمثل فى تلف فى الحويصلات الهوائية فى شكل ضيق فى الحويصلات الهوائية مع تعويض تمدد الحويصلات المجاوره مصحوبا بزياده فى سمك الحواجز بين الحويصلات و التهاب القصيبات الهوائية يظهر في شكل تمدد في القصيبات الهوائية وزياده في سمك جدارها وتسرُّب لكرات الدم الحمراء داخل تجويفه. احتقان في الاوعيه الدمويه وزيادة في سمك جدارها وارتشاح خلوى فى النسيج المجاور لها بالاضافة الى نزيف في بعض الانسجة.

أيضاً أظهرت دراسه التركيب الدقيق للنسيج الرئوى الدقيق أن الجرذان المسممه باللامبادا سيهالوثيرين اظهرت تغيرات على مستوى التركيب الدقيق تتضمن فجوات سيتوبلازميه وبكنزة في الانوية واختفاء للزُغَيْبَات الدقيقه فى النوع الثانى من الخلايا الرئوية اما النوع الاول من الخلايا الرئويه فأظهر انكماش فى النواة وفجوات سيتوبلازمية بالاضافة الى زيادة سمك الحاجز بين الحويصلات.

أظهرت الدراسة أيضا ان معاملة الجرذان المعرضة للامبادا سيهالوثيرين بالجنسنج قد أحدث تأثيرات محسنة في والرئة. هذا التحسن نتيجة تأثيره المضاد للاكسدة و القدرة الفائقة على التخلص من الشوارد الحرة من أنسجة الرئة. كلتا الجرعتين المختبرتين من الجنسنج ادت الى انخفاض بشكل ملحوظ فى مستوى mRNA ومستوى التعبير البروتيني لجين p٥٣ (المنشط لموت الخلايا المبرمج) وارتفاع في مستوى mRNA ومستوى التعبير البروتيني لجين Bcl-٢ (المثبط لموت الخلايا المبرمج) وكشفت عن تحسن كبير واستعادة الأنسجة الطبيعية و التركيب الدقيق للرئة ولكن جرعه (٢٠٠ ملجم/كيلوجرام من وزن الجسم) من الجنسنج أظهرت نتائج افضل.

الخلاصه: ونستخلص من هذه الدراسه ان المعالجه بالجينسنج له تأثير وقائي ضد جهد الأكسده الناتج من التعرض لمبيد لامبادا سيهالوثيرين وما يصاحبه من موت الخلايا المبرمج والتغيرات الهستوباثولوجية والتركيبية الدقيقة في رئه الجرذان البيضاء.