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Formalin Induces Alterations in Body and Lung Weights and Histological Structure of the Lung of Adult Male Albino Rats and Amelioration by Mint Aqueous Extract

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Abstract

Background: Anatomy departments utilize formalin to harden museum specimens, and cadavers. Moreover, it is a component of pressed wood goods, paper, textile fibers, adhesives, plastics, carpeting, foam insulation, disinfectants, nail hardeners, and some finger paints. Additionally, it is used to stop germs from spoiling fish, fruits, milk, beverages, ice cream, sweetmeat, and spices. Because the respiratory epithelial cells are damaged and lose their ability to function, it leads in acute lung injury, a cytotoxic reaction in the respiratory system. The purpose of this study was to assess how formalin affected the adult male albino rats' body and lung weights as well as the histological structure of their lungs, and how this effect was mitigated by an aqueous extract of mint leaves. **Materials and Methods:** For this experiment, thirty adult male albino rats were procured from the Libyan Medical Research Centre located in the city of Zawia. Three equal groups were formed out of the animals. As a control group, Group I was given drinking water. Group II was the formalin group; for 30 days, they were given solely formalin at a dose of 10 mg/kg BW. Groups III were treated as a formalin + mint group, receiving 10 mg/kg BW of formalin for one hour, followed by 30 days of daily dosages of 600 mg/kg BW of mint extract. Gastric tubes were used to deliver all samples orally. On the 30th day, the animals were weighed, anesthetized, then scarified by cervical dislocation, and dissected. This was done 24 hours after the last dose. The lungs were extracted, weighed, and some of the lung tissue was preserved for 72 hours in 10% neutral buffered formalin, followed by dehydration and paraffin embedding. Subsequently, 5µm slices were made and stained with hematoxylin–eosin for histological evaluations. The weight of the body and lungs was reported as mean ± SE. One-way analysis of variance was used to conduct multiple comparisons (ANOVA). A value of p less than 0.05 was deemed significant. **Results:** The adult male albino rats' body weight significantly decreased ($P<0.01$) following the administration of formalin and formalin+mint, according to the data. On the other hand, rats given formalin+mint for 30 days showed a significant ($P<0.01$) increase in body weight as compared to the formalin group. Rats given formalin for 30 days showed an increase ($P<0.01$) in lung weight as a percentage of body weight when compared to the controls. On the other hand, compared to the formalin group, adult male albino rats given formalin+mint for 30 days experienced a significant ($P<0.01$) decrease in lung weight as a percentage of body weight. Control rats' lung sections underwent histological examination, which revealed normal pulmonary tissue architecture. When rats were given formalin, their lung tissues changed significantly in comparison to the control group. Co-administration of mint extract with formalin caused improvement in the lung tissues and restored the histoarchitecture to near normal as in the control group. **Conclusion:** It can be concluded that administration of formalin to rats significantly decreased body weight and increased lung weight as a percentage of body weight and a severe histopathological changed in lung tissues in comparison to the control group. While, treatment with mint improved these changed.

Keywords: Formalin, Body weight, Lung weight, Histopthological alterations of the lung, Mint aqueous extract, Amelioration, Adult male albino rats.

Introduction:

The lung is the essential organ of respiration and the organ that receives the entire cardiac output. Also, the lung plays an important role in host defense and regulation of circulating levels of biologically active materials by extensive surface of pulmonary vascular bed (Fishman *et al.*, 1998, Mohamed *et al.*, 2012).

Formalin is used in the plastics industry, as a fumigating agent in operating rooms, as a disinfectant for the preservation of surgical and pathological specimens, as well as for the coloring and hardening of celluloid, museum specimens, and cadavers in anatomy departments (Verma *et al.*, 2016). It is utilized in products such as pressed wood items, paper, textile fibers, adhesives and plastics, carpeting, foam insulation, cosmetics, nail hardeners, disinfectants, and specific finger paints and cleaning solutions (Mamun *et al.*, 2014). Some dishonest vendors have treated perishable goods with formalin to prevent spoilage. In order to preserve fish fresh, vegetables (like tomato and cucumber), fruits (like apple and grapes), milk, drinks, sweetmeat, ice cream, and spices from deteriorating due to germs, formalin is often and illegally employed (Restani *et al.*, 1992).

In order to sell fish at auction or on the market, fishermen intentionally inject them with formalin (Kartikaningsih, 2008, Maramis *et al.*, 2015). Formalin does not fulfill safety criteria when used as a preservation because it can react with chemicals in cells to affect their function, injuring cells, tissues, organs, and even the organism itself (Mahdi, 2008, Maramis et al., 2015). Formalin can be obtained naturally or artificially and used as a food preservative in food and drink (Tomkins *et al.*, 1989, Mamun *et al.*, 2014). Exposure to formaldehyde induced a hepatotoxicity and haematotoxicity in human and experimental animals (Al-Sarraj and Al-Habity, 2013, Maramis *et al.*, 2015, Verma *et al.*, 2016, Elshaer and Mahmoud, 2017). Because formaldehyde is quickly absorbed from the gastrointestinal tract after ingestion and from the respiratory system after inhalation, it is dangerous to use as a preservative (Mamun et al., 2014). Verma *et al.* (2016) reported that individuals may encounter respiratory distress, rhinorrhea, and ocular discharge at direct exposure to formalin fumes.

A significant elevated risk of cancer, particularly nasopharyngeal carcinoma in humans, has been associated with formalin exposure at work (Hayes *et al.,* 1986). In isolated instances, high formalin concentrations have also been connected to gastrointestinal cancer (Takahashi *et al.,* 1986). Long-term formaldehyde exposure has been linked to a number of reproductive diseases, including aberrations that may result in infertility during sexual maturation, spermatogenesis, sperm viability, and count, as well as histologically harmful effects on testicular tissue (Razi *et al.,* 2013).

Formaldehyde induces cytotoxicity in the respiratory tract, in the form of acute lung injury, which is caused by respiratory epithelial cell damage and loss of function (Mohamed *et al.,* 2012). So, much attention is paid to the effects of formaldehyde on the respiratory system, especially, the histological alterations induced in the lung by exposure to formalin.

Herbal products enhance antioxidants. Natural antioxidant defenses are endogenous from reactive oxygen species and restore the ideal balance in the equation of reactive radicals (free radicals) (Al-Mamary *et al.,* 2002). Therefore, recent studies are directed to the protective effects of plants and foods rich in antioxidants, such as green tea, mint, Garlic, and rosemary. Peppermint (*Mentha piperita*) is a perennial aromatic herb belonging to the Labiatae family and the Mentha genus. It has tremendous medicinal uses in different parts of the world (Baliga and Rao, 2010) because it contains. It contains more than 40 chemical compounds, including flavonoids such as oxetine, phenolic acids such as caffeic acid and rosmarinic acid, and its essential oil is rich in menthol (Anonymous, 1999, Baliga and Rao, 2010). Its antioxidants contribute to the prevention and treatment of diseases associated with oxidative stress because they remove free radicals and neutralize peroxide-stimulating ions (Sharma *et al.,* 2007, Singh and Gupta, 2011). It is used peppermint relieves flatulence, nausea and vomiting, is an antispasmodic, carminative, antimicrobial, and treats intestinal inflammation and irritable bowel syndrome (Bouchra *et al.,* 2003).

Over recent years it has been demonstrated that both peppermint and its constituents induce antioxidant, antispasmodic, anticarcinogenic, antitumorigenic, antiallergic, antiinflammatory, antimutagenic, anticancer, antinauseant, antiseptic, antilipid peroxidation, antiheadache and antiobesity properties (Shah and D'Mello, 2004, Mimica-Dukic N, and Bozin, 2008, Jain *et al.,* 2011, Rita and Animesh, 2011, Yi and Wetzstein, 2011, de Cássia da Silveira *et al.,* 2013).

2. Objectives:

The current study aimed to evaluate the effect of formalin on body and lung weights and histological structure in the lung of adult male albino rats and amelioration by mint leaves aqueous extract.

3. Material and Methods

3.1 Chemicals:

Formalin was purchased from Sigma chemical company.

3.2. Plant Material

Mint (*Mentha piperita*) was purchased from Al-Zawiya market, and mint juice was made by mixing 20 g of mint leaves with 200 ml of distilled water in a blender, then straining and filtering it to obtain the purified mint extract. Mint juice was given orally at a dose of 0.6 g/kg body weight for 30 days (Barbalho *et al.,* 2011).

3.3. Animals

Forty-eight adult male albino Rats (180-200 g) obtained from Libyan Medical Research Centre in the city of Zawia were used for this experiment. The animals were maintained on 12 h light and dark cycle, at 25±2 ºC and 60%-70% humidity with standard pellets diet. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals published by Clark *et al.,* 1997.

3.4. Experimental design

The animals were equally divided into three groups Group I served as a control group and received drinking water. Group II served as a formalin group and received only formalin at (10 mg/kg) BW for 30 days. Groups III served as a formalin + mint group and received a formalin at 10 mg/kg BW, and after an hour were treated with the mint extract at doses of 600 mg/kg BW daily for 30 days. All samples were administered orally using gastric tubes.

After 24 hours of final dose on the 30th day, the animals were weighed, anesthetized, then scarified by cervical dislocation, and dissected.

3.5. Histopathological analysis

The lungs were removed, weighed, and a portions lungs were fixed in 10% neutral buffered formalin for 72 h, dehydrated, and embedded in paraffin. Later, 5μm sections were prepared followed by staining with hematoxylin–eosin for histological assessments.

3.6. Statistical analysis

Body weight and lungs weight were expressed as mean ± SE. Multiple comparisons were performed by one-way analysis of variance (ANOVA). Value of $p < 0.05$ was considered to be significant.

4. Results:

4.1 Effect of administration of adult male albino rats to formalin and formalin+ mint on the body weight and lung weight (gm/100gm of body weight):

A) Body weight

Body weight was markedly decreased in all groups under investigation after administration of formalin and formalin+ mint in adult male albino rats. The data recorded in table (1) and figure (1) indicate a marked decreased (*P<0.01*) in body weight in rats received formalin and formalin+ mint for 30 days compared with the controls. While, administration of formalin+ mint to adult male albino rats for 30 days caused a significant increase (*P<0.01*) in body weight compared with the formalin group.

B) Lung weight as a percent of body weight

Lung weight as a percent of body weight was increased (*P<0.01*) in rats received formalin for 30 days compared with the controls. While, administration of formalin+ mint to adult male albino rats for 30 days caused a significant decrease (*P<0.01*) in lung weight as a percent of body weight compared with the formalin group (Table .1 & Figure .2)

Table. 1: Effect of administration of adult male albino rats to formalin and formalin+ mint on the body weight and lung weight (gm/100gm of body weight).

Groups Parameters	Control Mean ± SE	Formalin Mean ± SE	Formalin + mint Mean ± SE
Body weight (g)	298.5 ± 4.60	34.8 ± 16.07**2	270 ± 2.63**##
Lung weight (g/100g B.W)	0.715 ± 0.069	0.951 ± 0.015**	0.787 ± 0.029##

*: significant at ($P<0.05$) compared to control group; **: significant at ($P<0.01$) compared to control group;
#: significant at ($P<0.05$) compared to formalin group; ##: significant at ($P<0.01$) compared to formalin group.

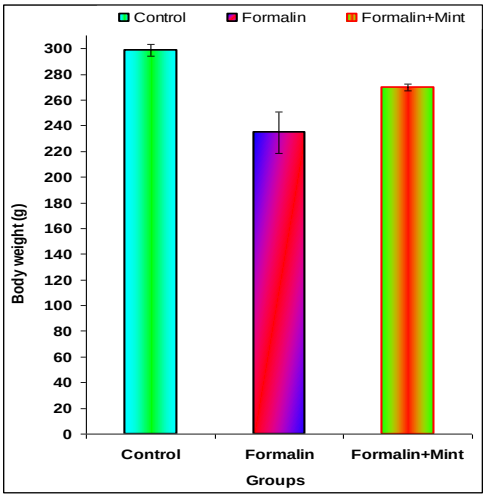

Figure. 1: Effect of administration of formalin and formalin+ mint to adult male albino rats on the body weight.

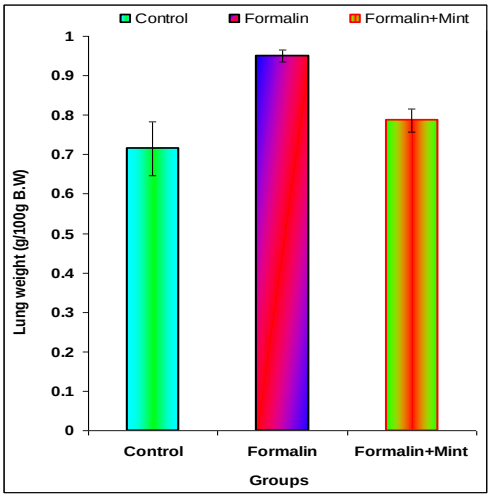

Figure. 2: Effect of administration formalin and formalin+ mint to adult male albino rats on the lung weight (gm/100gm of body weight).

4.2. Histological examination

4.2.1. Histological examination the lung of control rats

Lung sections from the control rats revealed a normal pulmonary tissue architecture with clearly visible patent bronchial passageways and alveolar cavities, which included the alveolar sacs, alveolar ducts, and alveoli. The alveoli were polyhedral chambers with narrow walls that were encircled by a single layer of squamous epithelium. There was a thin layer of connective tissue in between each alveolar. There are thin and thick sections of the alveolar septum. Both type I and type II alveolar cells made up the alveolar septum. Type I alveolar cells were thin, squamous, and flat, and they covered the majority of the alveolar lining surface in the lung tissue of the control samples. Type II cells, on the other hand, were cuboidal secretary cells that were present at the angular junctions of the alveolar walls, scattered among the type I cells but with a tendency to converge at the septal junction. In order to clear inhaled particulate debris, alveolar macrophages that were present in the alveolar wall also moved into the lumen. The distribution of pulmonary vessels throughout the pulmonary parenchyma was normal (**Figure 3**).

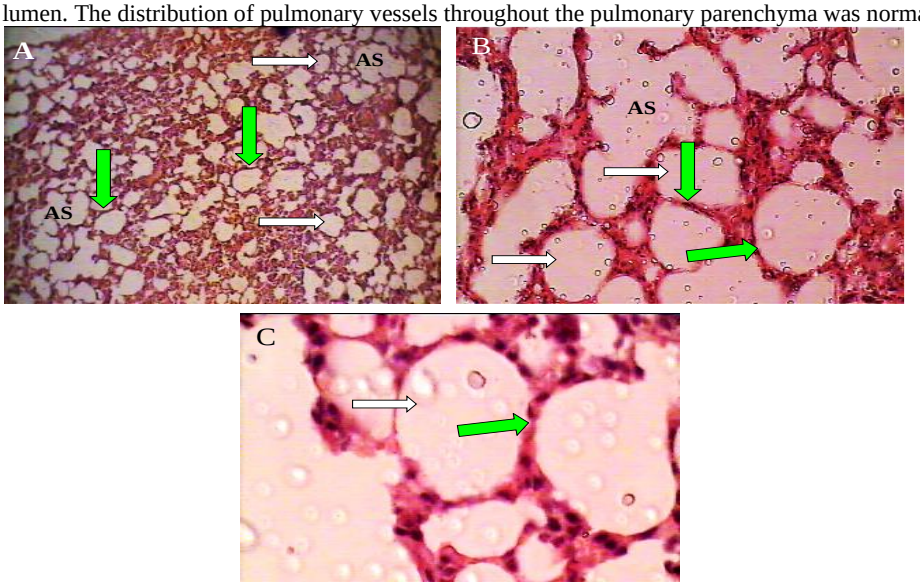

Figure. 3: Histological structure of the lung of control adult male albino rats. The alveoli (White arrows) were thin-walled polyhedral chambers surrounded by single layered squamous epithelium (Green arrows), and alveolar sacs (AS). **(A: X100, B: X400; C: X1000, H&E).**

4.2. 2. Histological examination the lung of formalin administrated rats

When rats were 2. given formalin, their lung tissues changed significantly in comparison to the control group. Lung sections revealed abnormalities in the pulmonary cytoarchitecture, such as the alveolar walls being thickened in certain areas and

obliterated in others. The alveolar septal veins also shown dilatation and congestion. Purulent exudates in the bronchioles, pulmonary fibrosis with thickened alveolar walls, enlarged alveolar walls, type II pneumocyte hyperplasia, cellular pyknosis, bronchiolar epithelia degeneration, a few collapsed alveoli, alveolar hemorrhage/edema, inflammatory cell infiltration, and foamy macrophage accumulation. Pneumocyte proliferation causes the alveolar wall to become more cellular, thickening the alveolar septa. Emphysema is demonstrated by the production of air space bullae and dilated interalveolar septal capillaries. Animals' lungs displayed vasculitis along with pulmonary blood vessel dilating (**Figure 4**).

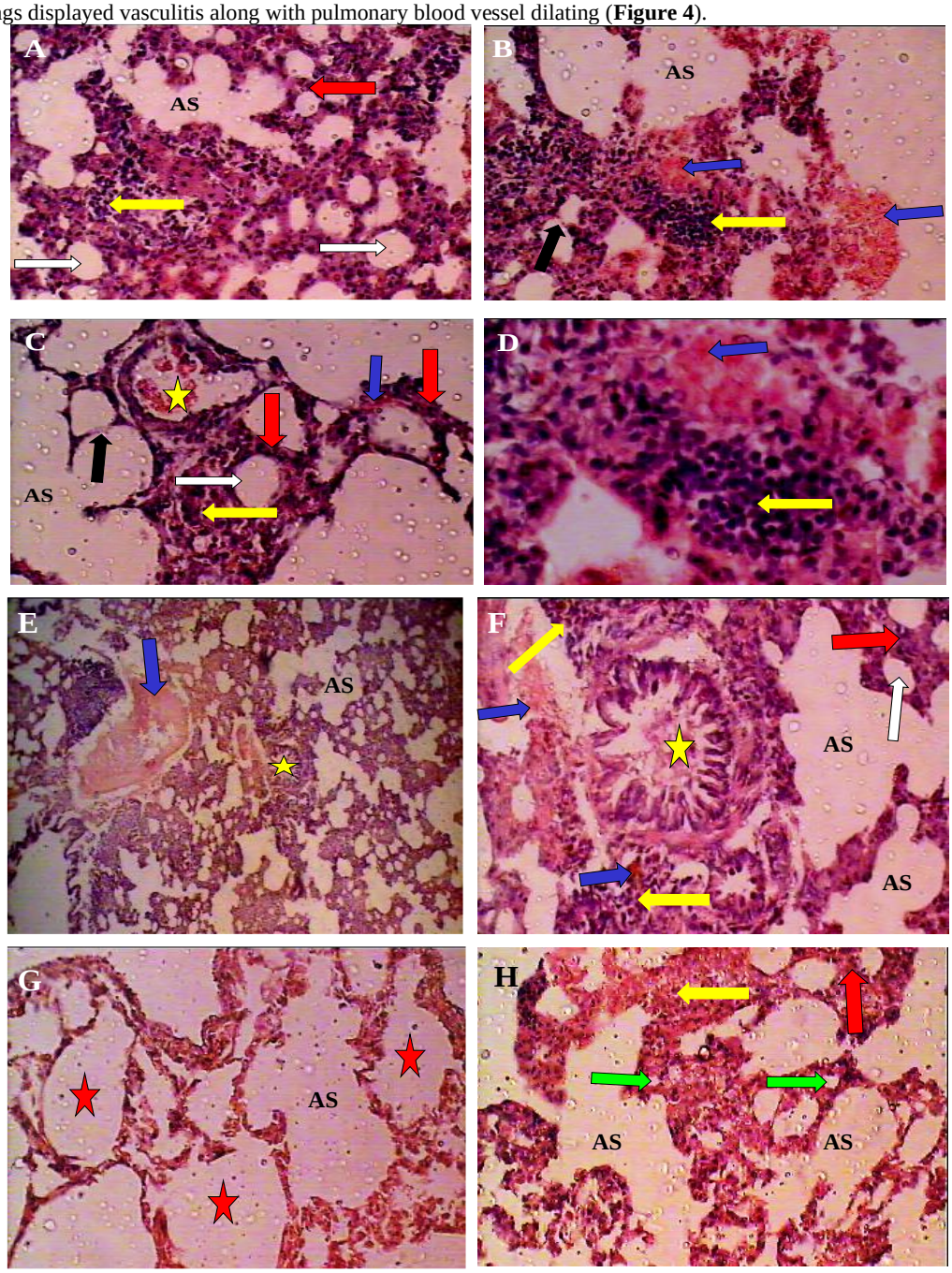

Figure. 4: Histological structure of the lung of formalin administrated adult male albino rats. The alveoli (White arrows) The alveolar hemorrhage (Blue arrows), thickening the alveolar septa (Red arrows), inflammatory cell infiltration (Yellow arrows), Purulent exudates in the bronchioles (Yellow stars), Emphysema (Red stars), few collapsed alveoli (Green arrows), and alveolar sacs (AS). (**E: X100, A, B, C, F, G& H: X400; D: X1000, H&E**).

4.2.2. Histological examination the lung of formalin + mint administrated adult male albino rats

When formalin+mint was administered to adult male albino rats, histological analysis of the lung sections revealed that the control group's pulmonary tissue architecture was almost normal. The alveoli were polyhedral chambers with narrow walls that were encircled by a single layer of squamous epithelium. There was a thin layer of connective tissue in between each alveolar. There are thin and thick sections of the alveolar septum. Both type I and type II alveolar cells made up the alveolar septum. Type I alveolar cells in lung tissue were thin, squamous, and flat, and they covered the majority of the alveolar lining's surface. Type II alveolar cells, on the other hand, were cuboidal secretary cells found at the alveolar walls' angle junctions, scattered among type I cells but with a tendency to converge at the septal junction. In order to eliminate inhaled particulate

debris, alveolar macrophages that were located in the alveolar wall also moved into the lumen. Alveolar minor infiltration of cells. There was evidence of dilatation and congestion in several alveolar septal veins (**Figure 5**).

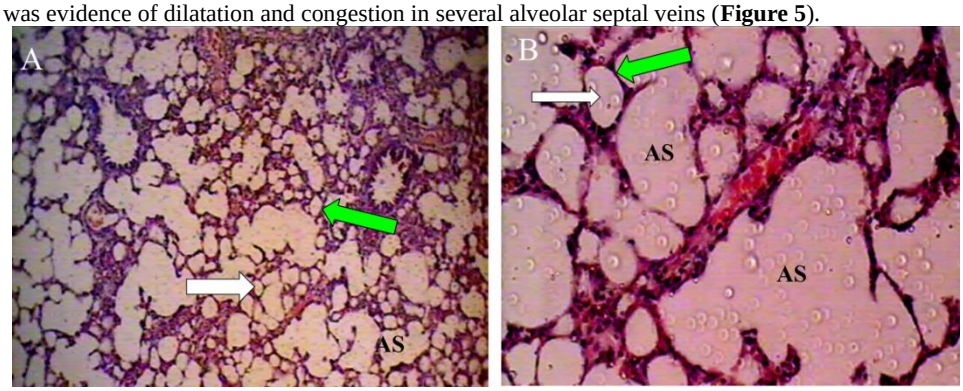

Figure. 5: Histological structure of the lung of formalin+mint administrated adult male albino rats. The alveoli (White arrows) were thin-walled polyhedral chambers surrounded by single layered squamous epithelium (Green arrows), alveolar sacs (AS), and alveolar minimal cellular infiltrate. Dilatation and congestion were found in some of the alveolar septal vessels **(A: X100, B: X400, H&E).**

5. Discussion

The current study was carried out to evaluate the effect of formalin on body weight and lung weight (g/100g of body weight) and histological structure in the lung of adult male albino rats and amelioration by mint aqueous extract.

Researches on humans and laboratory animals has demonstrated that both acute and long-term exposure to formaldehyde can cause cancer and respiratory damage (Lu *et al.,* 2008, Turkoglu *et al.,* 2008, ATSDR, 2010, Zhang *et al.,* 2013).

The results of the current study showed a significant decrease in body weight of rats that received formalin compared to the controls. These findings run parallel with study of Tobe *et al.,* 1989 who found that shown a reduction in the body weight of subjected to formalin. It is possible that this reduction in body weight was caused by insufficient consumption of food and water. Furthermore, Park *et al.* (2020) observed that mice exposed to 5.36 mg/m^3 formaldehyde saw a significant reduction in body weight gain between day 4 and day 15. Rats and mice studies have demonstrated that formaldehyde exposure at concentrations between 3 to 400 ppm reduces food and water intake and body weight, while concentrations above 6 ppm in human and animal models alter pulmonary function and reduce body weight (Tesfaye *et al.,* 2021). Egwurugwu *et al.* (2018) observed that the rats exposed to formalin displayed several physical symptoms, including altered eating patterns and decreased body weight. Compared to control F344 rats, the mean body weights of the F344 rats treated with formaldehyde were decreased (Ohtsuka *et al.,* 1997). When pregnant mice were given formaldehyde, their body weight decreased in comparison to the control group (Merzoug and Toumi, 2017). According to a study (Aydin *et al.,* 2015), rats exposed to formaldehyde at a level of 5.27 ppm experienced a drop in body weight relative to the control group; this drop in weight may have been caused by a decrease in hunger (Aydin *et al.,* 2015, Merzoug and Toumi, 2017).

Repeated exposure to toxic substances leads to chemical stress that activates the hypothalamic pituitary adrenal axis, which leads to increased secretion of cortisol from the adrenal glands (Friedman & Lawrence, 2002), and the response to stress in humans has led to a 30% decrease in food intake (Stone & Brownlei, 1994). In this state of stress, energy is used in the physiological processes necessary to reduce the poisoning resulting from formaldehyde to achieve internal balance, which may lead to a loss of body weight (Yau & Potenza, 2013).

The current study showed a significant increase ($P<0.05$) in lung weight (g/100g of body weight) in rats that received formalin compared to the controls. Similar result was obtained by Park *et al.,*2020 who found that the relative lung weight (lung weight/body weight × 100%) in mice was significantly higher in the 5.36 mg/m^3 formaldehyde exposure group than in the control group. Also, Lino-dos-Santos-Franco *et al.,* 2010 recorded that formaldehyde exposure cause a significant increase of pulmonary vascular permeability. These results may be related to increase relative lung weight of 5.32 mg/m^3 formaldehyde - exposed mice (Park *et al.,*2020).

The present study showed that formalin administrated to rats caused a severe change in the lung tissues compared with the control group. Lung sections showed distortions in pulmonary cytoarchitecture which includes the alveolar walls were destroyed in some places and thickened in other places, dilatation and congestion were prominent in the alveolar septal vessels. few collapsed alveoli, alveolar hemorrhage/Edema, inflammatory cell infiltration, foamy macrophages accumulation, cellular pyknosis, bronchiolar epithelia degeneration, presence of purulent exudates in the bronchioles, pulmonary fibrosis with thickened alveolar walls, enlarged alveolar walls and type II pneumocytes hyperplasia. There is increased cellularity of alveolar wall due to proliferation of pneumocytes resulting in thickened alveolar septa, emphysema evident from bulla formation of air spaces and dilated interalveolar septal capillaries. The lungs of animals showed dilatation of the pulmonary blood vessels accompanied by vasculitis. There was deposition of collagen in the extracellular matrix, due to pulmonary fibrosis, and subsequent reduction in the alveolar spaces. These results are consistent with the findings in rats by (Turkoglu *et al.,* 2008, Mohamed *et al.,* 2012), in the rabbit lungs after exposure to 40% formaldehyde (Neelam et al., 2011). Mohamed *et al.,* 2012 reported that light microscopic examination of the lung showed thickened alveolar septum, bronchiolar epithelial hyperplasia, proliferative capillary, pulmonary vasculitis, hyperplastic parabronchiloar lymphocytic aggregations, pulmonary fibrosis and precancerous changes (goblet cell metaplasia and bronchiolar epithelial dysplasia).

The mechanism of polymorphonuclear leukocytes inflammatory cells invasion induced by formaldehyde inhalation explained by Kimura *et al.,* 2010 who reported that inhaled formaldehyde rapidly increased vascular permeability in rat airway and produced microvascular leakage in the airway through stimulation of tachykinin NK1 receptors by tachykinins released from sensory nerves. Also, Lino-dos-Santos-Franco *et al.,* 2011, Seow *et al.,* 2015 mentioned that prolonged exposure to formalin

can induce many pathophysiological conditions, including inflammatory diseases by interfering in the level of T CD3+ cells, natural killer (NK) cells, TNF, IL-6 and IL1-b. Njoya *et al.*, 2009 reported that there was emphysema evident from bulla formation of air spaces in all animal exposed groups due to rupture of inter alveolar septa. The mechanism by which formaldehyde brought about the ulceration of the alveoli was by excavation and desquamation of the surface epithelium and derangement with distorted supporting tissues of alveolar wall. The observed histological alterations in the pulmonary epithelium ranged from acute interstitial inflammation, pulmonary fibrosis, acute purulent bronchitis, acute lung injury and chronic lung injury; similar to that reported by Kamta *et al.*,1996, Chinedum *et al.*,*2014*.

Reactive oxygen species (ROS) are formed continuously in cells as a consequence of external factors and they become harmful when they are produced in excess under abnormal conditions such as inflammation (Green *et al.*, 1989, Gutteridge and Halliwell, 1990, Turkoglu *et al.*, 2008). ROS may cause cell damage and are involved in the pathophysiology of inflammation, and cancer (Good *et al.*, 1996, Halliwell and Gutteridge, 1999, Turkoglu *et al.*, 2008). Formaldehyde has oxidant effects. Formaldehyde decreases SOD activity and increases MDA levels in the lung which is indicative of oxidative damage. In other words, Formaldehyde leads to oxidative damage in the lungs (Zararsiz *et al.*, 2004, Turkoglu *et al.*, 2008). Turkoglu *et al.*, 2008 observed that Formaldehyde caused histopathological changes in the lungs of the rats. Additionally, it plays oxidative role for respiratory structure including lungs.

Conclusion

It can be concluded that administration of formalin to rats significantly decreased body weight and increased lung weight as a percentage of body weight and a severe histopathological changed in lung tissues in comparison to the control group. While, treatment with mint improved these changed.

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