The Possible Protective Role of Echinacea and Ginger and both of them on the Lung of Diabetic Male Rats: Histological and Immunohistochemical Study

Original Article

Hekmat Osman Abdel-Aziz[1], Ghada Mohammed Ahmed[1], Mohamed Arafa Adly[2] and Hoda M. Elsayed[1]

[1]Department of Histology, Faculty of Medicine, [2]Department of Zoology, Faculty of Science, Sohag University, Egypt

ABSTRACT

Introduction: Diabetes is a systemic disease characterized by a state of hyperglycemia. Several changes in the respiratory functions had been detected in diabetic patients.

Aim of the Work: The current study estimated the efficiency of two herbal extract (ginger and echinacea) on the changes in the lung of diabetic animal model.

Materials and Methods: 50 adult male albino rats were used. They were divided into five groups. Control group (group I) received regular diet. Group II: diabetic group (diabetes induced by a single intraperitoneal injection of streptozotocin 60 mg/kg). Group III: diabetic rats treated with 100mg/kg echinacea orally for 30 days. Group IV: diabetic rats treated with 400mg/kg ginger orally for 30 days. Group V: diabetic rats treated with both 100mg/kg echinacea and 400mg/kg ginger orally for 30 days. Paraffin blocks were prepared for histological and immunohistochemical examinations. Morphometric study and statistical analysis were done for the thickness of the interalveolar septum, the area percentage of collagen fibers and number of positive caspase -3 cells and CD68 positive macrophages. Ultrathin sections were prepared for electron microscopic examination.

Results: Diabetes caused a significant increase in the thickness of the interalveolar septa, the area percentage of collagen fibers, the number of caspase-3 positive cells as well as the number of CD68 positive alveolar macrophages compared to the control group. Ultrastructurally, pneumocyte type II appeared with denser nucleus, numerous vacuoles and disorganized lamellar bodies.

Conclusion: Groups treated with echinacea or ginger or both showed a significant decrease in the thickness of the interalveolar septa, the area percentage of collagen fibers, the number of caspase-3 positive cells as well as the number of CD68 positive alveolar macrophages compared to the diabetic group. These groups also showed that pneumocyte type II restored their normal appearance.

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Key Words: Caspase-3, diabetes, echinacea, ginger, lung.

Corresponding Author: Ghada Mohammed Ahmed, PhD, Department of Histology, Faculty of Medicine, Sohag University, Egypt, **Tel.**: +20 11 4418 5629, **E-mail:** ghadam090@yahoo.com

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. It is caused by a disturbance in pancreatic beta cells[1].

Some diabetic patients showed changes in respiratory functions such as lung volume, pulmonary diffusing capacity, control of ventilation and bronchomotor tone[2].

Streptozotocin is used for induction of diabetes in experimental animals as it causes a selective necrosis of the pancreatic beta cells[3].

Echinacea is one of medical herbs. It had been used to treat several diseases as flu and infectious diseases. It contains several components that play a role in its therapeutic ef¬fects which include alkylamides, caffeic acid derivatives, glycoproteins, polysaccharides, polyacetylenes, phenolic compounds, essential oils and flavonoids[4]. Studies reported that echinacea compounds improved insulin sensitivity[5].

Ginger is one of the most used spices worldwide. It was used to treat many disorders as vomiting, pain, indigestion, and cold-induced syndromes. It was reported that ginger has anti-cancer, anti-inflammatory, and analgesic activities. Studies reported that ginger has beneficial effects in management of metabolic disorders and their complications[6].

MATERIALS AND METHODS

Animals

50 adult male albino rats were used in the experiment. They were purchased from animal house in Faculty of Science, Sohag university. Their weights ranged from 200-250 grams. They were kept with free access to water and chow.

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Chemicals

1. Streptozotocin from Sigma-Aldarich chemicals Co. St. Louis. Mo. USA. (was dissolved in cold saline solution).
2. Ginger powder from Sigma-Aldarich chemicals Co. St. Louis. Mo. USA.
3. Echinacea purpurea extract (in the form of Immulant syrup, 16.7 mg/ml) (manufactured by Mepaco Medifood company)[7].
4. Rabbit polyclonal anti CD68 antibody, E13920, Thermo scientific company, Neomarks, Fremont, USA. It was obtained from Sigma-Aldarich chemicals company.
5. Rabbit polyclonal cleaved caspase-3 antibody, RB-1197-P0(Ab4), Thermo scientific company, Neomarks, Fremont, USA. It was obtained from Sigma-Aldarich chemicals company.

Experimental design

50 rats were divided into five experimental groups (10 rats for each):

- **Group (I):** Control group (the normoglycaemic group).
- **Group (II):** Animals were induced to develop diabetes mellitus by a single dose of streptozotocin via intraperitoneal injection (60 mg/kg). After 72 h, blood glucose levels were measured in tail vein blood samples by glucometer after overnight fast 12-14h. A plasm glucose level greater than 200 mg/dl was confirmed by the occurrence of diabetes[3].
- **Group (III):** Diabetic rats were given echinacea daily 100mg/kg for 30 days orally via gastric gavage by[8].
- **Group (IV):** Diabetic rats were given aqueous extract of ginger daily 400mg/kg for 30 days orally via gastric gavage[9].
- **Group (V):** Diabetic rats were given aqueous extract of ginger daily 400mg/kg and echinacea daily 100mg/kg for 30 days orally via gastric gavage.

At the end of the experiment, the animals were anesthetized by inhalation of diethyl ether. They were sacrificed and their lungs were dissected out and processed for light and electron microscopy study.

Light microscopy

Specimens of the lung were obtained, rinsed in physiological saline and fixed in 10% formalin saline for 24 hours. The preserved organs were trimmed for processing, dehydration with alcohol, clearing with xylene, infiltration and embedding in paraffin wax. Paraffin blocks were sectioned at 5 um thickness using microtome (Leica RM 2125) and stained with:

1. Hematoxylin & Eosin: for general histological study.
2. Masson Trichrome staining: for demonstration of collagen fibers in the lung sections. All tissue processing and histological stains were done[10].
3. Immunohistochemical study: For detection of macrophages using CD68 polyclonal antibody [11] and apoptotic cells using caspase-3 polyclonal antibody[12].

Protocol for immunohistochemical reaction

1. Deparaffinization, rehydration and antigen retrieval was performed by boiling in 10 mmol/l citrate buffer (pH 6.2) in microwave oven for two cycles, 3 min each.
2. Blocking of the endogenous peroxidase by 2% hydrogen peroxide for 5 min.
3. Incubation overnight at 4 degrees refrigerator with the primary CD68 or caspase-3 antibody after dilution to 1:150.
4. On the next morning, the sections were subjected to biotinylated secondary antibody in a humid chamber.
5. Enzyme conjugate streptavidin was applied.
6. The slides were stained by substrate-chromogen mixture and then counterstained using Hematoxylin reagent.
7. The slides were rinsed in distilled water and dehydrated in ascending grades of alcohol .
8. Clearing and mounting with cover slip were done .
9. Negative control was done with omission of primary antibody[13]. Positive reaction for CD68 appeared as brown cytoplasmic deposits. Positive reaction for caspase-3 appeared as brown cytoplasmic deposits and may also nuclear deposits.

Electron microscopy

Specimens were fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for 2 hours at 4ºC, post fixed in 1% osmium tetroxide for one hour at 4ºC. The specimens were processed and embedded in resin. Semithin sections were done at 1um thickness and stained by toluidine blue[14]. Ultra-thin sections were performed using ultramicrotome and stained with uranyl acetate and lead citrate and then examined and photographed using a JEOL JEM 1010 electron microscope (JEOL Ltd, Tokyo, Japan) at the Electron Microscope Research Laboratory of the Histology and Cell Biology Department, Faculty of Medicine, Assuit University.

Morphometric Studies

The light microscopy Leica ICC50 Wetzlar (Germany) at Department of Histology, Faculty of Medicine, Sohag

University was used. Eight slides from different rats of each group were assessed. Ten fields in each section were taken, and analysis of each field using Image J software (version 1.46r)[15] was done as follow:

1. The area percentage for collagen fibers in lung sections were calculated in each examined field (power x200)[16].
2. The number of CD68 positive macrophages in lung sections was counted in each field (power x400)[17].
3. 3-The number of caspase-3 positive cells in lung sections was counted in each field (power x400)[18].
4. The thickness of interalveolar septa in semithin sections was calculated in each examined field (power x1000)[19].

Statistical Analysis

Statistical package SPSS for Windows, version 16.0 was used. Data were presented as means± standard deviation (SD) and the values were defined of statistical significance as *p value* ≤ 0.05. T test for independent variables was used to compare the results between groups of the recordings reported by two observers[20].

RESULTS

Hematoxylin and eosin stain (H&E)

Examination of lung sections of the control rats revealed normal structure of lung parenchyma. It is formed of intrapulmonary bronchus, bronchioles, alveolar ducts, alveolar sacs separated by interalveolar septa and blood vessels (Figure 1). Diabetic rats' sections examination revealed thickening of the interalveolar septa due to inflammatory cell infiltration with narrower alveolar lumen compared to the control rats (Figure 2). Peribronchiolar cell infiltration was also noticed (Figure 3). Some bronchioles showed desquamation in some parts of their epithelium. Blood vessels had thickened wall compared to the control rats (Figure 4).

Echinacea treated group showed an apparent decrease in the thickness of the interalveolar septa, less cellular infiltration with wider alveolar lumen compared to diabetic rats. Bronchiole showed no desquamated epithelial cells (Figure 5).

Ginger treated group revealed an apparent decrease in the thickness of interalveolar septa, less cellular infiltration and wider alveolar lumen. Bronchiole showed few desquamated epithelial cells compared to diabetic rats (Figure 6).

Group treated with both echinacea and ginger revealed thin interalveolar septa, wider alveolar lumen with bronchiole were relatively similar to control rats (Figure 7).

Masson trichrome stain

Control group (GI) showed scanty delicate collagen fibers around bronchioles and blood vessels (Figure 8A).

Diabetic group (GII) showed an apparent increase in the amount of collagen fibers around bronchioles, blood vessels and in the interalveolar septa compared to the control rats (Figure 8B).

Diabetic group treated with echinacea extract (GIII) showed apparently fewer collagen fibers around bronchioles, blood vessels and in the interalveolar septa compared to diabetic rats (Figure 8C).

Diabetic group treated with ginger extract (G IV) showed also less collagen fibers compared to diabetic rats (Figure 8D).

Diabetic group treated with both echinacea and ginger extracts (G V) showed scanty collagen fibers around bronchioles, blood vessels and in the interalveolar septa compared to diabetic rats (Figure 8E).

Immunohistochemical Findings

A- CD68 immunoreactivity

Control rats (G I) (Figure 9A) showed CD68 positive alveolar macrophages in the alveolar wall and in the interalveolar septa. Diabetic rats (G II) showed an apparent increase in alveolar macrophages in the interalveolar septa compared to the control group (Figure 9B). Diabetic rats treated with echinacea extract (GIII) showed an apparent decrease in alveolar macrophages in the interalveolar septa compared to diabetic rats (Figure 9C). Diabetic rats treated with ginger extract (G IV) showed also decrease in alveolar macrophages compared to diabetic rats (Figure 9D). Diabetic rats treated with both echinacea and ginger extracts (GV) showed apparently fewer macrophages in the interalveolar septa compared to diabetic, echinacea or ginger treated rats (Figure 9E).

B- Caspase-3 immunoreactivity

Control rats (G I) (Figure 10A) showed few caspase-3 positive cells in the alveolar wall. Diabetic rats (G II) (Figure 10B) revealed an apparent increase in positive cells in the alveolar wall compared to the control group. Diabetic rats treated with echinacea extract (GIII) (Figure 10C) revealed an apparent decrease in positive cells in the alveolar wall compared to diabetic rats. Diabetic rats treated with ginger extract (G IV) (Figure 10D) revealed also apparent decrease of positive cells compared to diabetic rats. Diabetic rats treated with both echinacea and ginger extracts (G V) (Figure 10E) revealed an apparent decrease in positive cells in the alveolar wall compared to diabetic, echinacea or ginger treated rats.

Toluidine blue stain

Examination of the Control rats (GI) showed that alveoli are lined with pneumocyte type I and pneumocyte type II with thin interalveolar septa. Pneumocyte type I has flat

nuclei and small amount of cytoplasm while pneumocyte type II has vesicular nuclei and vacuolated cytoplasm. Alveolar macrophages and blood capillaries were also seen (Figure 11A).

Diabetic rats (G II) showed increased thickness of the interalveolar septa compared to the control group. Some of pneumocytes type II had deeply stained nuclei and others had pyknotic nuclei. The cytoplasm appeared more vacuolated compared to the control rats. Many inflammatory cells as macrophages and lymphocytes in interalveolar septa with congested blood capillaries were also seen (Figure 11B).

Diabetic rats treated with echinacea extract (GIII) showed an apparent decrease in the thickening of interalveolar septa compared to diabetic rats. Pneumocyte type II appeared less vacuolated compared to diabetic rats (Figure 11C).

Diabetic rats treated with ginger extract (GIV) showed also decrease in the thickness of interalveolar septa. Pneumocyte type II showed less vacuoles compared to diabetic rats (Figure 11D).

Diabetic rats treated with both echinacea and ginger extracts showed significant improvement compared to diabetic, echinacea or ginger treated rats (Figure 11E).

Ultrastructural Findings

Control group revealed that pneumocyte type II appears as dome shaped with apical microvilli and euochromatic nucleus. The cytoplasm has many lamellar bodies (Figure 12). Diabetic rats showed many changes in pneumocyte type II such as heterochromatic and irregular nucleus, many cytoplasmic vacuoles and disorganized lamellar bodies (Figure 13). Diabetic rats treated with echinacea showed that pneumocyte type II appeared less vacuolated with more regular lamellar bodies compared to diabetic rats (Figure 14). Diabetic rats treated with ginger showed that pneumocyte type II appeared dome shaped with apical microvilli, the cytoplasm had apparently fewer vacuoles with more regular lamellar bodies compared to diabetic rats (Figure 15). Diabetic rats treated with echinacea and ginger showed that pneumocyte type II appeared dome shaped with apical microvilli. The cytoplasm had many lamellar bodies with absence of or few vacuoles (Figure 16).

Morphometric results

The mean area percentage of collagen fibers in lung It was found that the mean area percentage of collagen fibers in group (II) is significantly increased compared to the control group. In groups (III), (IV) and (V), it decreased significantly compared to that in group (II) (Table 1, Figure 8F).

The mean number of CD68 positive macrophages

The mean number of alveolar macrophages in group (II) significantly increased compared to the control group. In groups (III), (IV) and (V), it significantly decreased compared to that in group (II) (Table 2, Figure 9F).

The mean number of caspase-3 positive cells in lung

The mean number of positive alveolar cells in group (II) is significantly higher compared to the control group. In groups (III), (IV) and (V), it decreased significantly compared to that in group (II) (Table 3, Figure 10F).

The mean thickness of the interaveolar septa

The mean thickness of interaveolar septa in group (II) is significantly increased compared to the control group. In groups (III), (IV) and (V), it decreased significantly compared to that in group (II) (Table 4, Figure 11F).

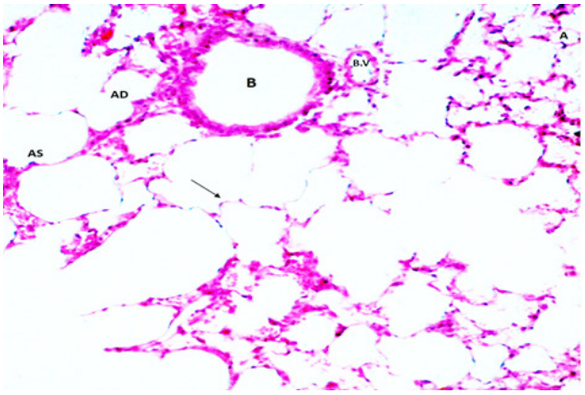

Fig. 1: A photomicrograph of a section in the lung of the control rat showing; alveolar sac (AS), alveolar duct (AD), alveolus (A), bronchiole (B) and blood vessels (B.V). Note, thin interalveolar septa (arrow). (H&E x200)

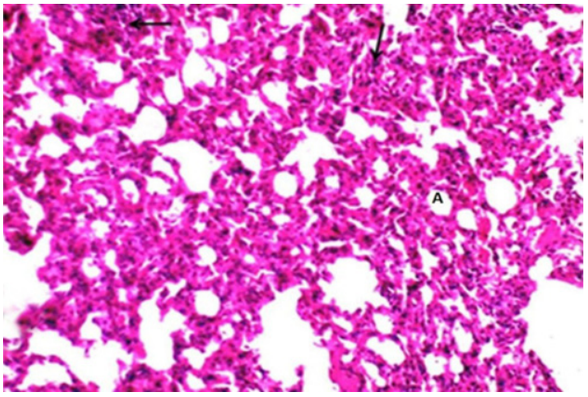

Fig. 2: A photomicrograph of a section in the lung of diabetic rat showing; thick interalveolar septa with inflammatory cell infiltration (thin arrow) and narrow alveolar lumen (A). (H&E x200)

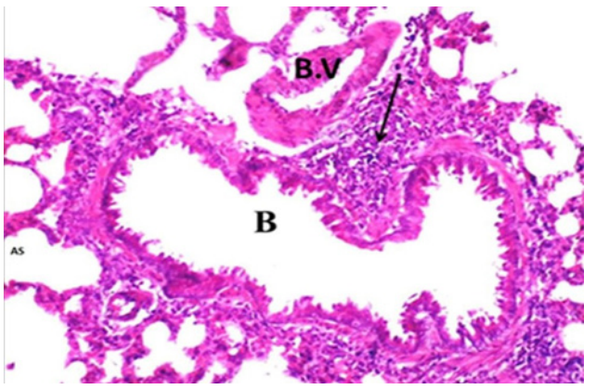

Fig. 3: A photomicrograph of a section in the lung of diabetic rat showing; peribronchiolar inflammatory cell infiltration (arrow) and thick walled blood vessel (B.V). Bronchiole (B), alveolar sac (AS). (H&E x400)

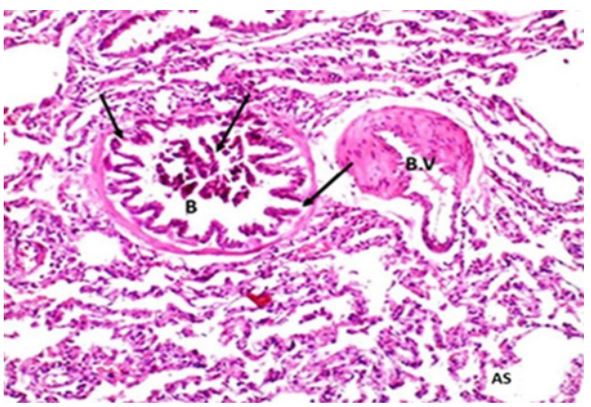

Fig. 4: A photomicrograph of a section in the lung of diabetic rat showing bronchiole (B) with epithelium desquamation (arrow) with cell debris in the lumen. Note thick walled blood vessel (B.V), alveolar sac (AS). (H& Ex200)

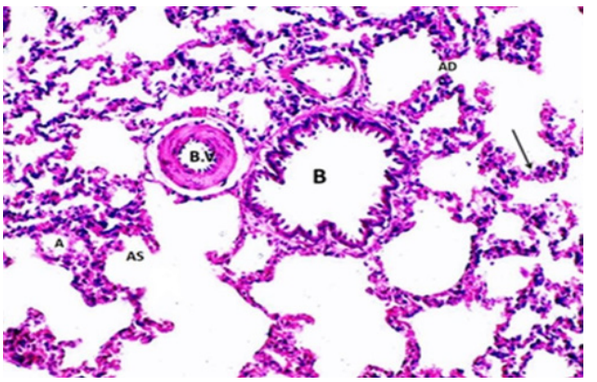

Fig. 5: A photomicrograph of a section in the lung of diabetic rat treated with echinacea showing thinner interalveolar septa (arrow) with patent alveolar lumen (A). Note, alveolar sac (AS) and alveolar duct (AD), bronchiole (B) and blood vessel (B.V). (H& Ex200)

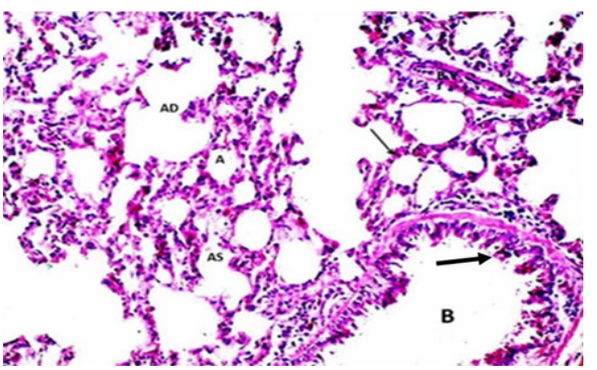

Fig. 6: A photomicrograph of a section in the lung of diabetic rat treated with ginger showing; relatively thin interalveolar septa (thin arrow) and patent alveolar lumen (A). Note few desquamated epithelial cells are still present (thick arrow), alveolar sacs (As), alveolar duct (AD), bronchiole (B) and blood vessel (B.V). (H&Ex200)

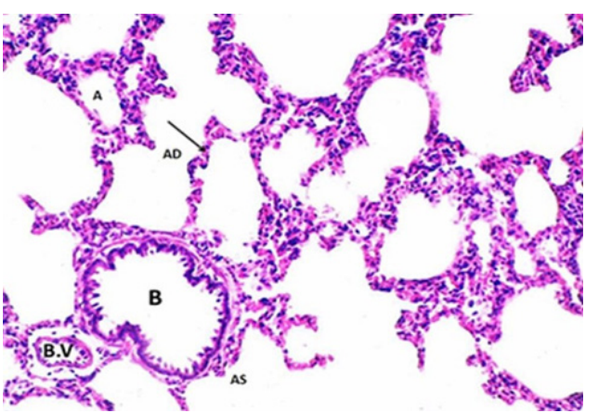

Fig. 7: Photomicrograph of a section in a lung of diabetic rat treated with both echinacea and ginger showing; relatively thin interalveolar septa (arrow), patent alveolar lumen (A) and bronchiole (B) with no desquamated epithelial cells. Note, alveolar sac (A), alveolar duct (AD), thin walled blood vessel (B.V) and bronchiole (B). (H &E x200)

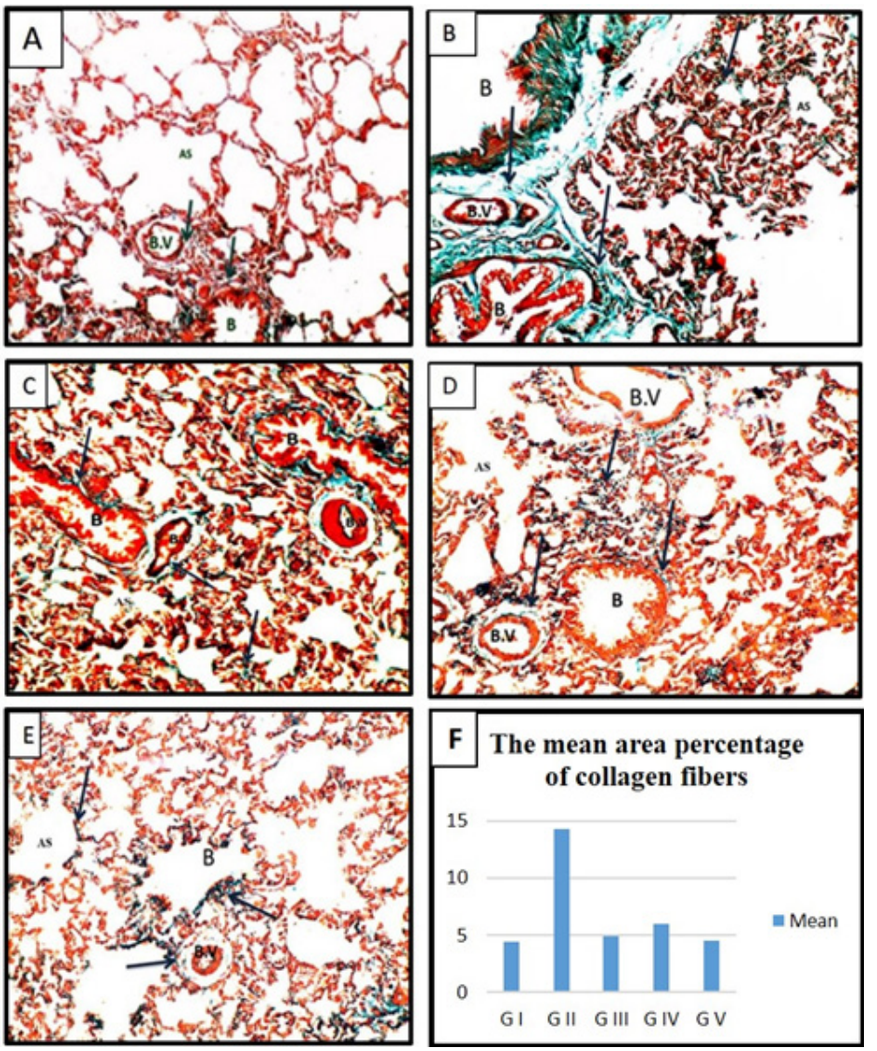

Fig. 8: Photomicrographs of a section in the lungs of; (A): control group, (B): diabetic group, (C): diabetic animals treated with echinacea, (D): diabetic animals treated with ginger, (E) : diabetic animals treated with echinacea and ginger. Note collagen fibers (arrow), alveolar sac (AS), bronchiole (B) and blood vessels (B.V). (F): Histogram showing the mean area percentage of collagen fibers in different groups. (Masson trichrome stain x200)

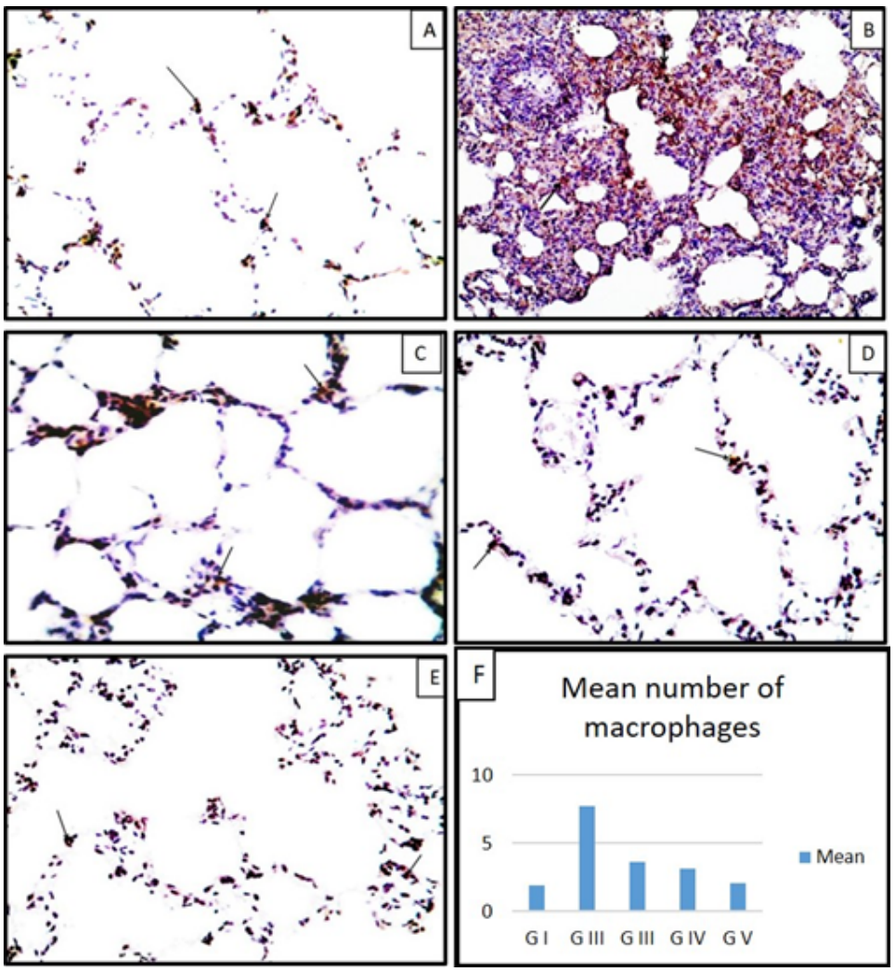

Fig. 9: Photomicrographs of a section in the lungs of; (A): control group, (B): diabetic group, (C): diabetic animals treated with echinacea, (D): diabetic animals treated with ginger, (E): diabetic animals treated with echinacea and ginger. Note alveolar macrophage (arrow). (F): Histogram showing the mean number of CD68 positive alveolar macrophages. (CD68 immunostain x400)

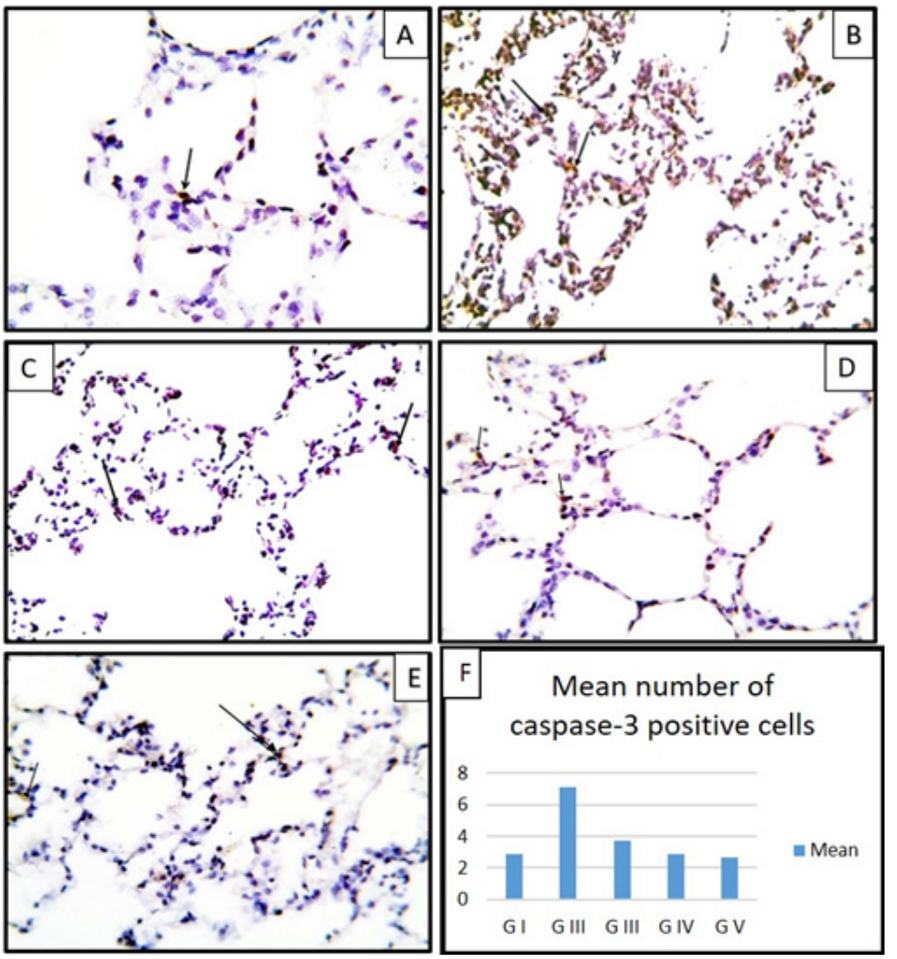

Fig. 10: Photomicrographs of a section in the lungs of; (A): control group, (B): diabetic group, (C): diabetic animals treated with echinacea, (D): diabetic animals treated with ginger, (E) : diabetic animals treated with echinacea and ginger. Note positive alveolar cell (arrow). (F): Histogram showing the mean number of caspase-3 positive alveolar cells. (Caspase-3 immunostain x400)

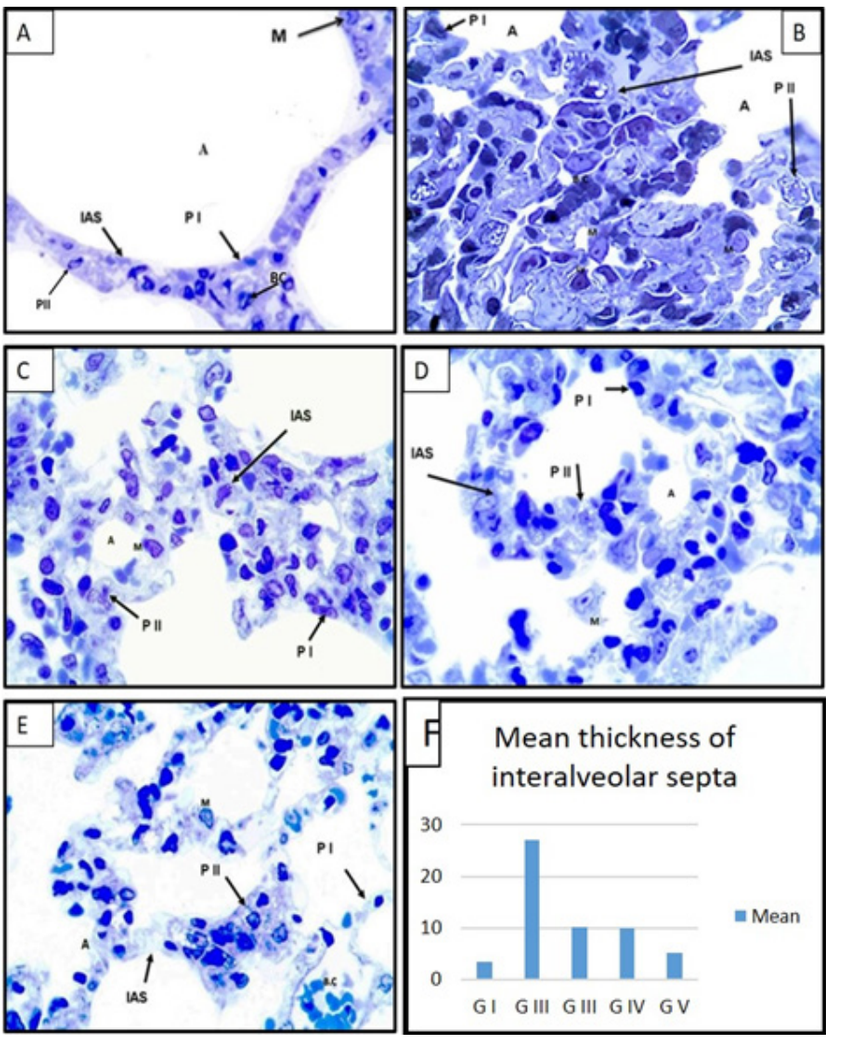

Fig. 11: Photomicrographs of a semithin section in lungs of (A) control group, (B) diabetic group, (C) diabetic animals treated with echinacea, (D) diabetic animals treated with ginger, (E) diabetic animals treated with echinacea and ginger. Note the alveoli (A), interalveolar septa (IAS), pneumocytes type I (PI), pneumocytes type II (PII), alveolar macrophages (M), lymphocyte (Ly) and blood capillaries (BC). (F): Histogram showing the mean of the thickness of interaveolar septa in different groups. (Toluidine blue x1000)

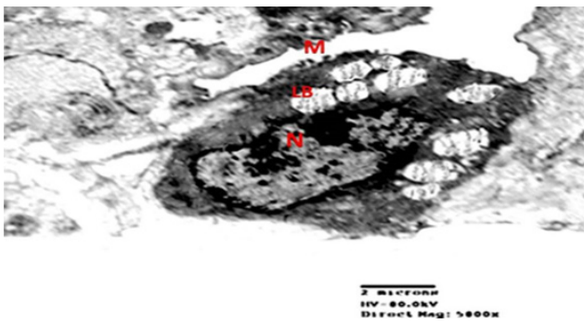

Fig. 12: An electron micrograph of ultrathin section of the lung of control rats showing pneumocyte type II dome-shaped cell with euchromatic nuclei(N), apical microvilli (M) and regular lamellar bodies (LB). (TEM x5800)

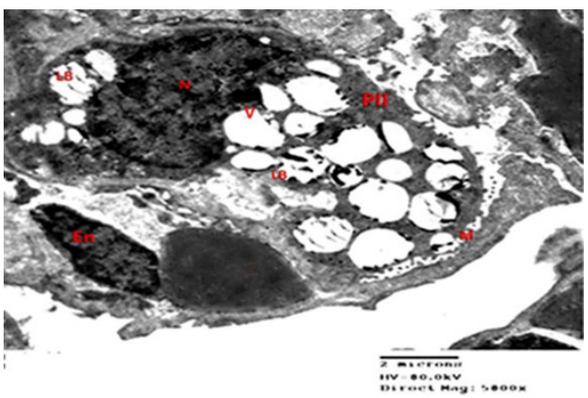

Fig. 13: An electron micrograph of ultrathin section in the lung of diabetic rats showing pneumocyte type II (PII) with apical microvilli (M), heterochromatic nucleus (N), disorganized lamellar bodies (LB) and cytoplasmic vacuoles (V). Note Endothelial cell (En) (TEM x5800)

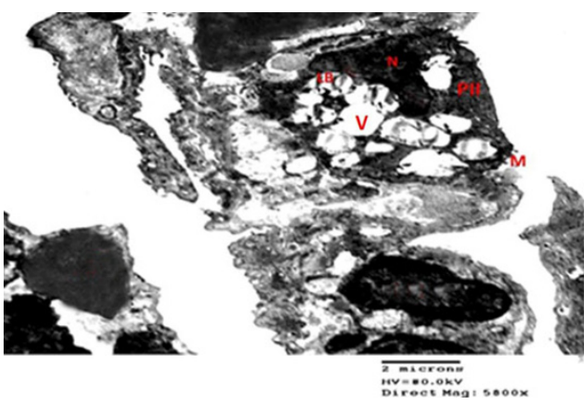

Fig. 14: An electron micrograph of ultrathin section in the lung of diabetic rats treated with echinacea showing pneumocyte type II (PII) with few apical microvilli (M), condensed nucleus (N), more regular lamellar bodies (LB) and less cytoplasmic vacuoles (V) compared to diabetic rats. (TEM x5800)

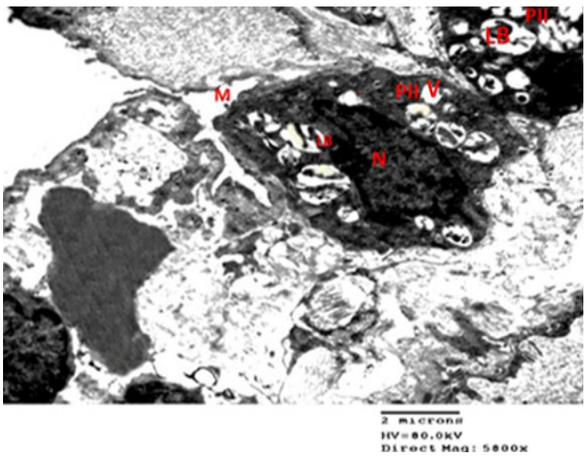

Fig. 15: An electron micrograph of ultrathin section in the lung of diabetic rats treated with ginger showing pneumocyte type II (PII) with apical microvilli (M) and condensed nucleus (N). The cytoplasm shows more regular lamellar bodies (LB) and less vacuoles (V) compared to diabetic rats. (TEM x5800)

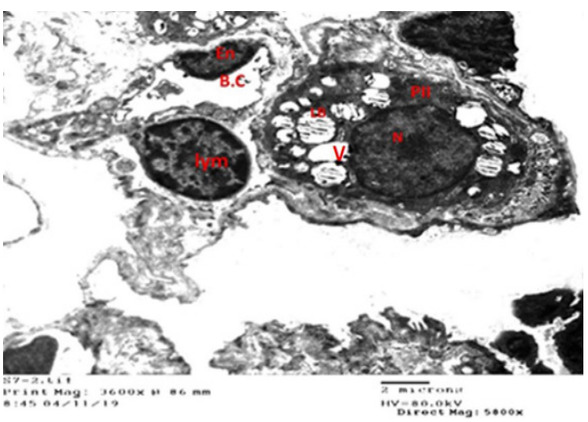

Fig. 16: An electron micrograph of ultrathin section in lung of diabetic rats treated with echinacea and ginger showing pneumocyte type II (PII) with condensed nucleus (N), regular lamellar bodies (LB) and few cytoplasmic vacuoles (V) compared to diabetic rats. Note blood capillary (B.C) with endothelial cell (En) and lymohocyte (lym). (TEM x5800)

Table 1: The mean area percentage of collagen fibers in lung

Group	Mean area percentage of collagen fibers (±SD)
Group (I)	4.43% ± 0.98
Group (II)	14.28% ± 1.51*
Group (III)	4.89% ±1.26#
Group (IV)	6.0% ± 0.01#
Group (V)	.5% ± 0.84#

*Significant as compared to G I (*P value*≤. 0.05), # Significant as compared to G II (*P value* ≤. 0.05)

Table 2: Mean number of alveolar macrophages

Group	Mean number of macrophages (±SD)
Group (I)	1.9 ± 0.99
Group (II)	7.7 ± 2.66*
Group (III)	3.6 ±1.42#
Group (IV)	3.1±1.44#
Group (V)	2.1±1.19#

*Significant as compared to G I (*P value*≤. 0.05), # Significant as compared to G II (*P value* ≤. 0.05)

Table 3: Mean number of caspase-3 positive cells in lung

Group	Mean number of caspase-3 positive cells (±SD)
Group (I)	2.9 ±1.19
Group (II)	7.1 ± 1.66*
Group (III)	3.7 ±1.63#
Group (IV)	2.9 ±1.66#
Group (V)	2.7± 1.33#

*Significant as compared to G I (*P value*≤. 0.05), # Significant as compared to G II (*P value* ≤. 0.05)

Table 4: Mean thickness of the interalveolar septa

Group	The mean thickness of interaveolar septa (±SD)
Group (I)	3.5 ±0. 06
Group (II)	27.1 ± 0.86*
Group (III)	10.1 ±0.36#
Group (IV)	9.9 ±0.5#
Group (V)	5.1 ± 0.14#

*Significant as compared to G I (*P value*≤. 0.05), # Significant as compared to G II (*P value* ≤. 0.05)

DISCUSSION

Diabetes mellitus is a systemic disease caused by disturbance in pancreatic β-cells function. The lung is a target organ for diabetic complications. Clinical abnormalities were reported in diabetic patients like decreased vital capacity and total lung capacity[21]. The current work was done to evaluate the possible protective effect of echinacea and ginger and both of them on the lung of diabetic animal model. Streptozotocin was used to induce diabetes via single intraperitoneal injection[22].

Lung of diabetic rats showed a significant increase in the thickeness of the interalveolar septa, narrowing of the alveolar lumen with peribronchiolar inflammatory cell infiltration and in the interalaveolar septa. Increase in the thickness of the wall of blood vessels and desquamation of bronchiolar epithelium were also observed. Similar findings were reported in the lung of diabetic rats by other study[23]. They found that diabetes induced lung fibrosis and inflammation with increased thickness of interalveolar septa.

The presence of infiltrating inflammatory cells could be explained by the presence of hyperglycemia that causes inflammation through cytokine secretion. Studies suggested that hyperglycemia caused oxidative stress that induces neutrophil accumulation in the lung. The accumulated leukocytes cause lung injury by the release of reactive oxygen metabolites, proteolytic enzymes and cytokines[24]. Thickness of the wall of blood vessels can be caused by changes in collagen and elastin as well as microangiopathy that is associated with diabetes[21]. Degeneration of bronchiolar epithelium caused by oxidative damage associated with hyperglycemia[24].

In the present study, diabetic group treated with echinacea exhibited partial improvement in pulmonary changes in the form of a significant decrease in the thickening of the interalveolar septa and blood vessels with widening of alveolar lumen. It was reported that echinacea improved pulmonary changes caused by infection with Pasteurella multocida serotype A. It decreased the thickening of the interalveolar septa and neutrophil infiltration. This could be attributed to its antioxidant effect[25].

The present study showed that ginger results in partial improvement of diabetic changes in the lung causing decreased the thickening of the interalveolar septa, widening of the alveolar lumen and decreased inflammatory cell infiltration and thickness of blood vessels wall. These findings are in agreement with other study[26]. They found that ginger improved changes in lung caused by diabetes such as inflammatory cell infiltration and degeneration of bronchiolar epithelium.

Studies proved the antioxidant effect of ginger on the liver of diabetic rats[22]. The ginger improved the changes on liver caused by diabetes as degenerative changes, lipid deposition in liver cells and portal area fibrosis.

In the current work, lung of diabetic animals exhibited a significant increase in the area percentage of collagen fibers in the interalveolar septa, around bronchioles and blood vessels. These results are in agreement with previous studies. They found an increase in the content of collagen fibers in both lung and spleen of diabetic rats[27]. Studies reported that hyperglycemia leads to non-enzymatic glycosylation of proteins in the lungs and chest wall that causes the collagen less liable to proteolysis. This leads to its accumulation in the lung. The collagen accumulation causes the increased stiffness of lung parenchyma and chest wall. This could explain the restrictive functional abnormality that presented in some diabetic patients[2].

In the current study, lung of diabetic rats treated with echinacea had a significant decrease in the area percentage of collagen fibers compared to diabetic rats. In agreement with this finding, researchers reported that echinacea decreased collagen accumulation in the spleen of diabetic rats[27]. Other study reported that echinacea reduced accumulation of collagen and elastin caused by testicular toxicity by magnetic nanoparticles[28]. Echinacea also increased expression of insulin-like factor

3 and testosterone levels. They reported that this effect of echinacea extract is due to its antioxidant properties.

In the present study, lung of diabetic rats treated with ginger showed a significant decrease in the area percentage of collagen fibers. Studies also found that ginger decreased collagen accumulation in lung of rats treated with ethanol[29]. Studies reported antifibrotic role of ginger in decreasing the synthesis of extracellular matrix[30].

In the present study, statistical analysis for CD68 immunoreactivity showed a significant increase in the mean number of alveolar macrophages of diabetic rats compared to the control group. A significant decrease in the mean number of alveolar macrophages in diabetic groups treated with echinacea, ginger or both compared to diabetic rats was also found.

The accumulation of alveolar macrophages could be attributed to the hyperglycemia that causes cytokine secretion which leads to inflammation. Studies had shown that cytokines were increased in the serum of diabetic patients[31]. Alveolar macrophages could release a chemotactic material special for neutrophils that produce proteases and toxic oxygen free radicals that enhance tissue injury[32].

Macrophage infiltration was observed in adipose tissue, pancreas and liver of diabetic animal models[33].These cells produce inflammatory cytokines such as TNF-α and IL-6. They act in paracrine and autocrine manner to induce the insulin resistance by interfering with insulin signaling in peripheral tissues through the induction of the N-terminal kinase and nuclear factor-kappa B pathways. These pathways that are induced in several tissues have an effect in tissue inflammation[34].

Researchers reported also a significant decrease in the level of TNF-α and IL-1β produced by macrophages in testis of diabetic rats treated with echinacea[35]. Studies proved anti-inflammatory effect of echinacea in patients of atopic eczema[36].

Other study reported the anti-inflammatory effect of ginger in prostate of diabetic rats[37]. In this study, diabetic rats treated with ginger revealed a decrease in inflammatory markers such as IL-6 and TNF-α. Studies reported that ginger consumption decreased inflammatory markers in diabetic patients[38]. Researchers reported that ginger decreased macrophages in rats with fructose-induced renal injury[39].

In the current work, statistical analysis for caspase-3 immunoreactivity revealed a significant increase in the mean number of positive cells in lung of diabetic rats compared to control group. It also revealed a significant decrease in the mean number of positive cells in diabetic groups treated with echinacea, ginger or both compared to diabetic rats.

Apoptosis resulted from production of cytochrome c from mitochondria, then cleavage and induction of the cytoplasmic enzymes; caspases[40]. Hyperglycemia induces generation of reactive oxygen species through the NADPH oxidase. These substances initiate apoptosis of cells[41].

Other study also reported that echinacea decreased apoptotic cells in the spleen of diabetic rats[27]. It could be attributed to the antioxidant effect of echinacea or its ameliorative effect in hyperglycemia. Echinacea was reported to ameliorate the level of apoptosis in testicular toxicity with magnetic nanoparticles[28].

Other study reported that ginger decreased apoptosis in tongue of diabetic rats[42]. Researchers reported a decrease in caspase-3 positive cells in kidney, liver and pancreas of diabetic rats treated with 6 shogaol (one of constituents of ginger)[43]. Decreased apoptotic cells could be attributed to the antioxidant effect of ginger. Antioxidant mechanism of ginger is the removal of free radical such as xanthine superoxide and xanthine oxidase[44].

In the present study, the ultrastructural examination of the lung of diabetic rats showed changes in pneumocytes type II. It revealed heterochromatic nucleus, disorganized lamellar bodies and cytoplasmic vacuoles. This could be due to oxidative stress that occurred with diabetes. These findings are in agreement with other study[21]. They found that diabetes induced degenerative changes in pneumocyte type II such as pyknotic nucleus, degenerated lamellar bodies and cytoplasmic vacuoles. The vacuoles may be degenerated lamellar bodies, lipid vacuoles or degenerated organelles[23].

In the current study, ultrastructural findings in diabetic rats treated with echinacea showed that pneumocyte type II had more regular lamellar bodies and less vacuoles. Echinacea has antioxidant and anti-inflammatory properties that prevent oxidative stress on lung tissue[25].

In the current work, ultrastructural findings in diabetic rats treated with ginger showed pneumocyte type II with more regular and euchromatic nucleus, more regular lamellar bodies and less cytoplasmic vacuoles. These could be due to to antioxidative and anti-inflammatory roles of ginger on lung[29].

In our study, diabetic rats treated with both echinacea and ginger extracts revealed more improvement in changes caused by diabetes on lung.

CONCLUSION

It is concluded that echinacea and ginger could have a protective role on pulmonary changes in diabetic animal model. It is also concluded that the use of both echinacea and ginger has more beneficial effect than use of echinacea or ginger alone. They can be used as an adjuvant treatment to minimize respiratory complications associated with diabetes.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدورالوقائى المحتمل للاشنيسيا والزنجبيل وكلاهما على رئة ذكور الجرذان المصابة بالسكرى: دراسة نسيجية وكيمياء النسيج المناعية

حكمت عصمان عبد العزيز[١]، غادة محمد احمد[١]، محمد عرفة عدلى[٢]، هدى محمد السيد[١]

[١]قسم الهستولوجى، كلية طب، جامعة سوهاج

[٢]قسم علم الحيوان،كلية علوم، جامعة سوهاج

مقدمة: مرض السكري هو مرض مزمن يتميز بحالة من ارتفاع السكر في الدم ويتسبب في ذلك خلل في إفراز الأنسولين، عمل الأنسولين أو حتى كليهما. وتعتبر الرئة عضو معرض لمضاعفات مرض السكرى وقد تم رصد العديد من الاضطرابات في وظائف الجهاز التنفسي عند المرضى الذين يعانون من مرض السكري. تعتبرالإشنيسيا نوع من الأعشاب المقتطفة والمكملات الغذائية و أظهرت الاشنيسيا خصائص مضادة للأكسدة، مضادة للالتهابات، مضادة للبكتيريا ومضادة للفيروسات. يعد الزنجبيل واحد من التوابل الأكثر استهلاكا في العالم. وهو يستخدم لعلاج العديد من الاضطرابات بما في ذلك القيء والألم وعسر الهضم والمتلازمات الناجمة عن البرد. والزنجبيل له تأثير على سكر الدم، و يعمل كمضاد للأكسدة وله دور تجديدى على أجهزة مختلفة مثل الكبد والطحال والكلى.

الهدف من البحث: والدراسة الحالية درست تأثير الإشنيسيا والزنجبيل والجمع بينهما على رئة ذكور الجرذان المصابة بمرض السكري التجريبى .

مواد وكرق البحث: وقد تم تقسيم ٥٠ من الجرذان الذكور البالغين الى ٥ مجموعات(١٠ جرذان لكل مجموعة): المجموعة الأولى هى المجموعة الضابطة والمجوعة الثانية هى مجموعة السكري. وقد تم الحث لتطوير مرض السكري عن طريق حقن واحد داخل الصفاق من الاستربتوزتوسين في جرعة من ٦٠ ملغ / كغ. بعد ٧٢ ساعة، تم قياس السكر في الدم للتأكد من الاصابة. المجموعة الثالثة هى جرذان مصابة بالسكرى وقد تلقت ١٠٠ ملغ / كغ من الأشنيسيا لمدة ٣٠ يوما عن طريق الفم. المجموعة الرابعة هى جرذان مصابة بمرض السكرى وقد تلقيت ٤٠٠ ملغ / كغ من الزنجبيل لمدة ٣٠ يوما عن طريق الفم. المجموعة الخامسة لجرذان مصابة بمرض السكرى وقد تلقت كلا من ١٠٠ ملغ / كغ من الأشنيسيا و ٤٠٠ ملغ / كغ من الزنجبيل لمدة ٣٠ يوما عن طريق الفم. بنهاية التجربة، تم تشريح الحيوانات بعد تخديرها واستخراج الرئتين وتثبيتها وتحضيرها للدراسة بالميكروسكوب الضوئى والاليكترونى. وتم عمل دراسة وتحليل احصائى لسمك الحاجز بين الحويصلات الهوائية والنسبة المئوية لألياف الكولاجين في الرئة وعدد الخلايا الموجبة لكاسبز-٣ والخلايا البلعمية فى رئة الجرذان.

النتائج: وكشفت نتائجنا أن: مرض السكرى أدى الى زيادة ذات دلالة احصائية فى سمك الحاجزبين الحويصلات الهوائية والنسبة المئوية للمساحة المشغولة بألياف الكولاجين فى الرئة. وكان هناك زيادة فى عدد خلايا الالتهاب حول الشعيبات الهوائية. وقد حدث تغييرات فى الخلايا الحويصلية من النوع الثانى مثل وجود فجوات بالسيتوبلازم والأجسام الصفحية الغير منظمة. المجموعة المعاجة بالأشنيسيا؛ فقد أظهرت الرئة انخفاضا ذا دلالة احصائية في سمك الحاجز بين الحويصلات الهوائية والنسبة المئوية للمساحة المشغولة بألياف الكولاجين. وقد اظهرت الخلايا الحويصلية

من النوع الثانى انخفاض فى الفجوات واكثر انتظاما للأجسام الصفحية بالمقارنة مع مجموعة السكرى. المجموعة المعالجة بالزنجبيل؛ فقد أظهرت الرئة انخفاضا ذا دلالة احصائية في سمك الحاجز بين الحويصلات الهوائية والنسبة المئوية للمساحة المشغولة بألياف الكولاجين. وقد اظهرت الخلايا الحويصلية من النوع الثانى انخفاض فى الفجوات وظهرت الأجسام الصفحية أكثر انتظاما بالمقارنة مع مجموعة السكرى. المجموعة المعالجة بالأشنيسيا والزنجبيل؛ فقد أظهرت الرئة تحسنا فى التغييرات التى سببها مرض السكرى. مرض السكرى أدى الى زيادة ذات دلالة احصائية فى عدد الخلايا البلعمية والخلايا الايجابية لكاسبز-٣ مقارنة مع المجموعة الضابطة. المجموعات المعالجة بالأشنيسيا أوالزنجبيل أو كليهما؛ أظهرت انخفاضا ذا دلالة احصائية فى عدد الخلايا البلعمية والخلايا الايجابية لكاسبز-٣ فى الرئة بالمقارنة مع مجموعة السكرى.

الخلاصة: خلص هذا العمل إلى أن مرض السكرى أدى الى تغيرات فى رئة الجرذان المصابة وأن الأشنيسيا و الزنجبيل كان لهما تأثير وقائي على النموذج التجريبي لداء السكري . وقد اثبتت الدراسة ان استخدام الاشيسيا والزنجبيل معا أكثر فاعلية من استخدامهما منفردين.