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Research Article

EFFECT OF STRESS DOSE OF EPINEPHRINE ON THE HISTOLOGY OF LUNG IN MALEMICE

Rand Abdulateef Abdullah[1], Mohammed Taib T. Jarjees[1] and Wahda Basheer Al-Youzbaki[2]

[1]Department of Anatomy, College of Medicine - University of Mosul, Iraq.
[2]Department of Pharmacology, College of Medicine - University of Mosul, Iraq.

ABSTRACT

Background: Modern life is full of hassles, deadlines, frustrations, and demands. For many people, stress is so common place that it has become a way of life. Long-term exposure to stress can lead to serious health problems. **Objective:** To evaluate the effect of daily exposure to stress on the lung, histology in male mice, and in relation with the duration of exposure to stress. Also to establish whether vitamin B-complex (B1, B6, B12) can reduce the bad effect of stress. **Methodology:** To achieve the aims of this study an interventionalnon randomized opened experimental study was conducted between the period of the 10th January 2013 and the 10th January 2014. Fifty healthy sexually matured male Albino mice aged between 6-8 weeks weighing 24-35gm were obtained from the animal house section of Mosul Medical College were included in this study. Epinephrine was used to prepare stress model. It was administered intraperitoneally (IP) daily to mice (200ng/kg body weight (BW)). The studied mice were divided into 5 groups, with 10 mice in each group as follows: Group G1 was the control group and received (normal saline (IP) for 12 weeks). Group G2 (epinephrine (IP) for 3 weeks). Group G3 (epinephrine (IP) for 12 weeks). Group G4 (epinephrine for 3 weeks plus B-complex 20mg/kg BW intramuscularly (IM) every 3 days for 3 weeks). Group G5 (B-complex (IM) as 20 mg/kg BW every 3 days for 3 weeks). The histological slides of lung were stained with Haematoxyline and Eosin.In addition to some histochemical stains that have been used (Periodic Acid Schiff's (PAS), Best's Carmine, Gomori's Alkaline phosphatase (ALP), and Orcein van Gieson (OVG). Slides were examined under light microscope. **Results**: The resultsof this study demonstrated that receiving a stress dose of epinephrine causing a significant higher mean lung weightsofthe studied mice of group G2 and G3of the studied mice in comparison with those of the other studied groups, but a non significant differences between those of the two groups. **The histological study of the lung sections** of group G2 revealed that the bronchioles were congested and infiltrated with chronic inflammatory cells with thickening of interalveolar septum. These changes were more pronounced in the lung sections of group G3 with collapse of many alveoli were noticed. The lung tissue of the studied mice of group G4 revealed a considerable degree of preservation of both lung tissue and alveoli except small focal areas of chronic inflammatory cells infiltrate were noticed. The histochemical OVG stain revealed that lung sections of the studied mice of group G2 showed more collagen formation around the bronchioles but this was more extensive in those of group G3 mice. **Conclusion:** This experimental study in mice concluded that stress can lead to lung damage, and the severity of damage depends on the duration of exposure to stress. Vitamins B- complex (B1, B6, and B12) canact as a protecting factor as they reduce the damaging effect of stress on this organ.

Keywords: stress, epinephrine, vitamin B, lung.

INTRODUCTION

Stress has been defined as the pattern of physiological reactions that prepares an organism for actionas exposure to hostile conditions (usually referred to as stressors) results in a series ofimportant adaptive responses that enable an organism to cope with a changing environment [1].

The sympathoadrenal and hypothalamic-pituitaryadrenocorticalsystems have complex interactions to maintain the internal environment during exposure of the organism to a wide variety of stressors[1,2,3,].Epinephrine is a hormone and neurotransmitter. When produced in the body it increases heart rate, contracts blood vessels and dilates air passages and participates in the "fight or flight" response of the sympathetic nervous system[2].

The release of catecholamine (CA) is a key initial event in responses to stressors and is followed by an increase in the expression of genes that encode catecholamine-synthesizing enzymes[4]. This process is mediated by transcriptional mechanisms in the adrenal medulla. The persistence of transcriptional activation depends on the duration and repetition of the stress. Stress involved in converting the transient increases in the rate of transcription into prolonged (potentially adaptive or maladaptive) changes in gene expression[5].

Stress has multiple effects on the physiology, neurochemistry, and behavior of humans[6] and animals[7].Not only is chronic stress unhealthy, also short-term stress episodes may damage some organs. Acute social stress insults, aggression between males to establish dominance, are a very common stressor in laboratory mice[8]. Few studies addressed the consequences of such a behavior on organ integrity[9,10].

Since1951 whenWitham and Fleming studied the effect of epinephrine injection on pulmonary vessels in human and found that it leads to increase pulmonary pressure and subsequent pulmonary hypertension[11]. Few other studies[12,13,14] found that psychological stress make the individual more prone to upper respiratory tract infection. Many animal studies found that epinephrine lead to pulmonary arteriolar vasoconstriction which leads subsequently to hypoxia in the dogs lung[15]or to cytoplasmic degenerative changes of the ciliated cells and damage of kinocilia on the apical surfaces of the ciliated cells of rabbit lung[16]. Or the accumulation of neutrophils in murine lungs which is responsible for developmentof neutrophil-mediated acute lung injury[17]or induce a 51% increase in oxidative stress in rat lungs as they are particularly susceptible to lesions by free radicals which induces lung tissue injury and disorders of their function[18].

Other three animal studies in mice the first revealed that the number of NK cells was decreased in the intraparenchymal region of the mice lungs after only two hours exposure to physical and psychological stress[19]. The second showed that social disruption in mice induced pulmonary inflammation and increase the percentage of neutrophils within the lungs 2-folds after social disruption with increasing of monocyte accumulation in the lungs. In addition to increase levels of other chemokines[20]while the third recognized that psychosocial stress evokes asthma exacerbation in ovalbumin-induced asthmatic mice[21].Also other two animal studies on guinea pigs revealed that repeated stress amplified bronchoconstriction and inflammatory response in distal airways and suggested that stress has an impact on asthmatic exacerbations[22], and the other study[23] found that repeated stress has increased distal airway constriction, and also induced an activation of cytokines, and oxidative stress pathways.

According to author knowledge, no study investigated the protective role of vitamin B-complex, on stress induced lung damage in mice, apart from the study of Das and Mukherjee(2012)[24] who proved that supplementation of trace amount of vitamin B-complex(thiamine (10 mg), riboflavine (10 mg), pyridoxine hydrochloride (3 mg), cyanocobalamin triturate (15 mcg)with lecithin for 24 weeks showed more protective effect in reversing the chronic ethanol-induced oxidative stress mediated effects. And a very recent study of Abu-Amara, *et al.*,(20013)[25]who concluded that sulpiride which is a neuroleptic drug, and has positive influence on the productive symptoms of psychosis and shows antidepressant activity[26]has no protective role against the marked damaging effects of noise stress on the lung and heart tissues of Adult Albino Rats. So the aims of this study were to prepare a stress model in male mice by intra peritoneal (IP) injections of epinephrine and to investigate its effects on the histology of lung, in relation to different duration of induced stress. Also to establish if there are a protective effects of vitamin B-complex against these effects.

METHODOLOGY

The approval of the study protocol by an ethic committee has been obtained from the local health committee of College of Medicine – University of Mosul – Iraq.

This is an Interventional Non Randomized Opened Experimental study conducted on mice in the animal room of the in the Department of Anatomy at the college of Medicine,University of Mosul, Iraq and part of the work at Electron Microscope Unit, Department of Anatomy at Al-Nahrain College of Medicine, Baghdad, Iraq, from the period between 10th January 2013 and the 10th January 2014.

Fifty sexually matured male Albino mice aged between 6-8 weeks weighing 24-35gm were obtained from the animal house section of Mosul Medical College. The animals kept in plastic mesh cages (12x20x10) cm, in small groups (10 mice/cage). The animals were housed at controlled environmental conditions (25±2ºC) and allowed free access to tap water and food ad Labium.The mice were kept for one month before starting the experiment for acclimatization under the same laboratory conditions(temperature, 12hour dark-light cycle, and humidity)and toexclude any possibility of abnormal behavior and disease[27], then they were divided into 5 groups, with 10 mice in each group as following:

Group G1: Received a daily injection of normal saline IP for 12 weeks and considered to be a control group.

Group G2: Received the stress dose of epinephrine by daily IP injection for 3 weeks.

Group G3: Received the stress dose of epinephrine by daily IP injection for 12 weeks.

Group G4: Received both the stress dose of epinephrine by daily IP injection of epinephrine for 3 weeks, and IM injection of vitamin B-complexevery 3 days for 3 weeks.

Group G5: Received IM injection ofvitamin B-complex every 3 days for 3 weeks.

Drugs used

1. Epinephrine

In present study epinephrine was used to prepare stress model. It was supplied byRenaudin Company(Itxassou, France) as epinephrine ampoule each contains 1 mg of epinephrine in 1 ml.

It was administered to mice 200ng/kg BW to induce stress in animal[8]. The drug was given by daily IP injection to the studied mice in group G2, G3 and G4 for 3 weeks (G2,G3) or for 12 weeks in those of group (G3).

Calculation of the stress dose for each mouse

The epinephrine ampoule contains 1 mg of epinephrine in 1 ml.

1 milligram=1000µg

1milliliter (ml) =1000 microliters (µl)

When we put it in 1000 ml of normal saline this means that in each one milliliter of normal saline there is 1 microgram (µg) of epinephrine.

1 µg=1000 nanogram(ng) 1µl= 1000 nanoliter(nl)

We took one milliliter from this solution and dissolve it in 9 milliliters of normal saline this means that each one milliliter contains 0.1µg which equals to 100ng.

The stress dose is 200 nanogm/kg BW[8].

so we give0.2 ng/KgBW of epinephrine IP daily.

Mantu syringes were used in this study. Dilution process was calculated as mentioned before now each ml of the solution made contains 100 ng, in mantusyring each unit contains 1ng.

2. Vitamin B-Complex

Neurorubine ampoule (vitamins B1, B6, B12) supplied by (Mepha LLC, Aesch-Basel, Switzerland), each amlpule contains 3 ml of solution which composed of 100 mg vitamin B1, 100 mg vitamin B6, and 1 mg vitamin B12. It was given to two groups (G4,G5) of mice IMM by the dose (20 mg/kg BW) vitamin B1 and B6, (0.2 mg/kg BW), and vitamin B12IM every three days[28].

Calculation of vitamin B-Complex dose for each mouse

Each ampoule of neurorubinecontains 3 ml, and each ampoule of 3 ml contains:B1(100 mg), B6 (100 mg), B12(1 mg).

Vitamin B-complex was given in a dose of 20 mg/kg BW[28]which equals to 0.02 mg/g.

3 ml = 300 unit (mantu syringe) = 100 mg of vitamins B1 and B6. We add 700 unit water for injection to 3 ml of ampoule. We get 1000 unit (mantu syringe) =100 mg. this means that each unit contains 0.1 mg of vitamins B1 and B6.We multiply the weight of mouse by 0.02 mg to get the total need of each mouse. Then the result will be divided by 0.1 mg to get the number of units in (mantu syringe) that must be given by IM.

After completion of the experimental treatment, the studied mice were killed by ether inhalation one week after the last injection. Dissection of the animal was done and the whole lungs were exposed for histological study.

The histological slides of lung were stained with Haematoxyline and Eosin.In addition to somehistochemical stains that have been used (Periodic Acid Schiff's (PAS), Best's Carmine, Gomori'sAlkaline phosphatase, and Orcein van Gieson (OVG)). Slides were examined under light microscope to study the histological changes of lung tissues in the different groups of the studied mice.

Statistical Analysis

The data obtained in the current study was analyzed using predictive analytic software (PASW) machine version 18.1 which was formally called SPSS.Standard statistical methods were used to determine the mean and standard deviation (SD).One-Way Analysis of Variance was used to estimate lung weights of different groups of the studied mice.P- value ≤ 0.05 was considered to be statistically significant.

RESULTS

Fifty healthy sexually matured male Albino mice aged between 6-8 weeks weighing 24-35gm were enrolled in this study and were divided into 5 groups, with 10 mice in each group, Epinephrine was used to prepare stress model. It was administered IP daily to mice (200ng/kg BW) and vitamin B-complex IM injections were given only to two groups (G4 and G5).

Table (1) illustrates the mean lung weights of the studied groups of mice, and it is obvious that ingroup G2 which received the stress dose of epinephrine for 3 weeks showed a significant high mean lung weights in comparison to that of the control group (G1) and with that which received the vitamin B-complex injections alone (G5) or with epinephrine (G4). Also group G3 mice which received the stress dose of epinephrine for 12 weeks showed a significant high mean lung weights in comparison with that of the control group (G1), G4 and G5, but non significant differences with those of group G2 mice which received the stress dose of epinephrine for shorter duration (3 weeks). Group G4 mice which received the stress dose of epinephrine together with vitamin B-complex injections for 3 weeks shows no significant differences in mean lung weights compared to those of control group (G1). While Group G5 mice which received only vitamin B-complex injections for 3 weeks appeared with no changes in mean lung weights as compared with that those of control group (G1) and group G4.

Table 1: Comparison of the mean lung weights among the studied groups of mice

The Studied Groups	Mean lung weights (gm) ±SD
G1	0.066 ± 0.005 (a)
G2	0.077 ± 0.011 (b)
G3	0.083 ± 0.007 (b)
G4	0.062 ± 0.008 (a)
G5	0.056 ± 0.015 (a)

Different letters vertically indicates significant differences

Histological and Histochemical findings of the lungs of the studied groups

Group G1 (control group)

The mice of this group received daily IP injections of normal saline for 12 weeks.

Histological Findings

Macroscopical Findings

The lung was pink color, spongy texture and smooth surface.The mean lung weight was (0.066± 0.005) **(Table 1**).

Microscopical Finding

The parenchyma of lung looked normal. Lung sections contained bronchi, which are secondary, tertiary bronchi and bronchioles, the smallest of which are called terminal bronchioles which is lined with low cuboidal epithelium. They give rise to respiratory bronchioles, which ultimately lead to the alveolar ducts, alveolar sacs and alveoli which are in close proximity to pulmonary capillaries **(Fig.1)**.

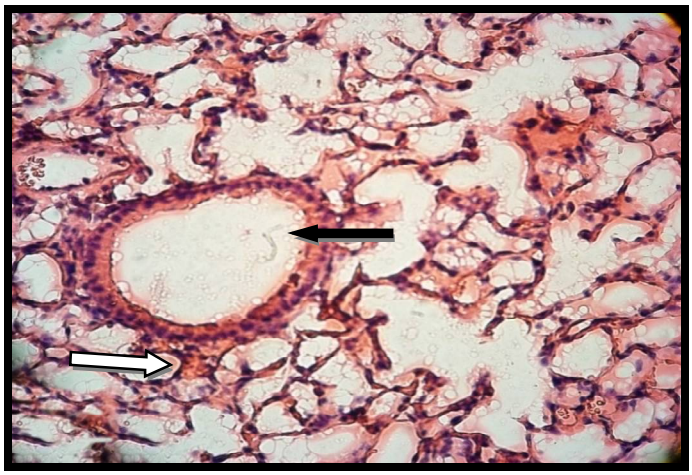

Fig. 1: Photomicrograph of lung sections of group G1of the studied miceshowing normal bronchioles (black arrow). Normal alveoli (white arrow) (H&E X 400)

Histochemical Finding

Staining of the lung sections of the studied mice of this group with Orcein Van Gieson showed mild positive reaction the elastic fibers in the connective tissue of lungs, and around blood vessels appeared brown in color. The collagen fibers appeared red in color (**Fig.2**).

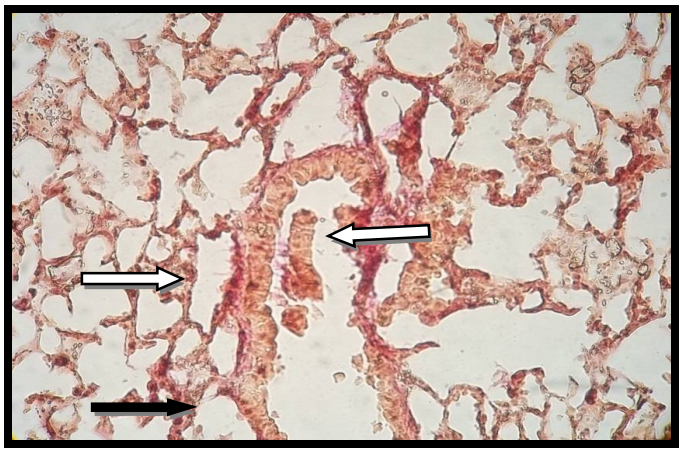

Fig. 2: Photomicragh of lung sections of control group (G1)of the studied mice showing mild positive reaction. The elastic fibers of the bronchioles wall appeared brown in color (black arrow). The collagen fibers looked red color (white arrows) (OVG X400)

Group G2

Histological Findings

Macroscopical Findings

The lung was slightly congested, spongy texture and smooth surface. The mean lung weights was (0.077±0.011)**(Table 1**).

Microscopical Findings

Most of the bronchioles appear normal but some bronchioles showed dilatation with peribronchial infiltration with chronic inflammatory cells **(Fig.3).** Some terminal bronchioles open directly to alveoli. The alveoli are normal with normal pneumocytes type 1 and 2. Thickening of interalveolar septum was observed **(Fig.4).**

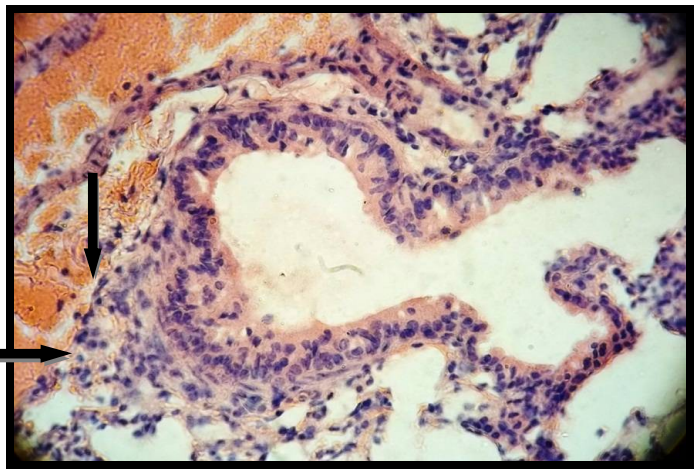

Fig. 3: Photomicrograph of lung sections of group G2 of the studied mice showing bronchiole (Br) were congested and infiltrated with chronic inflammatory cells (black arrows) (H&E X 400)

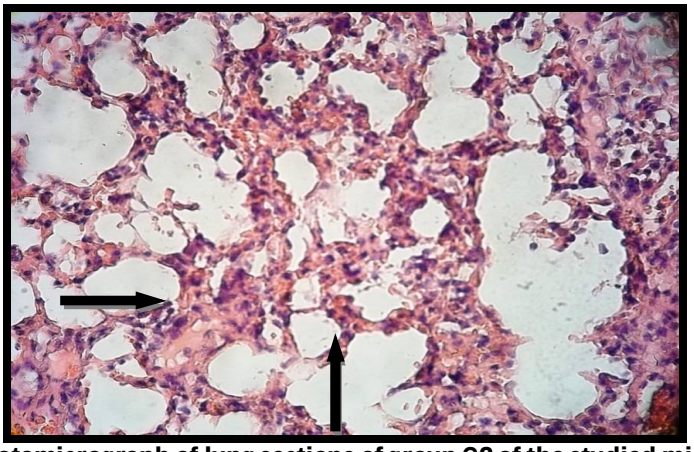

Fig. 4: photomicrograph of lung sections of group G2 of the studied mice showing more thickening of interalveolar septum and infiltration with chronic inflammatory cells(black arrows) (H&E X 400)

Histochemical Findings

Staining the lung tissues of mice of this group with Orcein Van Gieson showed moderate positive reaction. The elastic fibers in the connective tissue of lungs, and around blood vessels appeared brown in color. The collagen fibers appeared red in color and more abundant around the bronchioles **(Fig.5)**.

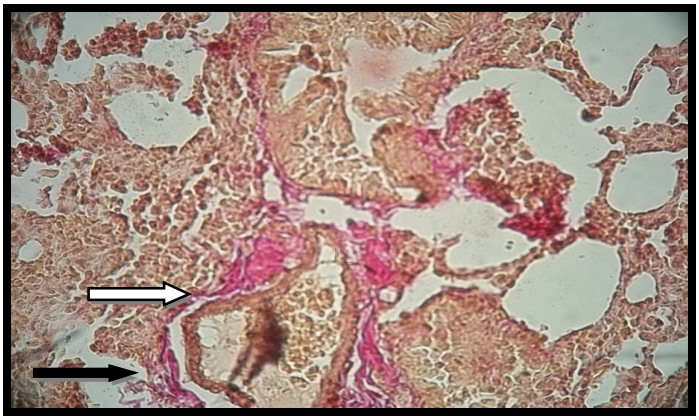

Fig. 5: Photomicragh of lung sections of group G2 of the studied mice showing moderate positive reaction. The elastic fibers of the bronchioles wall appeared brown in color (black arrow). The collagen fibers looked red color and more abundant (white arrow) (OVG X400)

Group G3

Histological Findings

Macroscopical Findings

The lung was deeply congested, spongy in texture with smooth surface.There was a significant high mean lung weight of this group (0.083±0.007)**(Table 1**).

Microscopical Findings

Examination of lung tissue of this group revealed severe peribronchial infiltration with chronic inflammatory cells (lymphocytes and plasma cells) and dilatation of the blood vessels **(Fig. 6).** Collapse of many alveoli and progressive thickening of interalveolar septum with heavy infiltration with lymphocyte were evident **(Fig. 7)**.

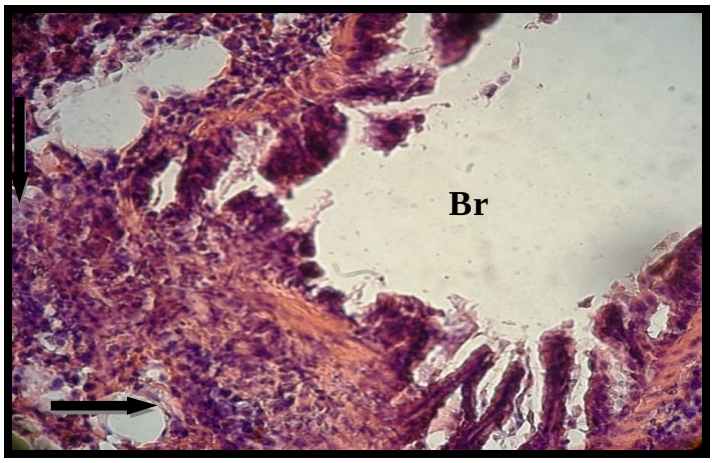

Fig. 6: Photomicrograph of lung sections of group G3 of the studied mice showing severe peribronchial infiltration with chronic inflammatory cells (lymphocytes and plasma cells) (black arrows)&Severe dilatation of bronchioles(Br) (H&E X 400)

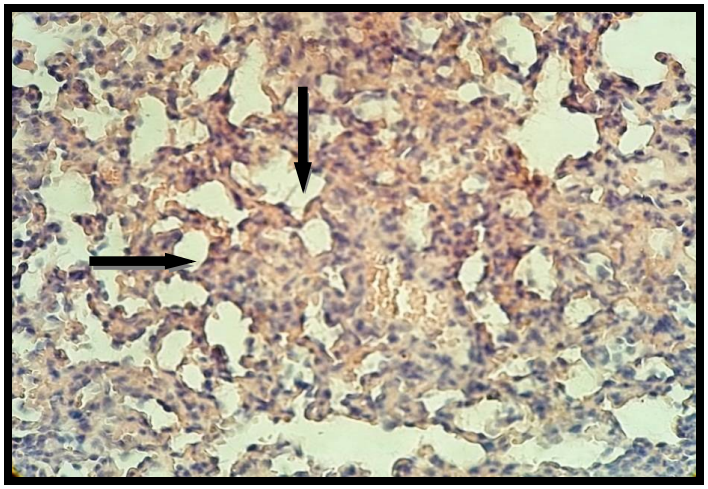

Fig. 7: Photomicrograph of lung sections of group G3 of the studied mice showing severe thickening of interalveolar septum and infiltration with chronic inflammatory cells(black arrows)&destruction of alveoli (A) (H&E X 400)

Histochemical Finding

Staining of lung tissues of this group of mice with Orcein Van Gieson showed strong positive reaction. The elastic fibers in the connective tissue of lungs, and around blood vessels appeared brown in color. The collagen fibers appeared red in color and more extensive around bronchioles and blood vessels than in other groups **(Fig. 8)**.

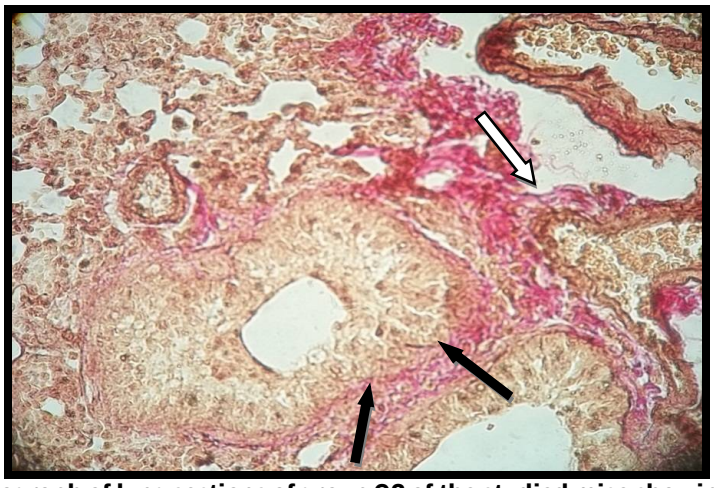

Fig. 8: Photomicrograph of lung sections of group G3 of the studied mice showing strong positive reaction. The collagen fibers looked red color and more abundant (black arrows) withthickening of the wall of blood vessel (white arrow) (OVG X400)

Group G4
Histological Findings
Macroscopical Findings
The lung was slightly congested, spongy texture and smooth surface. The mean lung weight was (0.062±0.008) **(Table 1**).

Microscopical Findings
The lung tissue of this group revealed a considerable degree of preservation of alveoli with normalinteralveolar septum **(Fig.9)**. Small focal areas of chronic inflammatory cells infiltrate were noticed **(Fig.10).**

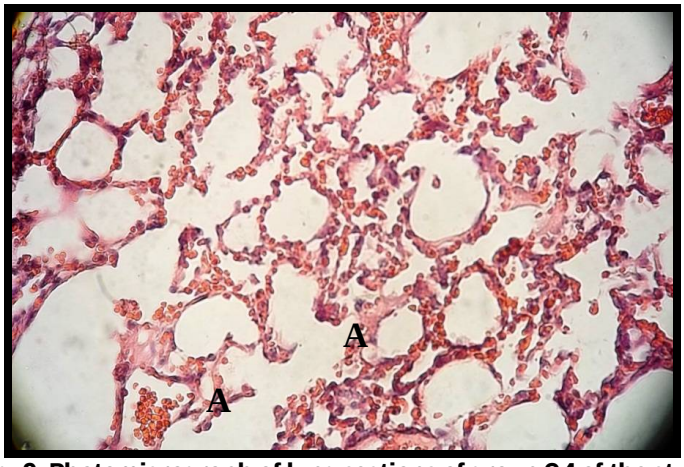

Fig. 9: Photomicrograph of lung sections of group G4 of the studied mice showing normal alveoli (A) (H&E X 400)

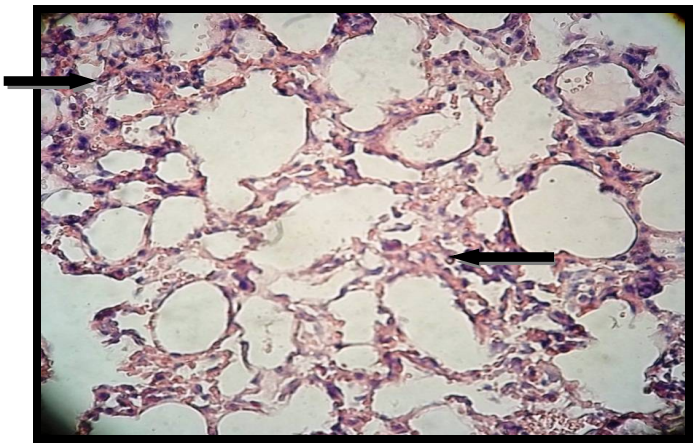

Fig. 10: Photomicrograph of lung sections of group G4 of the studied mice showing chronic inflammatory cellular infiltrate (black arrows) (H&E X 400)

Histochemical Findings

Staining the lung tissues of this group of the studied mice with Orcein Van Gieson gave mild positive reaction. The elastic fibers in the connective tissue of lungs, around the bronchioles and around blood vessels appeared brown in color. The collagen fibers appeared red in color around bronchioles and blood vessels **(Fig.11).**

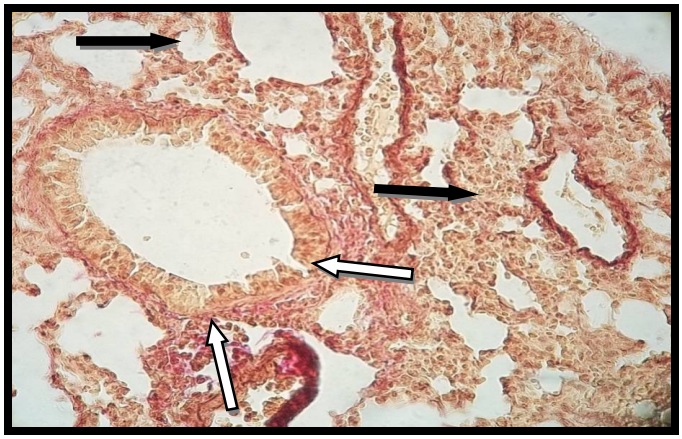

Fig. 11 : Photomicrograph of lung sections of group G4 of the studied mice showing mild positive reaction. The elastic fibers of the bronchioles wall appeared brown in color (black arrows). The collagen fibers looked red color (white arrows) (OVG X400)

Group G5

Histological Findings

Macroscopical Findings

The lung was pink color, spongy texture and smooth surface. The mean lung weight was (0.056 ±0.015) **(Table 1**).

Microscopical Finding

The histological structure of lung sections was looked normal. Consist of many bronchioles of various sizes and most of the lung is composed of thin-walled alveoli**(Fig.12).**

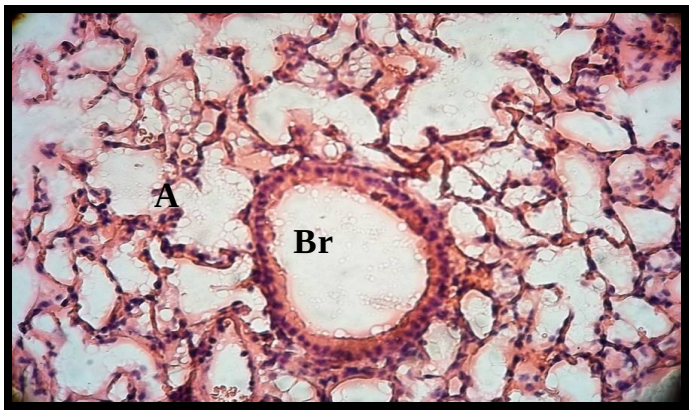

Fig. 12: Photomicrograph of lung sections of group G5 of the studied mice showing normal bronchiole (Br) & normal alveoli (A) (H&E X 400)

Histochemical Finding

Staining lung tissues of this group with Orcein Van Gieson showed mild positive reaction. The elastic fibers in the connective tissue of lungs, and around blood vessels appeared brown in color. The collagen fibers appeared red in color around blood vessels and bronchioles **(Fig.13).**

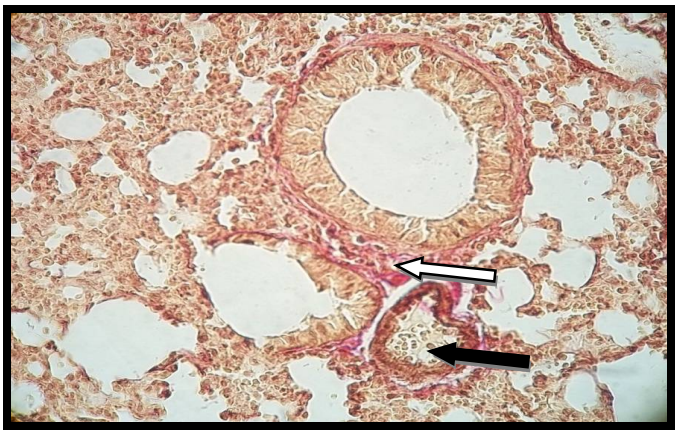

Fig. 13: Photomicrogragh of lung sections of group G5 of the studied mice showing mild positive reaction. The elastic fibers of the bronchioles wall appear brown in color (black arrows). The collagen fibers look red color (white arrows) (OVG X400)

DISCUSSION

The current study demonstrated that the studied mice of groups G2 and G3 suffered from significant higher lung weights compared to the other groups but this is more evident for group G3 as it was exposed to stress dose of epinephrine for longer duration (12 weeks). This is may be due to chronic inflammatory cells infiltration in the pulmonary parenchyma which was more evident in group G3 than group G2[15,17]. While group G4 which received the stress dose of epinephrine for 3 weeks together with vitamin B-complex injection showed normal lung weight compared with controlgroup (G1). This is may be due to the protecting effect of vitamin B-complex in lungs[24].

Histological changes in lung

This study found that there were interstitial inflammation with chronic inflammatory cells (lymphocytes and plasma cells) infiltration with thickening of interalveolar septum, in addition to collapse of many alveoli which was evident in lungs sections of group G3 of the studied mice that received the stress dose of epinephrine for longer duration (12 weeks).

Exposure to every day social stress in humans is associated with elevated inflammatory activity and with the up-regulated expression of genes that promote inflammation[29,30]. This will promote the development

and progression of many diseases. It can result in increased susceptibility to infection, increased disease severity, and the establishment of persistent infections[12-14]. Also long term exposure to emotional and physical stress in rats for more than 40 days induce a 51% increase in oxidative stress in the lungsas they are particularly susceptible to lesions by free radicals which induces lung tissue injury and disorders of their function[18].

The results of this study on the effect of stress dose of epinephrine for 12 weeks (group G3) in the studied mice, are in agreement with other studieson lung mice, one demonstrated that exposure to stress for 2 hours only lead to decrease number of NK cells in the intraparenchymal region[19] and the others found that social disruption in mice causes changes in immune cell reactivity and induce pulmonary inflammation with increased number of neutrophils and monocytes[20,31]. While Duan*et al.,* (2013)[21]recognized that psychosocial stress evokes asthma exacerbationin ovalbumin-induced asthmatic mice.

Besides that these results are coincides with other studies in different animal models of stress in rabbit[16] and in murine lung[17] which concluded that accumulation of neutrophils in lungs are responsible for lung injury, and repeated stress in guinea pigs induces inflammatory response and increase percentage of eosinophils and lymphocytes infiltration in distal airways[22].

Histochemical stain

According to author knowledge no article were found about the use of Orcein Van Giesonstain in the lung of mice exposed to stress. This study found that there is thickening of the bronchioland blood vessels walls which is due to increase production of collagen fibers seen by Orcein Van Giesonstain which was more evident in the lung sections of group G3 of the studied mice which received the stress dose of epinephrine for 12 weeks than those of group G2 mice which received the stress dose of epinephrine for 3 weeks. These results were due to hypoxia, as stress can induce inflammation, in which the immune cells may get overacted and cause collateral tissue injury ending up in tissue hypoxia[32]. Hypoxia induces extracellular matrix formation and maturation by human myofibroblasts and stimulate collagen synthesis and fibrous tissue formation[33].

These results were in agreement with results of a study in rat pulmonary circulation[34] which demonstrated that chronic hypoxia lead to generalized increase in wallthickness in small peripheral arterioles and a reduction in the number of peripheralarteries and the other studies [33,35-38]who found that hypoxia enhanced gene expression and protein production of extracellular matrix components in human myofibroblasts of cardiovascular tissue and also collagen synthesis increased at low oxygen concentrations.

Protective effect of vitamin B-complex on the lung tissue of the studied groups of mice:

The results of this study found that lung sections of the studied mice of Group G4 which received the stress dose of epinephrine together with vitamin B- complex injection for 3 weeks showed normal interalveolar septum only small focal areas of chronic inflammatory cells infiltrate were noticed. This meant that vitamin B complex reduced the bad effect of stress dose of epinephrine on lung histology.

According to author knowledge no article were found on the protective effect of vitamin B-complex on the lung after exposure to stress in mice, apart from the studies of the protective effects of vitamin B-complex in other animal from other toxic substances like the study ofDas and Mukherjee (2012)[24] who proved that administration of vitaminB-complex with lecithin can evidently produce a protective effect in reversing the chronic ethanol-induced oxidative stress mediated effects in the lungs of albino rats.While the study of Abu-Amara, *et al.,* (2013)[25], found thatusingsulpiride has no protective role against the marked damaging effects of noise stress on the lung and heart tissues of adult albino rats also.

CONCLUSION

The results of this study in mice concluded that stress canlead to lung damage, and the severity of damage depends on the duration of exposure to stress. Vitamins B-complex (B1, B6, and B12) canact as a protecting factor as they reduce the damaging effect of stress on this organ. This study **recommended** a better understanding of the relationship between stress and body pathology in human, and suggests that further studies on both human and animal models to beconducted.

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