Original Article

Effect of Bisphenol A on the Lung of Adult Male Albino Rats and the Possible Protective Role of Propolis: Light and Electron Microscopic Study

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ABSTRACT

Introduction: Bisphenol A (BPA), is known to be extensively utilized industrial chemical for manufacturing many products. It has a toxic, cumulative effect on human health. Propolis is a natural product with anti-inflammatory, anti-oxidant and anti-cytotoxic activities.

Objective: To study the effect of BPA exposure on adult male albino rats lung and to evaluate the possible protective role of propolis.

Material and Methods: Forty-five adult male albino rats were divided into four groups: Group I: control. Group II (BPA treated group): received BPA (500 mg/kg) for 8 weeks. Group III (recovery): received BPA as group II then left for 2 weeks without treatment for recovery. Group IV (Propolis and BPA treated group): received propolis at a dose of (50 mg/kg) and Bisphenol A (500mg/kg) for 8 weeks. All doses were given orally by gastric tube once daily. At assigned time of sacrifice, rats were anaesthetized and lung specimens were dissected and processed for biochemical, histological, immunohistochemical and ultrastructural studies.

Results: Bisphenol A caused alveolar collapse, distorted bronchiolar mucosa, inflammatory cellular infiltration and congested thickened blood vessels. By E/M; pneumocytes type II showed shrunken nuclei, empty lamellar bodies, and degenerated mitochondria. Oxidative stress markers showed significant increase of malondialdehyde (MDA) levels of groups II and III and significant decrease of superoxide dismutase (SOD) and reduced glutathione (GSH) levels compared to control group. Thickness of the interalveolar septum, area percentage of collagen fibers and number of positive (CD68) macrophages increased significantly with groups II and III compared with the control group. However, area percentage of B- cell lymphocyte-2 (BCL2) reactivity was reduced. The use of propolis improved these changes.

Conclusion: BPA exposure induced structural changes in the rat lung. These changes could be minimized by concomitant administration of propolis. So propolis is recommended to be used as a protective agent.

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Key Words: Bisphenol A., CD68, lung, propolis, reduced glutathione.

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INTRODUCTION

Rapid industrialization and improving living standards are in fact related to increased toxic hazards[1]. Bisphenol A (BPA) is an industrial manufactured chemical, exists as solid particles at room temperature having phenol odor[2]. Polyesters, plastic, synthetic rubber, and fire retardants are all made with it. These substances are used as a lacquer for coating metal items such as bottle caps, water pipes, cans as well as dental sealants and coats. Furthermore, Plastics are utilized in packaging meal and drinks[3].

Bisphenol A contamination results from consuming food in containers which have BPA in their component as baby bottles, table ware and cans for food preservation which are coated with epoxy resins producing a direct absorption as an exposure pathway. Bisphenol A is degraded in hot conditions allowing it to enter into food[4,5]. Recently, it has been revealed that BPA can be transmitted directly through the skin from some types of thermal printing paper, for example, cashier's receipts[2]. Human tissues have been proven to collect BPA, resulting in significantly higher levels of exposure[6]. This clearly indicates that daily human exposure to BPA at different ages is unavoidable and making it a reason for concern.

Bisphenol A is an endocrine-disrupting chemicals (EDCs)[7]. It is associated with a variety of diseases in humans, such as; increased coronary heart disease incidence, decrease thyroid hormone levels, reduce fertility, alter gene expression with subsequent neoplastic transformation and induce abnormal liver and kidney functions. So, consequently has received widespread attention[8,9]. On the other hand, studies performed on BPA dispersion in rats demonstrated that lung is the main site for its concentration. As a result of accumulation of BPA, recurrent exposure has an impact on lung functions[10].

Propolis or bee glue is a natural, adhesive, balsamic material with promising protective and therapeutic effects[11]. It is synthetized by honeybees by gathering the exudates of various plants then it is modified in the beehive by blending salivary secretion and wax to them[12].

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Propolis' biological properties are influenced by its chemical composition, geographical location, plant sources, and seasonality. Propolis contains flavonoids, polyphenols, essential fats, oils, pollen, and wax. Moreover, several vitamins (C, E, and B complex), minerals (Ca, Mg, Zn, Cu, Mn, and Fe) and trace elements are also, present in propolis[13].

In addition to, its anti-inflammatory and cyto-protective impact[14], propolis has antioxidant, anesthetic, anti-cancer[15,16], as well as anti-hepatotoxic characteristics[17].

Consequently, this study aimed to clarify the toxic effect of bisphenol A on lung tissue of adult male albino rats and to evaluate the possible protective role of propolis.

MATERIALS AND METHODS

Animals

The experiment employed 45 adult male albino rats of 12 weeks old weighting between 180 and 200 grams. Animals were purchased from animal house of Menoufia University, one week before to the commencement of the experiment to be acclimatized to laboratory conditions. Rats were kept in stain steel cages in a clean ventilated room with normal light-dark cycle. Laboratory diet was used to feed the animals. Handling and treatment of laboratory animals were performed in accordance with the medical research ethics committee of experimental animals at the Faculty of Medicine, Menoufia University.

Chemicals

- Bisphenol A (BPA) was purchased as a white powder (CAS registry no. 80-05-7, purity of 99%) from Sigma– Aldrich (St. Louis, Missouri, USA) from Sigma company.
- Propolis was obtained in the form of capsules (each contains 400 mg pure propolis) having “Bio propolis” as a brand name; from Sigma Pharmaceutical Industries for International Business Establishment Co. (IBE Pharma, Cairo, Egypt).
- Corn oil: It is used as a vehicle for BPA. It was obtained from local supermarket, Sheibin El-kom, Menoufia, Egypt.

Experimental design

The rats were randomly allocated into 4 groups:

Group I (control group) (n=15 rats) was divided into 3 subgroups included 5 animals for each:

- Group IA (Negative control): the animals in this group did not receive any treatment.
- Group IB (Propolis treated group): Each rat of this group received propolis at a dose of 50 mg/kg dissolved in distilled water orally using gastric tube once daily for 8 weeks[11].
- Group IC (Vehicle control group): Each rat of this group received 1 ml of corn oil (the vehicle of BPA) orally using gastric tube for 8 weeks.

Group II (BPA treated group) (n=10 rats): Rats were given 500 mg/kg body weight of bisphenol A (BPA)[18] once every day by oral route using gastric tube for 8 weeks. Each animal was given 1 ml of freshly prepared BPA solution, which was made by dissolving 2 grams of BPA in 20 ml of corn oil as a vehicle and administered orally through a gastric tube.

Group III (Recovery group) (n=10 rats): Rats of this group received BPA at a dose similar to group II for 8 weeks then they were kept for another two weeks without treatment for recovery.

Group IV (Propolis and BPA treated group) (n=10 rats): Propolis was given to each rat in this group at a dose of 50 mg/kg[11] once daily by a gastric tube for 8 weeks. Each animal received 1 ml of a solution prepared immediately before administration by dissolving 100 mg of propolis into 10 ml distilled water and Bisphenol A at a dose of 500 mg/kg body weight[18] orally by gastric tube once daily for 8 weeks.

At assigned time of sacrifice, the animals were anaesthetized by intraperitoneal injection of ketamine at a dose of 90mg/kg[19]. Then, 2.5 % glutaraldehyde with 0.1 mol/L phosphate buffer at pH 7.4 were used for intra-cardiac perfusion. lungs were dissected from each animal and rinsed in a 0.9% sodium chloride solution to remove any blood. Following that, they were processed for the following studies:

Biochemical assessment

Tissues from the left lower lung lobes were extracted, homogenized, and then centrifuged to assess the oxidant–antioxidant status of the rats by measuring the level of malondialdehyde (MDA), as well as the activity of superoxide dismutase (SOD) and reduced glutathione (GSH) in the lung tissue. The activity of MDA was measured using the Yeginsu and Ergin method[20]. The results were given in nanomoles per gram of protein. The method of Lu and Finkel[21] was used to measure SOD activity. Its findings were expressed in units per gram of protein. Reduced glutathione (GSH) was measured using Jurczuk *et al*. technique[22], with the results expressed in milligrams per gram of protein.

I- Light microscopic study

A- Histological study

Lower lobes of the right lungs of each animal were fixed, cleared, dehydrated, and embedded in paraffin wax. For standard histological analysis of general architecture of lung, five µm thickened sections were cut and stained with hematoxylin and eosin (Hx. and E.)[23], and Masson trichrome stain for identification of collagen fibers[24].

B- Immunohistochemical study

The paraffin sections of lung were cut on poly-L-lysin coated slides, deparaffinized, and rehydrated. After antigen retrieval, the sections were incubated with:

Anti-BCL2 antibody immunostaining: B-cell lymphoma 2 (BCL2) it is an anti-apoptotic marker. The primary antibody used was anti-BCL2 antibody at dilution of (1:1000) (rabbit polyclonal antibody, ab59348, Abcam), A cytoplasmic brown reaction was exhibited by positive cells. Human colon carcinoma tissue was used as a positive control[25].

Anti- CD68 antibody immunostaining: Cluster of differentiation 68 (CD68) is used for detection of alveolar macrophages. Mouse monoclonal antibody at a dilution of 1:200 (Code NCL-L-CD68; Leica Biosystems, Benton La, Newcastle Ltd, UK), was used as primary antibody. Brown cytoplasmic coloration was expressed as positive reaction. A section of the tonsil was used as positive control[26].

Negative control sections of both markers were prepared by the same procedure but with omitting primary antibody. Finally, Mayer's hematoxylin was used to counterstain the sections.

II- Electron Microscopic study

Tissues of lower lobes of right lungs were quickly cut into small pieces (1mm) and then was immediately fixed for 3 hours at 4°C in 3 % glutaraldehyde buffered with 0.1 mol/L PBS at PH 7.4. The tissues were then dehydrated in ascending grades of alcohol then embedding occurred in epoxy resin to cut semi-thin sections of 1ml thick. Finally, they were examined under a light microscope after being stained with toluidine blue[27]. Lead citrate with uranyl acetate were used to stain ultrathin sections that were assessed and photographed using a transmission electron microscope (TEM) (JEOL, JEM-2100, Tokyo, Japan)[28] at the faculty of science, Alexandria University, Alexandria, Egypt.

III- Morphometrical study

For quantitative evaluation, five different sections from five different animals in each group were measured employing a Leica DML B2/11888111 microscope supplied with a Leica DFC450 camera. The measured variance was estimated using the software version K1.45 of Image J. The measured data were undertaken using H&E, Masson Trichrome and immunohistochemical sections. For quantitative evaluation, the following parameters were calculated:

- Mean thickness of the interalveolar septum in H. and E.-stained sections (x400).
- Mean area percentage of collagen fibers with Masson trichrome stained sections (x 400).
- Mean area percentage of BCL2 immuno-positive intensity in BCL2 immuno-stained sections (x400).
- Mean number of CD68 positive alveolar macrophages in CD68 immuno-stained sections (x400).

This was done in the Anatomy Department, Faculty of Medicine, Menoufia University, Egypt.

Statistical analysis

All biochemical and morphometric data from the experiment were recorded and displayed as mean ± SD. The varied parameters obtained from separate groups were compared using both the one-way variance analysis (ANOVA) and the Bonferroni's post-hoc test. Findings were tabulated and graphed[29].

RESULTS

General observations

- No rat deaths were recorded during the experiment.
- Because the control subgroups (IA, IB, and IC) had similar biochemical, histological, immunohistochemical, and morphometrical results, they were grouped together as the control group.

Biochemical results

Compared to the control group, MDA level in groups II and III was increased significantly. Non-significant difference was found in MDA levels between group II and III. Treatment with propolis (group IV) showed non-significant difference in MDA levels in group IV compared to the control group and a significant decrease compared to groups II and III.

The activity of SOD and GSH showed a significant decrease in groups II and III compared to the control group. While their levels in groups II compared to group III showed non-significant difference. Treatment with Propolis (group IV) resulted in a non-significant difference in their levels compared to the control group. However, a highly significant increase compared to groups II and III was detected. (Table 1, Diagram 1) were used to s*et al*l of the settings.

Light microscopic results

Histological results

1- Hematoxylin and Eosin stain (H. and E.)

Control lung sections, demonstrated variable sized alveoli and alveolar sacs separated by delicate interalveolar septa housing blood capillaries. Patent bronchiolar passages were noticed (Figures 1,2). Bronchioles were lined with columnar cells arranged in folded mucosa, surrounded by thin smooth muscle fibers coat (Figure 1).

The alveolar walls were lined by pneumocytes type I; squamous epithelial cells with flattened nuclei and pneumocytes type II that appeared large cuboidal (rounded) cells with large rounded nuclei, located at the angular junction of the alveolar walls (Figure 2).

On examination of BPA treated group (II); consolidation of the lung interstitium, distorted bronchiolar mucosa, heavy inflammatory cellular infiltration was detected. Most of the alveoli were collapsed while others were dilated (Figure 3).

Discontinuity of both bronchiolar mucosa and bronchiolar muscular coat were noticed. The bronchiolar lumen contained sloughed bronchiolar mucosal cells with dark small nuclei, bathed in eosinophilic exudate. Heavy peribronchiolar and perivascular inflammatory cellular infiltration was also, noticed (Figures 4,5).

Congested blood vessels with thickened muscular wall were observed. Their endothelial cells lining showed vacuolated cytoplasm and pyknotic nuclei (Figures 3,5). Acidophilic homogenous material was detected in the lung interstitium (Figure 5). Dark brown particles were seen deposited in the interstitium and in the alveolar cavities (Figure 6). Alveolar cavities were also occupied by extravasated RBCs and inflammatory cells as neutrophils and eosinophil. Macrophages are also observed within the alveolar cavities (Figure 7). Interalveolar septum appeared thickened containing congested dilated capillaries with inflammatory cells (Figures 6,7).

While, lung sections of the recovery group (III) revealed areas of patent alveoli side by side with collapsed ones and consolidated parts (Figure 8). Inter alveolar septa appeared with variable thickness. Intact bronchiolar mucosa with continuous muscular layer was noticed. Stand still congested blood vessels and inflammatory cellular infiltrations were noticed (Figure 9).

By using propolis with BPA in group (IV), lung tissue reclaimed almost all its normal histological features. Intact continuous bronchiolar layers were noticed (Figure 10). Patent alveoli bordered by pneumocytes type I and pneumocytes type II appeared. However, few congested blood capillaries were still present within thin Interalveolar septa (Figure 11).

2- Toluidine Blue stain

On examination of Toluidine blue stained lung semi-thin sections of the control group (I), Patent alveoli bordered with pneumocytes type I having flattened nuclei and pneumocytes type II having spherical nuclei and vacuolated cytoplasm were seen (Figure 12).

While, sections of the lung of BPA treated group (II) showed lung interstitium occupied with macrophage cells that possess lightly stained nuclei, many granules, and pseudopodia. Pneumocytes type I were noticed having irregular oval nuclei. Pneumocytes type II with many vacuoles in their cytoplasm and small shrunken nuclei were also, seen. Many congested blood capillaries were noticed (Figure 13).

The recovery group (III) lung semi-thin sections showed many pneumocytes type I having irregular small nuclei. Pneumocytes type II appeared with irregular nucleus. Narrow alveolar spaces were noticed (Figure 14).

By the co-administration of propolis with bisphenol in group (IV); the alveoli appeared patent, bounded by pneumocytes type I with their flattened regular nuclei and pneumocytes type II with their characteristic rounded large nuclei (Figure 15).

3- Masson's Trichrome stain

Using Masson's trichrome stain; few thin collagen fibers deposited around bronchioles, blood vessels, and in the interalveolar septa were seen within group (I) lung sections (Figure 16). In the BPA treated group (II) a noticeable increase in the collagen fibers accumulation in lung sections was detected (Figure 17). Excess amounts of collagen fibers were noticed in the interalveolar septa, peribronchiolar and perivascular in the recovery group (II) (Figure 18). A significant reduction in collagen fibers deposition in lung sections of the propolis and bisphenol treatment group (IV) was noticed (Figure 19).

Immunohistochemical results

1- BCL2 immunostaining

lung sections of control group showed strong +ve cytoplasmic immunoreaction for BCL2 in alveolar and bronchiolar cells (Figure 20). Faint immunoreaction for the same marker was observed in BPA treated group (II) lung sections (Figure 21). Lung sections of the recovery group (III) showed moderate cytoplasmic reaction for BCL2 in bronchiolar cells and minimal +ve reaction in the alveolar walls (Figure 22). Strong +ve cytoplasmic immunoreactivity for BCL2 was detected with lung sections of group (IV) (Figure 23).

2- CD 68 immunostaining

A very few macrophages gave +ve immunoreaction for CD68 in the lung sections of the control group (I) (Figure 24). Many macrophage cells with +ve brown cytoplasmic reaction for CD 68 was found in different lung sections of BPA treated group (II) (Figure 25). Also +ve immunoreaction for CD 68 was detected in many macrophage cells in the lung sections of group III (Figure 26). While in group IV few scattered positively stained macrophage for CD 68 was detected (Figure 27).

Electron microscopic results

Examination of ultrathin lung sections of the control group patent alveoli were lined by pneumocytes type I having thin scanty cytoplasm and flat nuclei. Type II pneumocytes were recognized with their rounded euchromatic nuclei, apical microvilli, lamellar bodies and plenty of mitochondria (Figures 28,29).

On examination of lung sections of BPA treated group (II), pneumocytes type I had irregular, fragmented, and heterochromatic nuclei with dilated perinuclear space. Their cytoplasm showed many vacuoles with degenerated mitochondria. Pneumocytes type II showed shrunken nuclei, empty irregular lamellar bodies, degenerated mitochondria, and few apical microvilli. The interalveolar septa appeared thickened having collagen

fibers. the alveolar spaces were occupied with eosinophil (Figures 30,31,32).

Ultrathin lung sections of the recovery group (III) revealed pneumocytes type I with cytoplasmic vacuoles and irregular nuclei. Pneumocytes type II showed heterochromatic nuclei, empty lamellar bodies, mitochondrial degeneration and few microvilli. Interalveolar septum appeared thickened having bundles of collagen fibers. Congested capillaries were also, noticed (Figure 33,34).

Group (IV) showed pneumocytes type I having oval euchromatic nuclei and thin rime of cytoplasm. Pneumocytes type II possessed rounded nuclei, lamellar bodies, and rounded mitochondria. Wide alveolar space bounded with thin interalveolar septum having few collagen fibers were also, noticed (Figures 35,36).

Morphometrical results

The mean thickness of interalveolar septum increased significantly in groups II and III compared to the control. Group III showed non- significant difference in mean thickness of interalveolar septum when compared to group II. Co-administration of propolis with BPA (group IV) showed a highly significant decrease in the mean thickness of interalveolar septum when compared with groups II and III and a non-significant difference when compared to the control group (Table 2, Diagram 2).

Regarding the mean area percentage of collagen fibers in Masson trichrome-stained sections, a highly significant increase in its area % was recorded in groups II and III when compared to the control. While a non-significant difference in area % of collagen fibers was found when group II compared to group III. Group IV had a non-significant difference in the mean area % of collagen fibers compared to the control group, but a highly significant decrease in the same parameter was recorded when compared to groups II and III as shown in (Table 2, Diagram 3).

On assessment of the mean area percentage of BCL2-positive immunoreactive in BCL2-stained sections, a highly significant decrease in its area was noted in groups II and III as compared to the control. The recovery (group III) revealed a non-significant difference in mean area percentage of BCL2 positive immunoreactive area compared to group II. While, group IV exhibited non-significant difference in mean area percentage of BCL2-positive immunoreaction compared with the control, and highly significant increase compared with groups II and III as seen in (Table 2, Diagram 4).

The mean number of CD68 positive alveolar macrophages in CD68 stained sections, exhibited a highly significant increase in groups II and III as compared to the control group. Group III showed a non-significant difference in mean number of CD68 positive alveolar macrophages as compared to group II. Non-significant difference in mean number of CD68 positive alveolar macrophages was recorded in group IV compared to the control group, and a highly significant decrease as compared to groups II and III as seen in (table 2, Diagram 5).

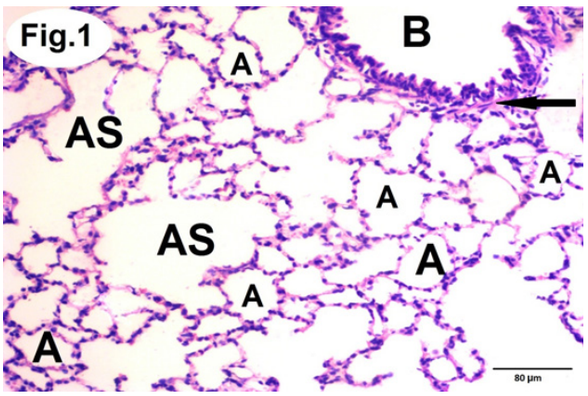

Fig. 1: A photomicrograph of lung section of control group (I) showing bronchiole (B) lined by simple columnar epithelium surrounded by thin layer of smooth muscles (→). Alveoli of variable sizes are noticed (A), alveolar sac (AS) where many alveoli open into are also, seen. (H. &E. X 100)

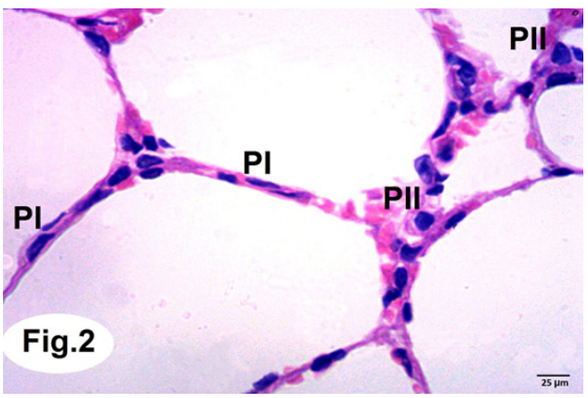

Fig. 2: A photomicrograph of lung section of control group (I) showing alveolar epithelium formed of pneumocytes type I (PI); thin flat cells with flat nuclei and pneumocytes type II (PII); large rounded cells positioned at the angles of the alveoli, with large rounded nuclei. (H. &E.X400)

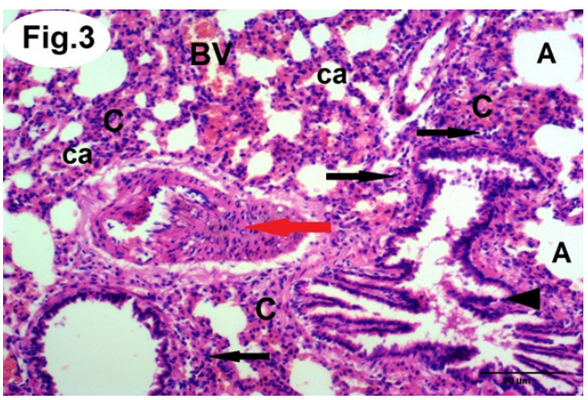

Fig. 3: A photomicrograph of lung section of BPA treated group (II) showing interstitial lung consolidation (C), distorted bronchiolar epithelium (►) and inflammatory cells infiltration (→). Some alveoli are collapsed (ca) while others are dilated (A). Notice the congested blood vessels (BV) as well as the blood vessel with thickened muscular wall (red arrow). (H. &E. X 100)

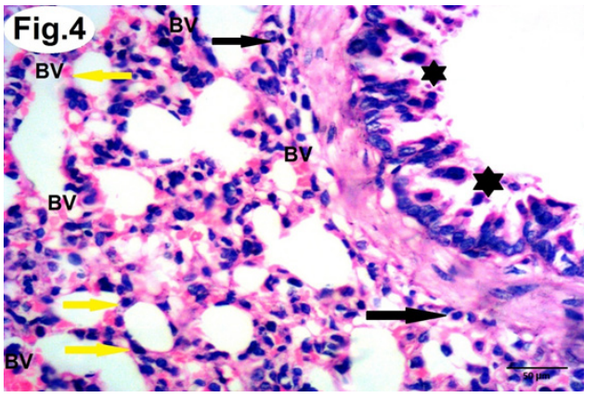

Fig. 4: A photomicrograph of lung section of BPA treated group (II) showing bronchiolar lumen contains sloughed bronchiolar mucosal cells with dark small nuclei (*). Peribronchiolar inflammatory cells infiltration (black arrow) is also seen. Thickened interalveolar septa (yellow arrow), housing congested blood capillaries (BV) are noticed. (H. &E. X200)

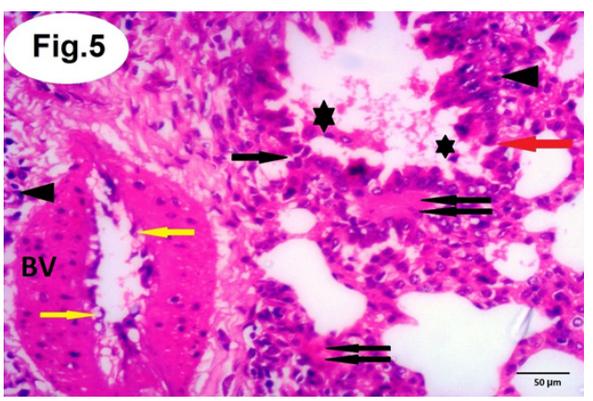

Fig. 5: A photomicrograph of lung section of BPA treated group (II) showing discontinuity of both bronchiolar mucosa (red arrow) and bronchiolar muscular coat (black arrow). Sloughed bronchiolar mucosal cells with dark small nuclei, bathed in esinophilic exudate are noticed (*). Acidophilic homogenous material is seen in the interstitium (↑↑). Dilated blood vessel (BV) with thickened wall is seen. Its endothelial lining has vacuolated cytoplasm and pyknotic nuclei (yellow arrow). Notice the inflammatory cellular infiltration around the bronchiole and the blood vessel (►). (H. & E. X 200)

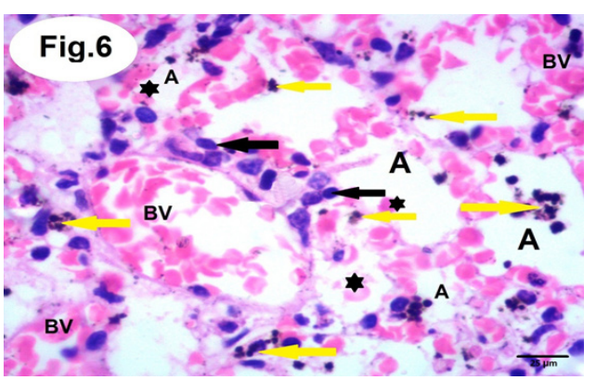

Fig. 6: A photomicrograph of lung section of BPA treated group (II) showing alveolar lumen (A), containing dark brown particles (yellow arrow) and RBCS (*). Thickened interalveolar septa with dilated congested blood capillaries (BV) and inflammatory cells (black arrow) are noticed. (H. & E. X 400)

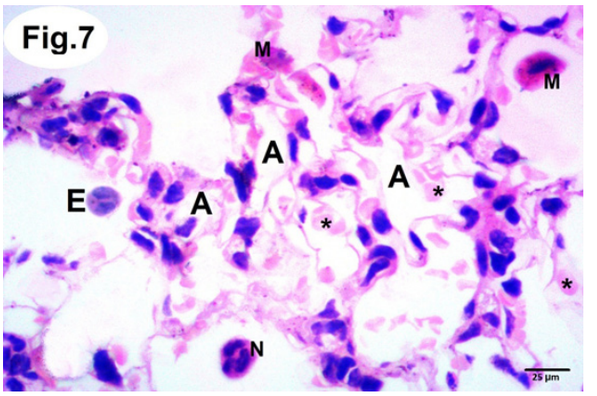

Fig. 7: A photomicrograph of lung section of BPA treated group (II) showing alveolar lumen (A) containing RBCS (*) with inflammatory cells like neutrophils (N) and eosinophil (E). Macrophage (M) cells are also, noticed. (H. & E. X 400)

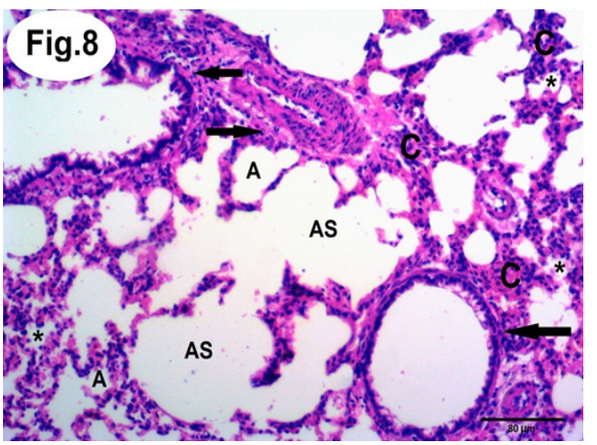

Fig. 8: A photomicrograph of lung section of recovery group (III) showing collapsed alveoli (*), patent alveoli (A), alveolar sac (AS), and consolidated interstitium (C). Perivascular and peribronchiolar inflammatory cells infiltration (→) are noticed. (H. & E. X 100)

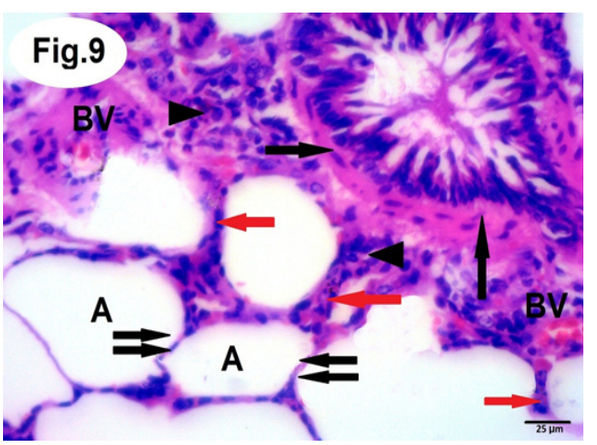

Fig. 9: A photomicrograph of lung section of recovery group (III) showing some alveoli (A) with thin interalveolar septum (s) and other with thickened septum (red arrow). Intact bronchiolar mucosa with continuous muscular layer (black arrow) is present. Inflammatory cellular infiltration (►) and congested blood capillaries (BV) are noticed. (H. &E. X 400)

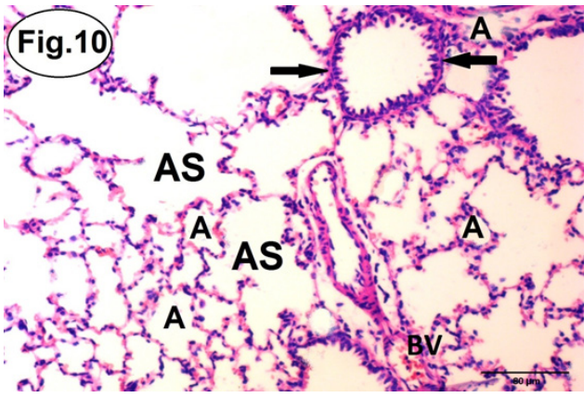

Fig. 10: A photomicrograph of lung section of group (IV) showing patent alveoli (A) & alveolar sacs (AS). Intact continuous bronchiolar layers are noticed (→). Notice the presence of dilated congested blood capillary (BV). (H. &E. X 100)

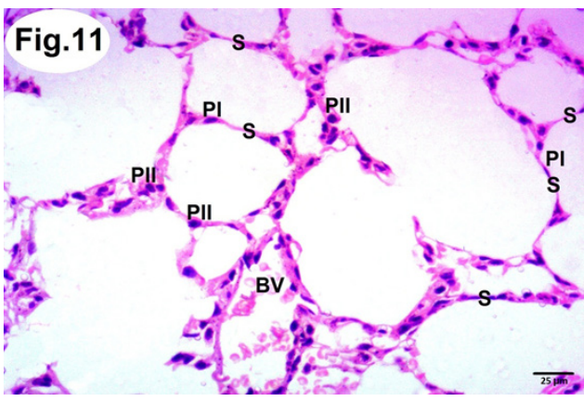

Fig. 11: A photomicrograph of lung section of group (IV) showing alveoli lined with pneumocyte type I (PI) and pneumocyte type II (PII). Interalveolar septa (S) appear thin with few congested blood capillaries (BV). (H. &E. X 400)

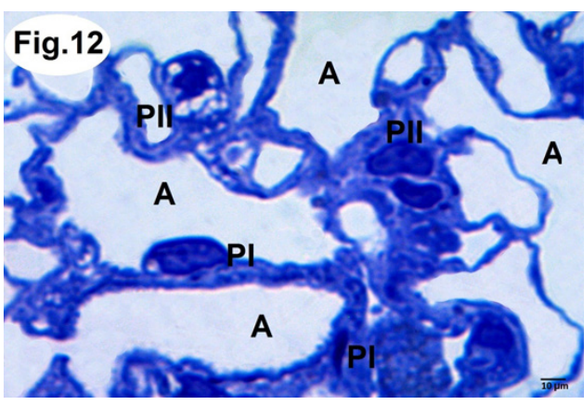

Fig. 12: A photomicrograph of lung section of control group (I) showing patent alveoli (A) lined with pneumocytes type I (PI) with flat nuclei, pneumocytes type II (PII) with rounded nuclei and vacuolated cytoplasm. (Toluidine blue X 1000)

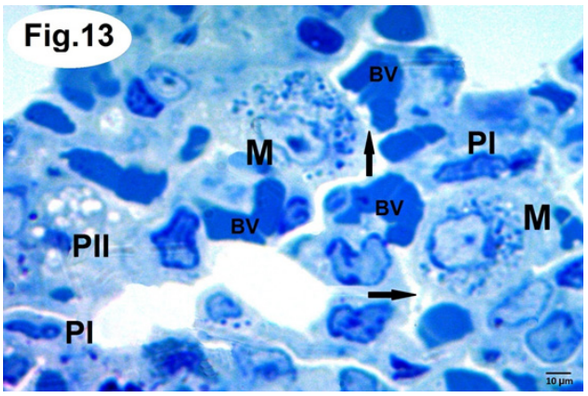

Fig. 13: A photomicrograph of lung section of BPA treated group (II) showing lung interstitium occupied with macrophage cells (M) with lightly stained nuclei, many granules, & pseudopodia (→). Pneumocytes type I (PI) showing irregular oval nuclei. Pneumocytes type II (PII) with vacuolated cytoplasm and small shrunken nuclei are noticed. Many congested blood capillaries (BV) are present. (Toluidine blue X 1000)

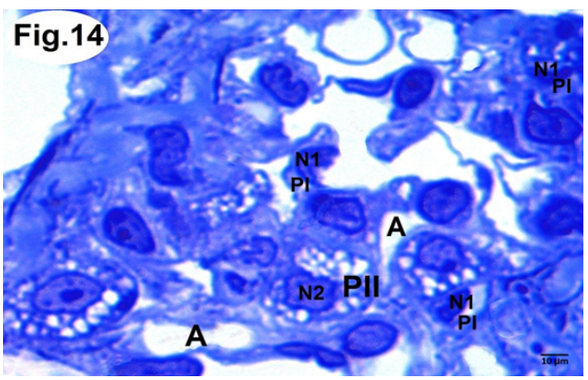

Fig. 14: A photomicrograph of lung section of recovery group (III) showing narrowed alveolar space (A). Many pneumocytes type I (PI) with irregular small nuclei (N1) are noticed. Pneumocytes type II (PII) appears with irregular nuclei (N2). (Toluidine blue X 1000)

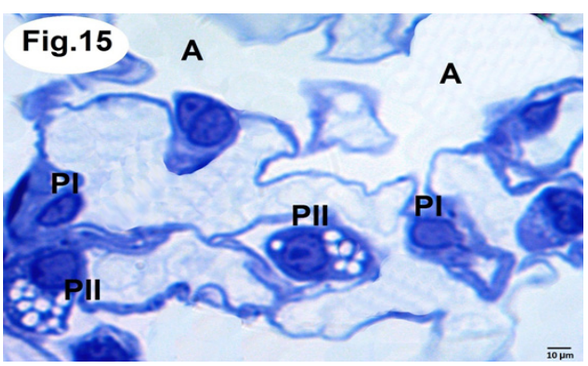

Fig. 15: A photomicrograph of a lung section of group (IV) showing patent alveoli (A), lined with pneumocytes type I (PI) and pneumocytes type II (PII). (Toluidine blue X 1000)

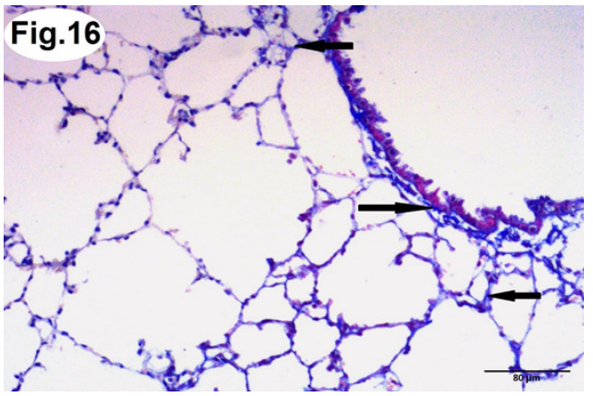

Fig. 16: A photomicrograph of a lung section of the of the control group (I) showing minimal collagen deposition (→) in the interalveolar spetae, around blood vessels and around bronchioles. (Masson's Trichrome X 100)

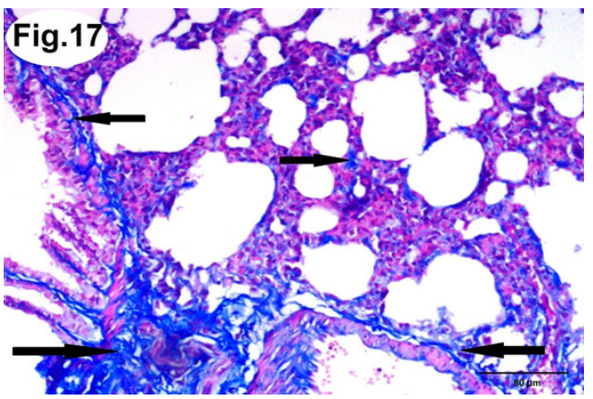

Fig. 17: A photomicrograph of lung section of BPA treated group (II) showing apparent increased collagen fibers deposition (→) in the thickened interalveolar septa and around bronchioles. (Masson's Trichrome X 100)

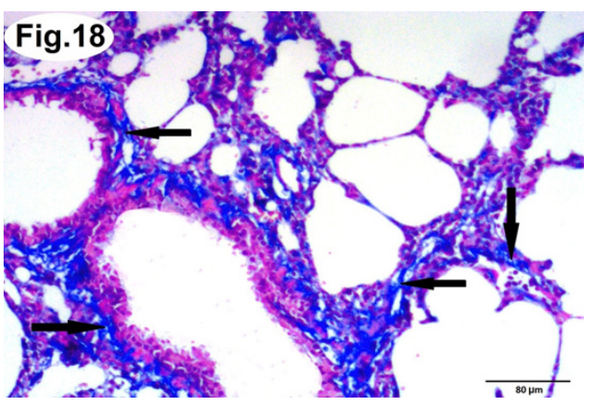

Fig.18: A photomicrograph of lung section of recovery group (III) showing apparent increased collagen fibers deposition (→) in the interalveolar septa, perivascular and around bronchioles. (Masson's Trichrome X 100)

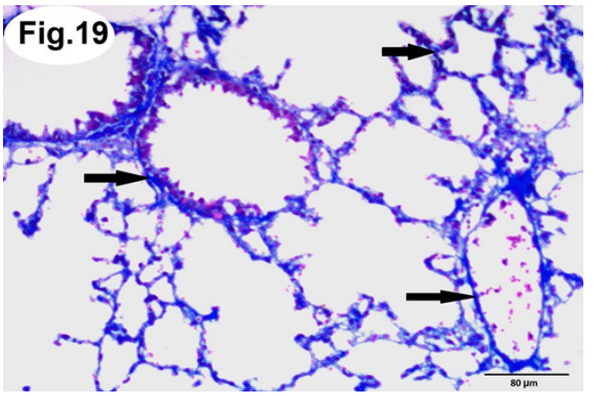

Fig. 19: A photomicrograph of lung section of recovery group (III) showing apparent increased collagen fibers deposition (→) in the interalveolar septa, perivascular and around bronchioles. (Masson's Trichrome X 100)

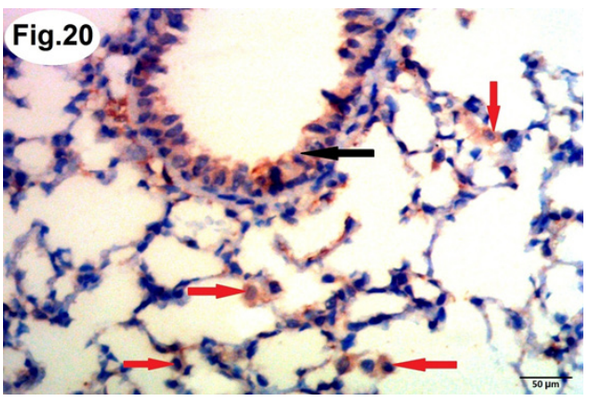

Fig. 20: A photomicrograph of lung section of control group (I) showing strong +ve immunoreaction for BCL2 in the form of brown cytoplasmic reaction on both bronchiolar cells (black arrow) and pneumocyte type II (red arrow) in the alveolar wall. (BCL2 X 200)

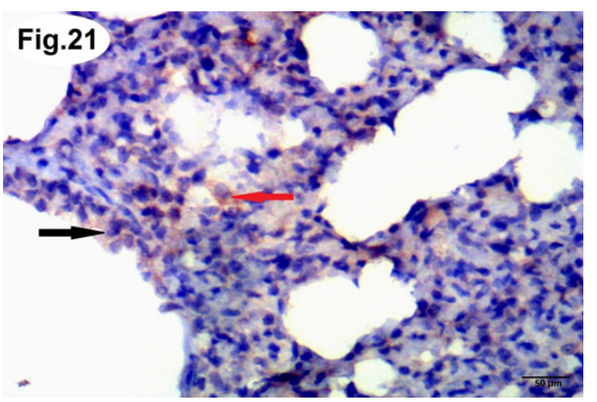

Fig. 21: A photomicrograph of lung section of BPA treated group (II) showing faint reaction for BCL2 in the bronchiolar cells (black arrow) and in lung interstitium (red arrow). (BCL2 X200)

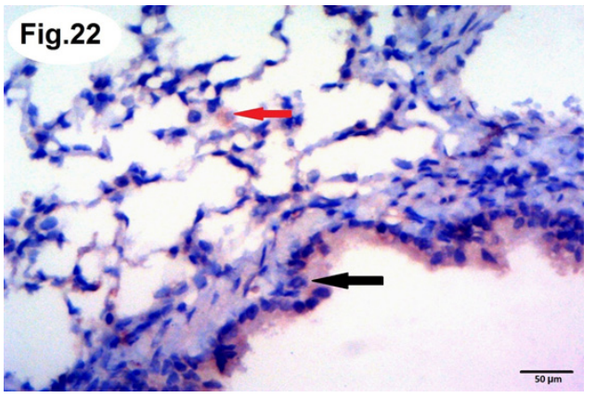

Fig. 22: A photomicrograph of a lung section of the recovery group (III) showing moderate cytoplasmic reaction for BCL2 in bronchiolar cells (→). Also minimal +ve reaction in the alveolar walls (red arrow) are noticed. (BCL2 X 200)

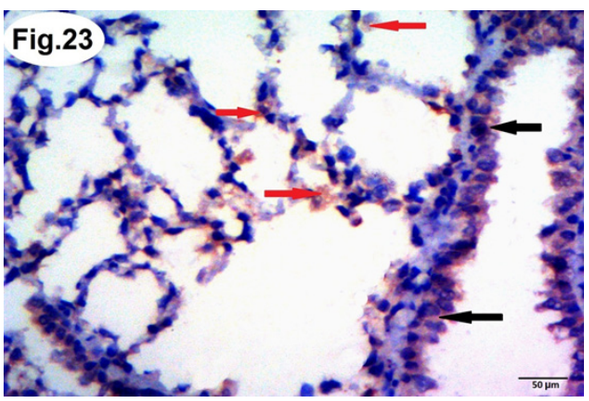

Fig. 23: A photomicrograph of a lung section of group (IV) showing strong +ve immunoreaction for BCL2 in bronchiolar cells (→) and pneumocyte type II (red arrow) in the alveolar wall. (BCL2 X 200)

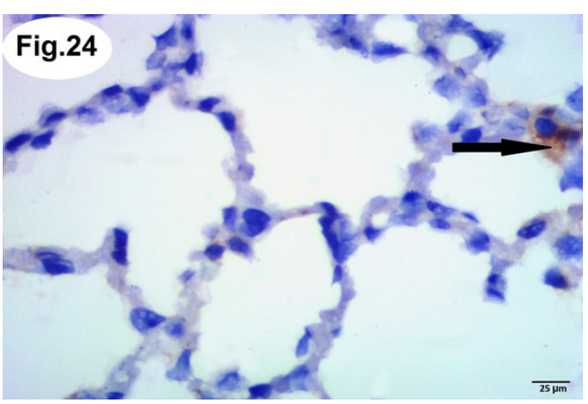

Fig. 24: A photomicrograph of a lung section of the control group (I) showing very few cells (macrophages) with +ve reaction for CD68 in the form of brown cytoplasmic deposits (→). (CD68 X400)

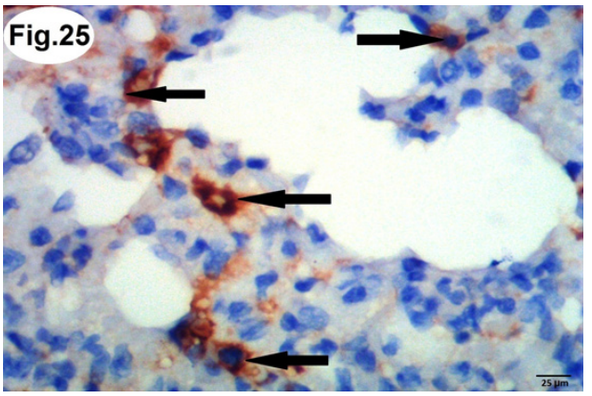

Fig. 25: A photomicrograph of a lung section of BPA treated group (II) showing plenty of cells (macrophages) (→) that give strong +ve cytoplasmic reaction for CD68. (CD68 X400)

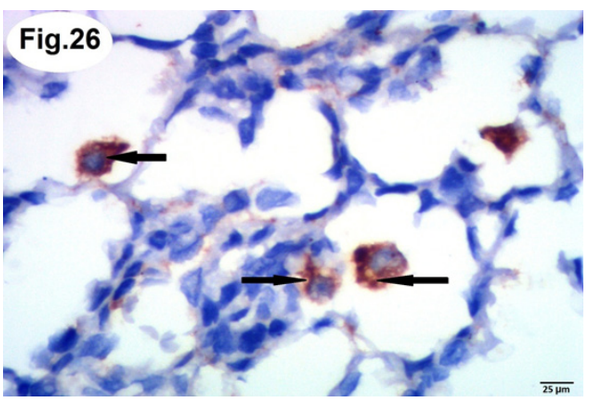

Fig. 26: A photomicrograph of a lung section of the recovery group (III) showing many cells (macrophages) (→) with +ve cytoplasmic reaction for CD68. (CD68 X400)

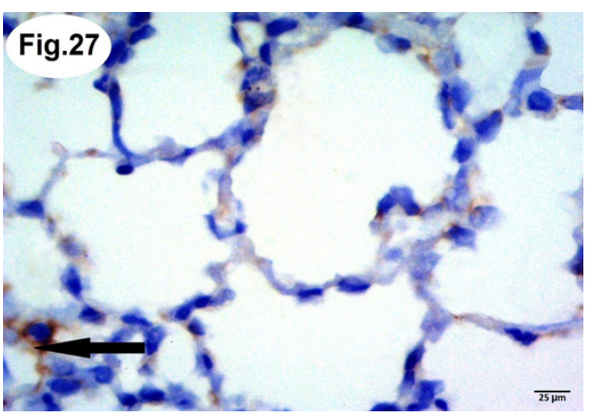

Fig. 27: A photomicrograph of a lung section of group (IV) showing scanty cells (macrophages) (→) with +ve reaction for CD68. (CD68 X400)

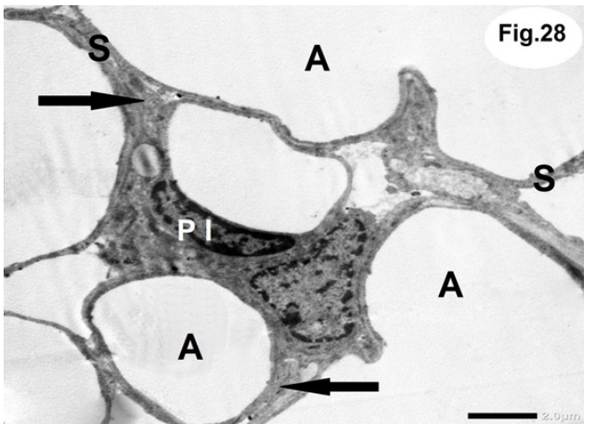

Fig. 28: An electron micrograph of the control lung showing patent alveoli (A) lined with pneumocytes type I (P I). Notice thin interalveolar septum (S) with few collagen fibers (→). X200O

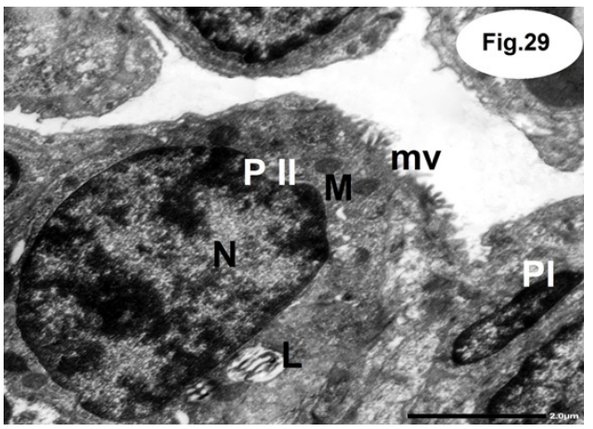

Fig. 29: An electron micrograph of the control lung showing pneumocyte type II (P II) with rounded euchromatic nucleus (N), provided with apical microvilli (mv). Its cytoplasm contains lamellar bodies (L) and numerous mitochondria (M). Pneumocyte type I (PI) with flat nucleus and thin scanty cytoplasm is also seen. X4000

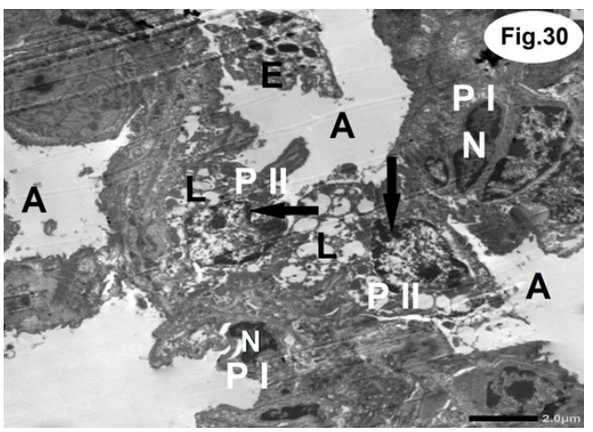

Fig. 30: An electron micrograph of the lung of BPA treated group (II) showing alveolar space (A) occupied with esionphile (E). Pneumocytes type I (P I) shows fragmented, heterochromatic and irregular nuclei (N). Pneumocytes type II (P II) shows irregular nuclei (→) and empty irregular lamellar bodies (L). X2000

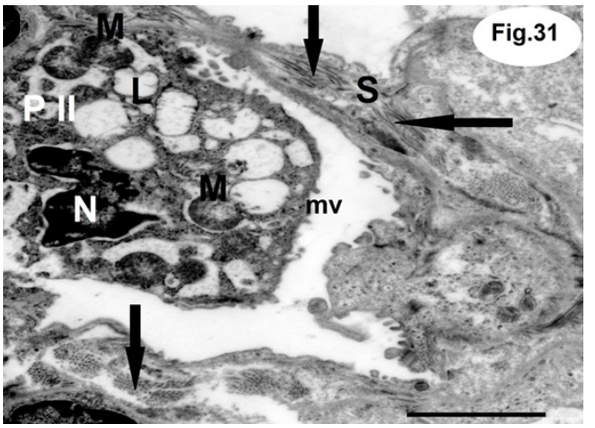

Fig. 31: An electron micrograph of the lung of BPA treated group (II) showing pneumocytes type II (P II) with shrunken irregular nucleus (N), empty lamellar bodies (L), degenerated mitochondria (M), and few apical microvilli (mv). The interalveolar septum (S) appears thickened by increased amount of collagen fibers (→). X4000

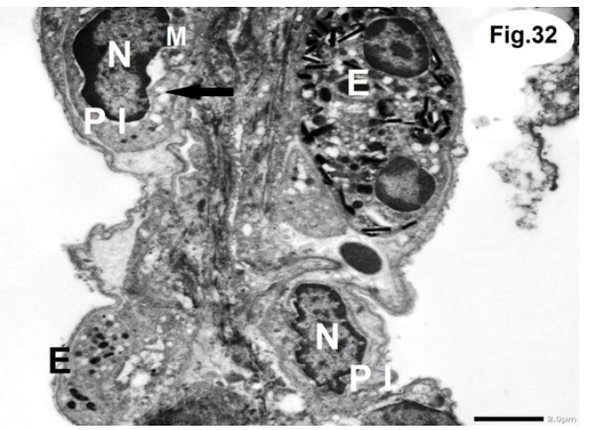

Fig. 32: An electron micrograph of the lung of BPA treated group (II) showing 2 pneumocytes type I (P I), their nuclei are irregular (N) with dilated perinuclear space. Many vacuoles (→) and degenerated mitochondria (M) are observed. Notice the esionphile cells (E). X2000

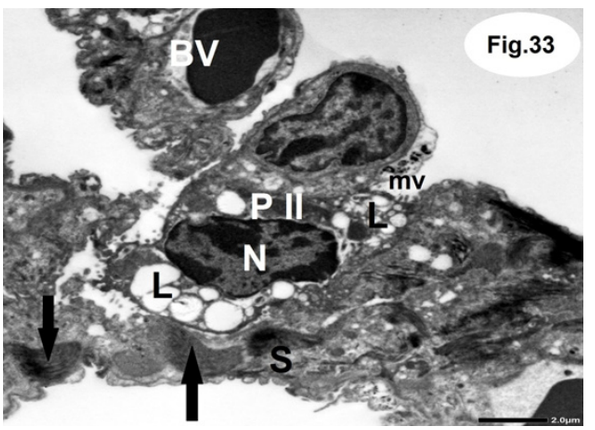

Fig. 33: An electron micrograph of lung of the recovery group (III) showing thickened interalveolar septum (S) with bundles of collagen fibers (→). Pneumocyte type II (P II) appears with heterochromatic nucleus (N), empty lamellar bodies (L) and few microvilli (mv). Notice the congested capillary (BV). X2000

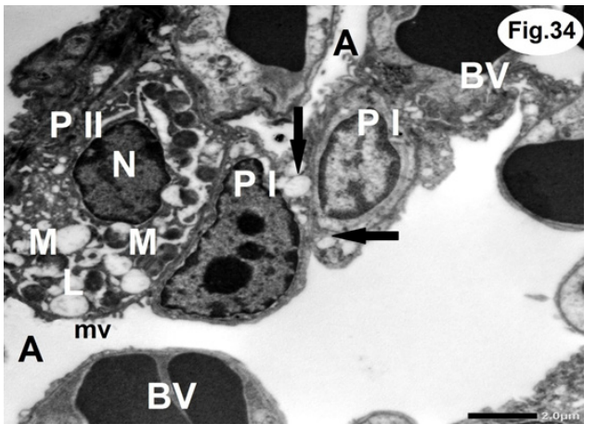

Fig. 34: An electron micrograph of lung of recovery group (III) showing alveolar space (A). Pneumocytes type I (P I) with cytoplasmic vacuoles are seen (→). Pneumocytes type II (P II) shows small rounded nucleus (N), few microvilli (mv), degenerated mitochondria (M) and empty lamellar bodies (L). Many congested blood capillaries (BV) are noticed. X2000

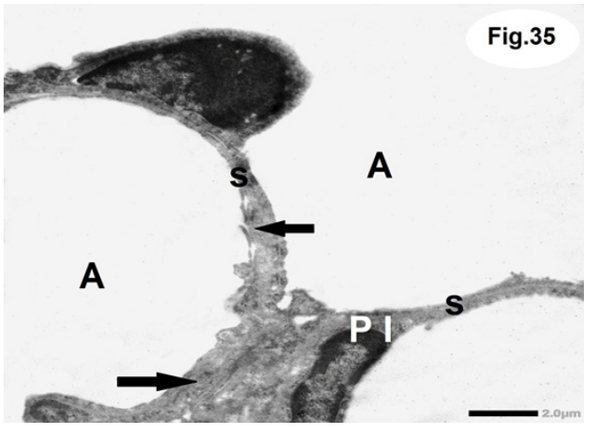

Fig. 35: An electron micrograph of the lung of group (IV) showing wide alveolar space (A) bounded with thin interalveolar septum (S) that contained few collagen fibers (→). Notice pneumocytes type I (P I) with flat oval nucleus (N) and thin rime of cytoplasm. X2000

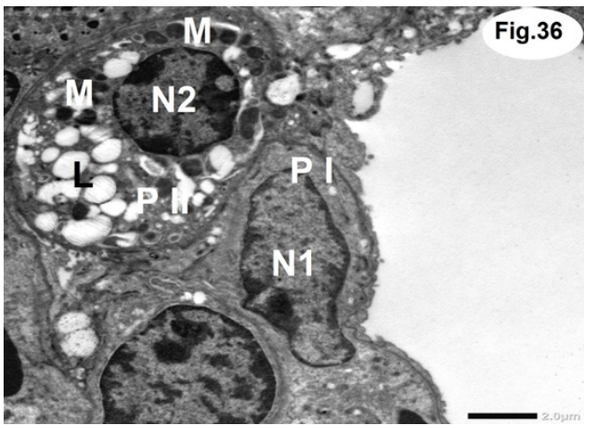

Fig. 36: An electron micrograph of the lung of group (IV) showing type I pneumocyte (P I) with oval euchromatic nucleus (N1) and thin rime of cytoplasm. While pneumocyte type II (P II) shows cytoplasm occupied by rounded nucleus (N2), lamellar bodies (L) and rounded mitochondria (M). X2000

Table 1: Comparison between different groups as regard MDA, GSH, and SOD levels within the lung tissue

Parameters	Mean ±SD				*P-value*
	I	II	III	IV	
MDA (nmol/gm)	1.3±0.1	3.8±0.2	2.4±0.1	1.9 ±0.3	P1=0.000 P2=0.000 P3=0.816 P4=0.069 P5=0.000 P6= 0.000
GSH (mg/gm)	39.1±0.3	19.1±0.3	16.9±0.4	36.9±0.5	P1=0.000 P2= 0.000 P3= 0.076 P4= 0.868 P5= 0.000 P6= 0.000
SOD (U/gm)	70.9±0.8	40.8±0.7	38.9±1.0	68.1±0.9	P1=0.000 P2=0.000 P3=0.822 P4=0.447 P5=0.000 P6=0.000

Table 2: comparison of the histological parameters in the lung in the different groups

Parameters	Mean ±SD				*P-value*
	I	II	III	IV	
Thickness of interalveolar septum (µm)	89.8±4.0	375.6±6.4	356.8±4.5	93.7±5.4	P1=0.000 P2=0.000 P3=0.718 P4=0.069 P5=0.000 P6= 0.000
Area percentage of collagen fibers	2.3±0.3	55.4±3.0	45.7±3.4	4.7±0.1	P1=0.000 P2=0.000 P3= 0.613 P4= 0.069 P5= 0.000 P6=0.000
Area percentage of BCL2 Positive immunoreactivity	51.4±0.7	28.2±0.6	37.9±0.5	47.6±1.1	P1=0.000 P2=0.000 P3=0.073 P4=0.636 P5=0.000 P6=0.000
No. of CD68 positive alveolar macrophages/field	7.3± 1.8	21.7±2.2	17.3±1.8	10.02±1.3	P1=0.000 P2=0.000 P3=0.964 P4=0.089 P5=0.000 P6=0.000

- P1: Comparison was done between group II and control.
- P2: Comparison was done between group III and control.
- P3: Comparison was done between group IV and control.
- P4: Comparison was done between group III and group II.
- P5: Comparison was done between group IV and group II.
- P6: Comparison was done between group IV and group III
  - *P value* > 0.05 means "non-significant".
  - *P value* < 0.05 means "significant".
  - *P value* < 0.001 means "highly significant".

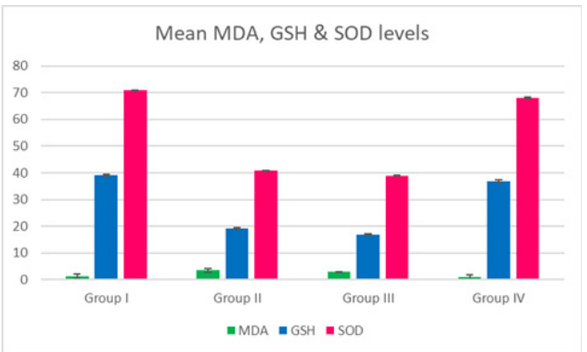

Diagram 1: comparison between different groups as regard mean levels of MDA, GSH and SOD

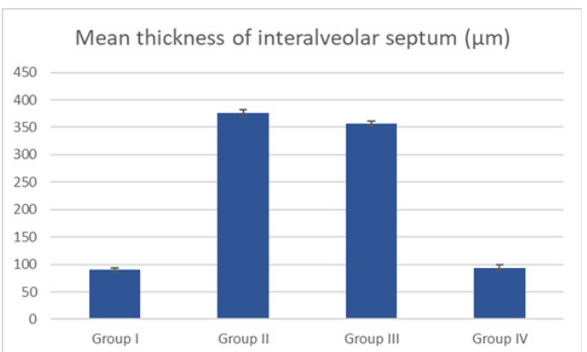

Diagram 2: Comparison between different groups as regard mean thickness of interalveolar septum (µm)

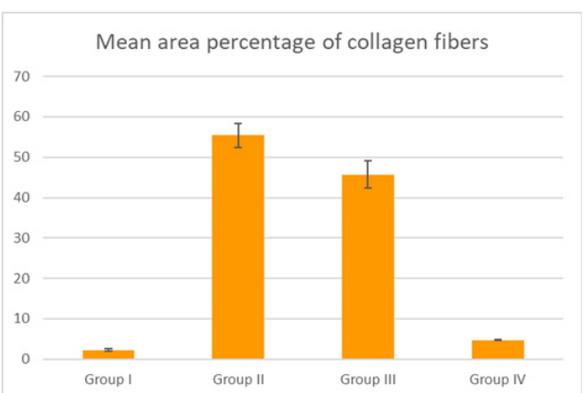

Diagram 3: Comparison between different groups as regard mean area percentage of collagen fibers in Masson-stained sections

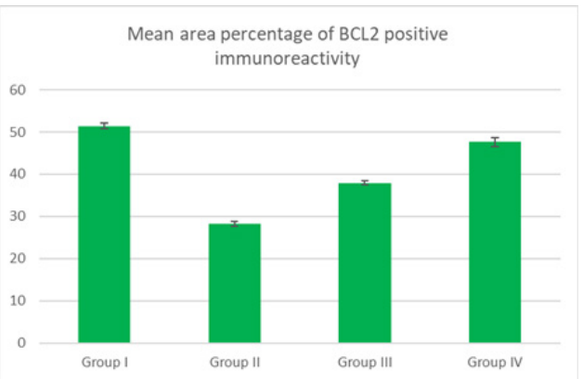

Diagram 4: Comparison between different groups as regard mean area percentage of BCL2 positive immunoreactivity in BCL2 immuno-stained sections

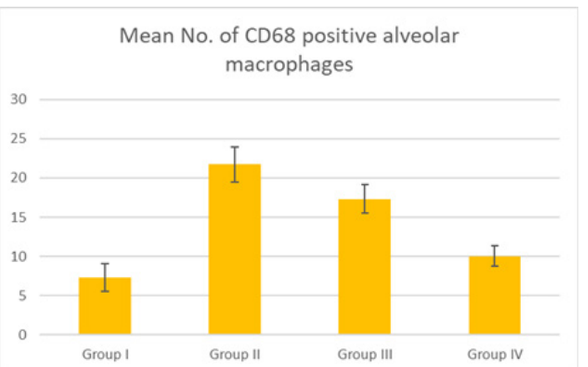

Diagram 5: Comparison between different groups as regard mean number of CD68 positive alveolar macrophages in CD68 immuno-stained sections

DISCUSSION

Bisphenol A is commonly applied industrial substance. It has been proven that exposure to BPA results in various disorders, including reproductive, pulmonary, cognitive, metabolic, and neurological abnormalities, as well as cancers[7].

Recently, the number of industrial applications of BPA products was increased. So, the human exposure to BPA is inevitable, likely to continue and even increase. So, BPA has attracted greater worldwide attention. However, studies

regarding the toxic effect of Bisphenol A on the lung and the protective measures for reduction of these effects are limited.

As a result, this research was designed to clarify the biochemical, histological and immunohistochemical changes in adult male albino rat's lung exposed to BPA, as well as the possible protective role of propolis.

In the present study, lung treated with bisphenol A showed disrupted lung architecture, consolidation of lung interstitium, collapsed alveoli with compensatory dilation of other alveoli. Pneumocytes type II showed shrunken heterochromatic nuclei, vacuolated cytoplasm, empty irregular lamellar bodies, degenerated mitochondria, and few apical microvilli. Also, Pneumocytes type I had heterochromatic nuclei, and vacuolated cytoplasm. Acidophilic homogenous material and dark brown particles were detected in between alveoli and in the interstitium. Congested thickened blood vessels with inflammatory cellular infiltration, thickening of Interalveolar septa were also, detected. The bronchi were distorted with discontinuity of their wall.

These results were in accordance with the findings of some scientists[3] and[18], who discovered congested thickened pulmonary arteries with extravasated red blood cells, inflammatory cell infiltration and collapsed alveoli in tissues of lung treated with BPA.

These recorded changes could be due to increased oxidative stress, hazardous free radical generation, and reactive oxygen species formation. These oxygen radicals' byproducts are cytotoxic agents as they attack biomolecules like membrane lipids, DNA, RNA, and proteins leading to progressive oxidative destruction, resulting in cell death and apoptosis, as some researchers had previously reported[30,31].

Other researchers[32,33] assumed that BPA administration resulted in increased MDA content within the tissues resulting in oxidative damage with decreased antioxidants such as superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase. These antioxidants share by a critical role in destruction of the formed ROS due to oxidative stress. Therefore, ROS overproduction and antioxidants depletion occurs resulting in disturbance of endogenous antioxidant defenses leading to tissue damage. This might be enforced in the current study by detection of high proportion of MDA with low levels of SOD and GSH in lung of group II treated with BPA as compared with control group.

Additionally, it has been documented that BPA-induced alteration in the activities of ATPase in cells leads to a decrease in ATP production causing cellular necrosis and damage. Bisphenol A accumulates within the mitochondria resulting in its dysfunction[34].

Another explanation for BPA's negative impacts is that it alters the dynamics of ion channels like Cl, Ca2+, and K+ in distinct types of cells via unique outer membrane receptors. Bisphenol A inhibited Cl release in respiratory epithelial cells by inhibiting basolateral K+ channels. Low-viscosity mucus formation requires transcellular active transport of CL through epithelial cells, preceded with water and Na+ transport, resulting in antiseptic lung environment. As a result of Cl transport failure, thick viscous mucus plugs accumulate, resulting in respiratory problems, as some scientists had previously concluded[35,36].

The presence of congested blood vessels in lung tissue treated with BPA was similar with the findings of[37], who attributed these alterations to an elevation in the proportion of oxygen demand to oxygen supply, resulting in a rise in the amount of adenosine generation. This causes vessel dilation with blood flow increase, restoring oxygen to normal levels.

The thickened blood vessel wall noticed with the BPA; might be due to the effect of ROS on blood vessel smooth muscle that resulted in release of cyclophilin A, which stimulated cell division as stated previously by[38,39].

Dark brown particles seen within the alveoli and in the interstitium could be hemosiderin granules formed as a result of ruptured RBCs. Oxidative stress occurring with BPA treatment leads to damage of RBCs cell membrane as previously documented by[33].

Inflammatory cells infiltration detected in the current study might be mediated by proinflammatory cytokines, especially tumor necrosis factor α (TNF-α), that results in changes on the effects of other cytokines, such as interleukin 1β (IL-1β). Reactive oxygen species (ROS) and free radicals produced due to oxidative stress induced by BPA result in proinflammatory cytokines TNF-α and IL-1β upregulation[40]. Increased (ROS) and proinflammatory cytokines activate nuclear factor kappa B (NF-kB) through kinase pathways. Nuclear factor kappa B (NF-kB) regulates chronic inflammation by influencing the transcription of several inflammatory genes, including cytokines and chemokines, which are responsible for the inflammatory changes seen in lung exposed to BPA[41,42].

Acidophilic homogenous substance detected within the alveoli of BPA treated group might be due to edema occurring as a result of inflammation. This result is in harmony with[43].

In the present study, BPA-treated group had a significant increase in the mean thickness of the interalveolar septum when compared with the control group. This could be related to an increase in the infiltration of numerous blood components such as macrophages and neutrophils, as some researchers had previously documented[44] that BPA activates macrophages to release inflammatory cytokines that enhance neutrophil accumulation and migration outside blood vessel. This could explain the presence of neutrophils noticed in the current study.

In the present study, BPA exposure caused marked collagen fibers deposition around bronchioles, blood vessels and within the alveolar wall in Masson trichrome

stained sections and electron microscopic ultrastructure examination; that was enforced statistically by significant increase of collagen fibers percentage in group II as compared to control. This result was in accordance with other researchers' work[10]. They demonstrated that BPA exposure results in increased fibroblast activity with their hyperplasia leading to increased collagen production.

Apoptosis detected within the lung tissue of BPA treated rats was demonstrated histologically by vacuolated cytoplasm and irregular, shrunken, and pyknotic nuclei. It was confirmed immunohistochemically by Faint BCL2 immune expression and enforced statistically by significant decrease in its area percentage compared to control. These findings were confirmed in a former study[45]. The BCL2 protein is critical for achieving a balance between apoptosis suppression and activation. An anti-apoptotic protein (BCL2) is responsible for keeping the outer mitochondrial membrane intact. Bisphenol A exposure induces a decrease in Bcl-2 expression, leading to change in permeability of mitochondrial membrane, leading in discharge of cytochrome c into cytosol and caspases stimulation. Caspase activation leads to apoptosis in cells[46].

Additionally, other scientists[47] postulated the downregulation of BCL2 to disruption in protein production within the cell due to oxidative stress resulting from exposure to BPA leading to disturbance in cellular homeostasis with occurrence of cellular degeneration and apoptosis.

This work showed strong positive immunohistochemical staining of CD68 marker within the lung tissue of rats of group II. It was authenticated morphometrically by highly significant increase in the alveolar macrophage number, and enforced by detection of macrophage in lung semi-thin sections. These findings were in harmony with the results reported by some researchers[10]. The increased number of macrophages could be due to recurrent division or stimulation of blood monocytes by numerous chemotactic substances secreted by the injured tissue. Macrophages are the essential cells in the lung responsible for innate immune responses. They encounter allergens and other threats to homeostasis. Macrophages possess anti-inflammatory and immunogenic properties[48,49]. They aid in the repair of injured tissues by promoting the synthesis of chemokines and growth factors[50].

Recovery group lungs sections showed mild improvement. However, some histological alterations were still present as consolidation of lung interstitium and thickened interalveolar septum. Stand still inflammatory cellular infiltration with congested blood vessels were seen. Pneumocytes type I showed vacuolated cytoplasm with irregular heterochromatic nuclei. Pneumocytes type II had empty lamellar bodies, mitochondrial degeneration and few microvilli. These histological alterations were confirmed by the immunohistochemical and biochemical results. These findings run in accordance with other scientists[51], who documented partial improvement after recovery from BPA treatment.

In the present study, group IV treated with propolis and BPA revealed a marked improvement in the biochemical, histological and immune-histochemical results. This is denoting the protective role of propolis against BPA's harmful effects on lung tissue. These findings support the findings of former studies who assumed that propolis has a protective effect against lung toxicity[50,52].

Propolis is among the most promising natural substances, with curative and protective characteristics without reported side effects. Propolis is believed to exert its biological activities due to synergistic action of its constituents. It contains over 300 polyphenol compounds including flavonoid, and phenolic acid, as well as their esters[53].

Propolis` anti-inflammatory and immunomodulatory properties were explained in a previous study by impairing neutrophil infiltration, suppressing proinflammatory mediators such as interleukins and tumor necrosis factor-α, all of which influenced immune defense mechanisms[54]. Furthermore, propolis has been shown to activate cellular immune response by increasing interferon-y, mRNA and activating synthesis of cytokines[55].

Moreover, propolis acts as an antioxidant drug by decreasing the extent of lipid peroxidation that was featured in this research by decreasing MDA level and increasing SOD and GSH enzymatic antioxidants activities. Moreover, it is a powerful ROS scavenger enhancing detoxification of free radicals[12]. Other investigators documented the role of propolis in reducing cellular hydrogen peroxide and nitric oxide levels which play the main role in cellular death. They also, added that propolis antioxidant activity might be contributed to other components such as caffeic acid phenethyl ester (CAPE) which prevents generation of reactive oxygen species (ROS) in many systems[56].

Propolis enhances the activity or expression of nuclear factor erythroid 2 (Nrf2), a critical intracellular transcription factor that restores antioxidant function under oxidative stress. As a result, free rad¬ical-scavenging enzymes are released, which neutralize, eliminate, and detoxify hazardous oxidants[57].

Moreover, the cyto-protective activity of propolis might be attributed to its ability to prevent the permeabilization of the internal mitochondrial permeability transition port (m PTP). The restoration of mitochondrial membrane potential may also give a mechanism for cells to avoid apoptosis[58]. Some investigators[54], suggested that propolis ameliorates fibrosis by its antioxidant characteristics and its modulatory effect on cytokine TGF-β1 that play an essential role in inducing fibrosis.

CONCLUSION

Propolis is a natural product with anti-inflammatory, anti-oxidant and anti-cytotoxic activities that could protect the lung tissue from the toxic effects induced by exposure to bisphinol A. We recommend the co-administration of propolis during BPA exposure as a protective agent.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. He, M.; Ichinose, T.; Yoshida, S.; Takano, H.; Nishikawa, M.; Shibamoto, T.; and Sun, G. (2016):" Exposure to bisphenol A enhanced lung eosinophilia in adult male mice". Allergy, Asthma and Clinical Immunology. 12:16(1-9).
2. Omran, B.H.F, Abdallah, E.A.A. and Abdelwahab, M.M. (2017):" Study of probable toxic effects of bisphenol A and the protective role of vitamin E on testes and prostate of adult male albino rats". Ain Shams Journal of forensic medicine and clinical toxicology. 29: 7-18.
3. Kattaia, A.A and Abdel Baset, S.A. (2014):" Effect of bisphenol A on the lung on adult male albino rats and the possible protective role of geraniol: a histological and immunohistochemical study". Egyptian journal of histology. Egyptian Journal of Histology 37(1):24-35.
4. Welshons, W.V.; Nagel, S.C. and Vom Saal, F.S. (2006):" Large effects from small exposures: III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure". Endocrinology. 146: S56:S69.
5. Heindela, J.; Newboldb, R.; Bucherb, J. *et al*., (2015):" NIEHS/FDA clarity-BPA research program update". Reproductive Toxicology. 58:33-44.
6. Stahlhut, R.W.; Welshons, W.V. and Swan, S. H. (2009):" Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both". Environ. Health Perspect. 117:784-789.
7. Hijazi, A; Guan, H.; Cernea, M. and Yang, K. (2015):" Prenatal exposure to bisphenol A disrupts mouse fetal lung development". The FASEB Journal. 29:4968-4977.
8. Meeker, J.D. and Ferguson, K.K. (2011):" Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007-2008". Environ Health Perspect. 2011; 119:1396-1402.
9. Sewelam and Mokhtar (2019):" Effect of Perinatal Exposure to Low Dose Bisphenol A on Hepatic and Renal Tissues of Male Albino Rat Offspring: Histological, Immunohistochemical and Morphometric Studies". Egyptian journal of histology. 42 (4):974-1000.
10. Mohammed, H.O; Al-sayed, A.M.; Abulfadle, K.A. and Farag, A.I. (2019):" Potential Protective Role of Vitamin E in Lung of Adult Male Albino Rat Exposed to Bisphenol A". Med. J. Cairo Univ. 87(8): 4901-4915.
11. Mahmoud, O.M.; N.A. Salem, N.A.; and Al Badawi, M. H. (2020):" Protective effect of propolis on manganese chloride neurotoxicity of olfactory bulb in adult male albino rat". Folia Morphol.79 (4): 672–680.
12. Kassab, A.A. and El-kaliny, H. H. (2017):" The Possible Role of Propolis in Ameliorating Paclitaxel-Induced Peripheral Neuropathy in Sciatic Nerve of Adult Male Albino Rats". Egyptian Journal of histology. 40 (2): 141-155.
13. El Bakary, R.H. and Sadek, M. T. (2018):" Possible Protective Effect of Propolis on Cardiac Muscle Injury Induced by Liver Fibrosis in Adult Male Albino Rat: Histological Study". Egyptian Journal of Histology. 41 (4): 559-569.
14. Sforcin, J.M., Bankova, V., Kuropatnicki, A.K., (2017):" Medical benefits of honeybee products". Evidence-Based Complementary and Alternative Medicine. Volume 2017. Article ID 2702106.
15. Rajpara, S.; Wilkinson, M.S.; King, C.M.; Gawkrodger, D.J.; J.S. English, J.S.; Statham, B.N.; Green, C.; Sansom, J. E.; M.M.; Chowdhury, M.M.; and Horne, H.L. (2009):" The importance of propolis in patch testing a multi-center survey Contact Dermatitis". 61: 287-290.
16. Sforcin, J. M. (2016):" Biological properties and therapeutic applications of propolis". Phytother. Res., 30: 894-905.
17. Toreti, V. C.; Sato, H.H.; Pastore, G.M.; and Park, Y.K. (2013):" Recent progress of propolis for its biological and chemical compositions and its botanical origin". Evidence-Based Complement. Alternat. Med. Volume 2013. Article ID 697390.
18. Abedelhaffez, A.S.; Abd El-Aziz, E.A.; Abdel Aziz, M.A.; and Ahmed, A.M. (2017):" Lung injury induced by Bisphenol A: A food contaminant, is ameliorated by selenium supplementation". Pathophysiology. 24(2):81-89.
19. Kalleny, N.K. and Soliman, N.B. (2011):"Light and electron microscopic study on the effect of topically applied hyaluronic acid on experimentally induced corneal alkali burn in albino rats". Egyptian journal of histology. 34: 829-848.
20. Yeginsu, A. and Ergin, M. (2009): "Antioxidant enzymes and lipid peroxidation in cold ischemic lung preservation". Experimental and clinical transplantation. 2: 94-98.
21. Lu, T. and Finkel, T. (2008): "Free radicals and senescence". Experimental Cell Research.314(9):1918-22.
22. Jurczuk, M.; Brzóska, M.M.; Moniuszko-Jakoniuk, J.; Gałazyn-Sidorczuk, M. and Kulikowska-Karpińska, E. (2004):" Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol". Food Chem Toxicol.42(3):429-38.

23. Bancroft, J.D and Layton, C. (2010): "In: Theory and Practice of histological techniques". 7th edition. London: Churchill Livingstone, 173-214.

24. Bancroft, J.D and Gamble, M. (2008): "Connective tissue stains. In: Bancroft, JD, Gamble, M. editors. Theory and practice of histological techniques". 6th ed. London, New York and Philadelphia: Churchill Livingstone. P: 150-155.

25. Jackson P, Blythe D. (2013): Immunohistochemical techniques [chapter 8]. In: Suvarna SK, Layton C, Bancroft JD, editors. Theory and practice of histological techniques. 7th ed. Philadelphia: Churchill Livingstone of Elsevier. 381–434.

26. Damoiseaux, J.G.; Yagita, H.; Okumura, K. and Vriesman, P.J. (1998): "Costimulatory molecules CD80 and CD86 in the rat; tissue distribution and expression by antigen presenting cells". Journal of Leukocyte Biology. 64(6):803-809.

27. Dykstra, M.J. and Reuss, L.E. (2003):" Staining methods for semi thins and ultra-thins. In: Biological electron microscopy, theory, techniques and troubleshooting. 2nd ed. Kluwer Academic Publishers/ Plenum Publishers. pp.175- 196.

28. Tizro, P.; Cboi, C. and Khanlou, N. (2018): "Sample preparation for transmission electron microscopy". Biobanking: methods and protocols.417-424.

29. Peat, J. and Barton, B. (2005): "Medical statistics. A guide to data analysis and critical appraisal". First edition. Wiley- Blackwell. 113-119. 41(2):546-558.

30. Attia, Z.I.A.; Mostafa, H.A. and Allam, Z.S.M. (2021):" Ameliorative effect of curcumin on the toxicity induced by bisphenol A on brain of male albino rats". Nat. Sci.19(1):19-24].

31. Amjad, S.; Rahman, S. and Pang, M.G. (2020):" Role of Antioxidants in Alleviating Bisphenol A Toxicity". Biomolecules, 10: 1008:1105.

32. Eweda, S.M.; Newairy, A.; Abdou, H.M. and Gaber, A.S. (2020):" Bisphenol A induced oxidative damage in the hepatic and cardiac tissues of rats: The modulatory role of sesame lignans". Experimental and therapeutic medicine. 19: 33-44.

33. Kamel, A.H.; Foaud, M.A. and Mousa, H. M. (2018):" The adverse effects of bisphenol A on male albino rats". The Journal of Basic and Applied Zoology. 79:6.

34. Fawzya, E.I.; El Makawyb, A.I.; El-Bambya, M.M.; Elhamalawya, H.O. (2018):" Improved effect of pumpkin seed oil against the bisphenol-A adverse effects in male mice". Toxicology reports. 5: 857–863.

35. Nadal, A.; Ropero, A.B.; Laribi, O.; Maillet, M. and Fuentes, E. (2000): "Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor α and estrogen receptor β". Proc Nat Acad. Sci USA. 97:11603–11608.

36. Ito, Y.; Sato, S.; Son, M.; Kondo, M.; Kume, H.; Takagi, K. and Yamaki, K. (2002):" Bisphenol A inhibits Cl− secretion by inhibition of basolateral K+ conductance in human airway epithelial cells". JPET 2002; 302:80–87.

37. Tolba, A.M. and Mandour, D. A. (2018):" Histological effects of bisphenol-A on the reproductive organs of the adult male albino rat". Eur. J. Anat. 22 (2): 89-102.

38. Satoh, K.; Nigro, P., *et al*. (2010): "Oxidative stress and vascular smooth muscle cell growth: A mechanistic linkage by cyclophilin A." Antioxidants and Redox Signaling, 12 (5): 675-682.

39. AL-Gabri, N.A.; Ali, A.; AL-Attar, E. and Hamed, M. (2017): "Pathological Study on the Role of Thymoquinone in Experimentally Induced Acute Lung Injury in Rats". Zagazig Veterinary Journal. 45 (3):228-237.

40. Ali, H.M.; Al-Rasheed, N.o.M.; Fadda, L.M.; Al-Rasheed, N.A.M. and Yacoub, H.I. (2016): "Down-Regulation of NF-κB, Bax, TGF-β, Smad-2mRNA expression in the Livers of Carbon Tetrachloride Treated Rats using Different Natural Antioxidants". Braz Arch Biol Technol; v.59: e16150553.

41. Igor, O.M.; Fabiane, F.; Camila, R.C. and Giuseppina, L. (2016): "Antioxidant Activity of γ-Oryzanol: A Complex Network of Interactions". Inter J of Mol Sc; 17 (8): 1107.

42. Muhammed, M.H.; Mostafa, R.M.; Said, M.A.; Afnan I. Elgndy, A. I. and Hend E. Elwakel, H.E. (2021):" Protective Effect of Thymoquinone on Bisphenol A-Induced Hepatotoxicity in Male Rats, Targeting the Role of Associated Pro-Inflammatory Cytokines and NF-kB". Benha medical journal vol. 38, academic issue.

43. Omorodion, N.T.; Atoigwe-Ogeyemhe, B.E.; Achukwu, P.U. and Odigie, E, B. (2019):" Histopathological changes associated with exposure of some viscerals and testicular tissues to bisphenol A and di(2-ethylhexyl) phthalate". Tropical journal of pharmaceutical research. 18(6): 1213: 1218.

44. Wetherill, Y.B.; Akingbemi, B.T., *et al*. (2007): "In *vitro* molecular mechanisms of bisphenol A action". Reproductive Toxicology, 24 (2): 178-198.

45. Wang, C.; He, J.; Xu, T.; Han, H.; Zhu, Z.; Meng, L.; Pang, Q. and Fan, R. (2021):" Bisphenol A(BPA), BPS and BPB-induced oxidative stress and apoptosis mediated by mitochondria in human neuroblastoma cell lines". Ecotoxicology and environmental safety. Vol. 207: 111299.

46. Lin, Y.; Sun, X. ; Qiu, L.; Wei, J.; Huang, Q.; Fang, C.; Ye, T.; Kang, M.; H Shen, H. and Dong, S. (2013):"Exposure to bisphenol A induces dysfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma (INS-1) cells". Cell Death and Disease. Vol. 4, page: 460.

47. Mohamed, H.K. and Rateb, A. (2019):" Histological and Biochemical Study on the Toxic Effects of Bisphenol A on the Thyroid Gland of Adult Male Albino Rats and the Possible Protection by Selenium". Egyptian journal of histology. 42(3):667-685.

48. Mosser, D.M. and Edwards, J. P. (2008):" Exploring the full spectrum of macrophage activation". Nat Rev Immunol. 8(12):958–69.

49. Draijer, C.; Robbe, P.; Boorsma, C.E.; Hylkema, M.N.; Melgert, B.N. (2013):" Characterization of macrophage phenotypes in three murine models of house-dust mite- induced asthma". Mediators Inflamm.632049. https://doi.org/10.1155/2013/632049.

50. Almansour, M.I. and Jarrar, B. M. (2017):" Protective Effect of Propolis Against Pulmonary Histological Alterations Induced by 10 nm Naked Gold Nanoparticles". Chiang Mai J. Sci. 2017; 44(2): 449-461. http://epg.science.cmu.ac.th/ejournal/.

51. Ibrahim, H.H. and Morsy, M. M. (2019):" Hazards of Bisphenol A on the Thyroid Gland of Adult Male Albino Rats and Possibility of Recovery after its Withdrawal". Med. J. Cairo Univ., Vol. 87, No. 5, September: 2945-2953, 2019 www.medicaljournalofcairouniversity.net.

52. Bilgin, G.; Kismet, K.; Kuru, S.; Kaya, F.; M Senes, M.; Bayrakceken, Y.; Yumusak, N.; Celikkan, F.T.; Erdemli, E.; Celemli, O.G.; Sorkun, K. and Koca, G. (2016):" Ultrastructural investigation of the protective effects of propolis on bleomycin induced pulmonary fibrosis". Biotechnic & Histochemistry, 1–9. http://dx.doi.org/10.3109/10520295.2015.1123294.

53. Kaya, E.; Yılmaz, S. and Ceribasi, S (2019):" Protective role of propolis on low and high dose furan-induced hepatotoxicity and oxidative stress in rats ". J Vet Res 63, 423-431. DOI:10.2478/jvetres-2019-0054.

54. El-Mahalaway, A.M.; Selim, A.A. and Mahboub, F.A. (2015):" The potential protective effect of propolis on experimentally induced hepatitis in adult male albino rats. Histological and immunohistochemical study". Journal of Histology & Histopathology. Volume 2 | Article 14:1-9. http://www.hoajonline.com/journals/pdf/2055-091X-2-14.pdf.

55. Wagh, V.D. (2013):" Propolis: A Wonder Bees Product and Its pharmacological Potentials". Advances in Pharmacological Sciences. Volume 2013, Article ID 308249, 11 pages. http://dx.doi.org/10.1155/2013/308249.

56. Abu Almaaty, A.H.; Abd El-Aziz, Y.M.; Omar, N.A.; Abdeen, A.M.; Hala Afifi, H.; Ibrahim, T.S.; Elhady, S.S. and Khedr, A.I.M. (2020):" Antioxidant Property of the Egyptian Propolis Extract Versus Aluminum Silicate Intoxication on a Rat's Lung: Histopathological Studies". Molecules. Vol. 25: 5821.

57. Erejuwa, O.O.; Sulaiman, S.A. and Ab Wahab, M. S. (2012):" Honey: a novel anti¬oxidant. Molecules". 17: 4400-4423.

58. Chaa, S.; Boufadi, M. Y.; Keddari, S. and Benchaib, A.H. (2021):" Protective effect of propolis from Tigzirt on epirubicin-induced cardiotoxicity and nephrotoxicity". Journal of Pharmacy & Pharmacognosy Research, 9 (4), 549-562, 2021. ISSN 0719-4250 http://jppres.com/jppres.

الملخص العربى

تأثير مادة البسفينول أ علي رئة الجرذان البيضاء البالغة و تقييم التأثير الايجابي المحتمل لشمع العسل: دراسة بالمجهر الضوئي و الإلكتروني

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الخلفية والهدف من البحث: تعتبر مادة البسفينول- أ من اكثر المواد شيوعا في الاستخدام الصناعي في العالم وذلك بالرغم من تأثيرها التراكمي السام علي صحة الانسان. ولهذا من المفيد استخدام المواد الطبيعية مثل شمع العسل بما لها من خصائص مضادة للالتهاب ومضادة للأكسدة. يهدف هذا البحث لدراسة تأثير مادة البسفينول- أ علي رئة الجرذان البيضاء البالغة والدور الوقائي المحتمل لشمع العسل لمحاربة هذه التأثيرات.

المواد و الطرق المستخدمة: استخدم في هذا البحث ٤٥ من ذكور الجرذان البيضاء البالغة والتي تم تقسيمها الي ٤ مجموعات وهي كالاتي: المجموعة الضابطة والمجموعة المعالجة ب البسفينول- أ والتي اخذت البسفينول- أ)٥٠٠ مجم / كجم) لمدة ثمانية اسابيع والمجموعة الثالثة وهي مجموعة النقاهة التي عولجت بالبسفينول-أ لمدة ثمانية اسابيع ثم تركت بلا علاج لمدة اسبوعين اخرين والمجموعة الرابعة والتي عولجت بمادة البسفينول-أ (٥٠٠ مجم /كجم) وشمع العسل (٥٠ مجم/كجم) معا. وبعد انتهاء المدة المحددة للتجربة تم استخلاص انسجة الرئة لعمل التحاليل الكيمائية، وتجهيز العينات للفحص تحت المجهر الضوئي والإلكتروني ولعمل الدراسات المورفومترية.

النتائج : مادة البسفينول- أ تسببت في ضرر بالغ لأنسجة الرئة علي مستوي الخلية متمثلا في تقلص الحويصلات الهوائية واختلال في النسيج المبطن للشعب الهوائية مع انتشار لخلايا الالتهاب المختلفة. كذلك اظهر الفحص بالميكروسكوب الالكتروني تضرر العضيات مثل الميتوكندريا والاجسام الصفائحية الموجودة في الخلايا الرئوية من النوع الثاني. وظهرت علامات الإجهاد التأكسدي في صورة ارتفاع لمستوي MDA للمجموعتين الثانية والثالثة بينما انخفضت مستويات (SOD) و(GSH). زاد سمك جدار الحويصلات الهوائية وكذلك نسبة مساحة ألياف الكولاجين وعدد الخلايا الموجبة ل CD68 بشكل ملحوظ مع المجموعتين الثانية والثالثة مقارنة مع المجموعة الضابطة. ولكن انخفضت النسبة المئوية للاستجابة المناعية الإيجابية لـ BCL2 في المجموعة الثانية والثالثة. وقد أدى استخدام شمع العسل (المجموعة الرابعة) إلى تحسين التغييرات الرئوية السابقة.

الاستنتاج: مادة البسفينول ـ أ لها تأثيرات ضارة علي انسجة الرئة والتي يمكن تحسينها عن طريق استخدام شمع العسل