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Cypermethrin-Induced Lung Damage in Albino Rats: The Preventive Impact of *Moringa oleifera*

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Abstract

Cypermethrin (CYP) is a popular environmental toxicant because of its wide-ranging use as a broad-band insecticide. The purpose of this research was to explore the potential impact of *Moringa oleifera* leaves extract (MOLE) as a protective agent against cypermethrin-induced lung toxicity in male albino rats. Forty experimental animals were allocated into four groups. G1 served as the negative control, G2, G3 and G4 were orally treated with MOLE (250 mg/kg body weight), CYP (26.15mg/kg body weight) and CYP + MOLE, respectively. CYP oral administration for 28 days elevated the levels of oxidative stress biomarkers like Malondialdehyde, superoxide dismutase, catalase and glutathione S-transferase and caused a decline in the content of reduced glutathione in the lung tissue of the rats. Lung injury was verified by histopathological changes evidenced by interstitial pneumonia, marked perivasculitis, focal pulmonary hemorrhage, haemosidrosis and pulmonary edema in CYP-exposed rats. Co-administration of MOLE mitigated the induced oxidative stress and histopathological alterations. As a result, *Moringa oleifera* can prevent cypermethrin-induced lung toxicity due to its free radical scavenging and antioxidant properties.

Keywords: Pyrethroid; Cypermethrin; *Moringa oleifera*; Lung; Oxidative stress; Histopathology.

1. Introduction

Pesticides persistence in the environment results in unescapable human and animal exposure to these poisonous compounds that pollute food, soil, water and air **[1].** Subsequently, heavy pesticide use increases the possible health hazards of human beings, comprising chronic, acute and subacute poisonings **[2].** Whereas lung inhalation and skin absorption tend to be the most critical workplace exposure routes, oral ingestion is the main route of pesticide exposure in non-working environments **[3].** In contrast to other exposures such as air and water, pesticide exposure from food intake is considered 5 times higher **[4].**

Cypermethrin (CYP) is a synthetic type II pyrethroid and has potent insecticidal properties. While CYP is the most effective insecticide, its usage has increased at an alarming pace, resulting in many unfavorable effects on non-target species, including humans. CYP exposure causes a variety of negative effects including kidney problems, hepatic fibrosis, defective blood coagulation, anemia, brain damage, birth defects, genetic disorders, infertility and cancer **[5].**

Oxidative stress refers to the disparity between reactive oxygen species (ROS) generation and body antioxidant defense systems. It is a deleterious process that can damage all cell structures leading to cell death. Also, it can result in several diseases like degenerative diseases, metabolic diseases, acute respiratory distress syndrome, asthma, idiopathic pulmonary fibrosis, hypertension, diabetes, ischemia, chronic obstructive pulmonary disease, atherosclerosis, neurological disorders and cancer **[6].** Moreover, it implicated in the toxicity of pesticides **[7].** Lipids, carbohydrates, proteins and nucleic acids are the focal components susceptible to free radical harm and oxidative stress. Consequently, this can impair the structure and the function of the cell resulting in cell death **[8].**

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Phytomedicine is becoming popular around the world because of its potency, cost-effectiveness and low side effects and many plants have medicinal effects against various diseases and defensive effect against the chemical contaminants hazards **[9].**

Moringa is a small trees-like agricultural plant. It is the single genus of around 13 known species in the monotypic Moringaceae family. Among Moringa species, *Moringa oleifera* Lamarck (Lam.) is the most prevalent **[10].** The therapeutic and nutritional benefit of *Moringa oleifera* has been well documented in the literature. *Moringa oleifera*, particularly its leaves, was found to provide an excellent source of naturally occurring antioxidants, comprising, minerals, ascorbic acid, sterols, α-tocopherol, carotenoids, vitamins, kaempferol, polyphenols, essential amino acids containing sulphur like cysteine and methionine, glucosinolates, and flavonoids, and other compounds known to have beneficial health consequences **[11].**

The various parts of the *Moringa oleifera* tree have been used since Roman, Greek and Egyptian times as a natural medication for thousands of years to enhance health and cure diseases. There have been records of different parts and preparations of *Moringa oleifera* used in conventional medicine, various disorders cure **[12]** and also used as dietary supplements **[13].** The phytochemical analysis of the ethanolic extract of *Moringa oleifera* leaves showed the presence of high total phenolic content, antioxidant capacity, alkaloids, flavonoids, saponins, steroids and tannins **[14-16].**

Moringa oleifera treats many health problems such as headaches, abnormal blood pressure, diabetes, nervous disorders, skin infections, psoriasis, eye and ear infections, cholera, scurvy, anemia, blood impurities, anxiety, catarrh, conjunctivitis, fever, sore throat, respiratory disorders, tuberculosis, bronchitis, chest congestion, cough, asthma **[17].** Additionally, previous studies have shown the value of *Moringa oleifera* extracts as a cerebroprotective, cardioprotective, neuroprotective, hepatoprotective, immunomodulatory effector, anti-inflammatory, and antioxidant **[18].** Therefore, the present study was carried out to evaluate the protective effects of *Moringa oleifera* leaves extract (MOLE) against CYP-induced lung toxicity in rats

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2. Materials and Methods

2.1. Chemicals

Cypermethrin (20% Emulsion Concentration "EC") was purchased from Kafr El Zayat Pesticides and Chemicals Company, Egypt. *Moringa oleifera* leaves powder was purchased from the Egyptian Scientific Society for *Moringa* at National Research Centre, Dokki, Giza where the plant was identified by Professor Dr. Aboelfetoh M. Abdallah, and a voucher sample number (MO19) was kept in the Horticulture and Crops Technology Department, National Research Centre, Egypt. All the chemicals used in this study were obtained from Sigma Chemical Company (USA).

2.2. Animals and housing

Healthy forty albino rats (adult males weighing 160±10g) were obtained from the animal house of the New Veterinary Office, Giza, Egypt. They were housed in the Animal House of Central Agricultural Pesticides Laboratory. The rats spent two weeks acclimating to laboratory conditions before beginning the experimental work. They were housed under controlled laboratory conditions of 12h light/12h dark cycle at room temperature 25 °C. Rats were maintained on the standard pellet diet and water ad. libitum.

2.3. Preparation of *Moringa oleifera* leaves extract

Moringa oleifera leaves powder was extracted with dehydrated alcohol for 24 hours at room temperature. The obtained extract was filtered using Whatman filter paper No.5 and vacuum dried at 40-50 °C then the rough concentrate was suspended for final use in double-distilled water **[19,20].**

2.4. Experimental design

The rats were distributed into four groups of ten animals each. G1(negative control): received 1ml distilled water by oral gavage. G2(positive control): received 250 mg/kg body weight MOLE for 14 days before the experiment and during the experimental period (28 days), G3 (CYP-treated group): orally administered 26.15mg/kg/day (1/10th LD_{50}) cypermethrin pesticide for 28 days and G4 (CYP + MOLE-treated group) orally administrated the same dose of CYP for 28 days plus 250 mg/kg body weight MOLE as G2.

The dose of MOLE (250 mg/kg body weight) was chosen according to **[21,22]** based on acceptable safety and efficacy. The dose of CYP that was chosen based on determined acute LD_{50} according to the protocol of Organization for Economic Cooperation and Development (OECD) guideline 401 for testing chemicals. " Acute oral toxicity" **[23].** This research was executed according to the standard procedures intended by OECD guidelines 407"Repeated Dose 28

Day" oral toxicity study in rodents **[24].** This study approved by the Ethics Committee for Institutional Animal Care and Use Committee (CU-IACUC) at Cairo University (approval number is CU/I/F/59/18).

2.5. Specimen collection

The rats were euthanized and killed by cervical dislocation. Lung tissues were removed and washed with saline. Part of the lung tissue fixed in neutral buffered formalin for histopathological investigation and the other part kept frozen at -20°C for biochemical parameters assay.

2.6. Preparation of lung tissue homogenate

The lung was homogenized in 10 ml ice-cold 50mM potassium phosphate buffer, pH 7.5 containing 1mM EDTA and potassium chloride (1.17% KCl) per gram tissue using a chilled Glass-Teflon potter-Elvehjem tissue homogenizer. The homogenate was then centrifuged for 15 minutes at 4°C at 10,000 x g, and the supernatant was collected and deposited at -20°C for later use.

2.7. Biochemical analysis

2.7.1. Determination of protein concentration

Protein concentration was measured according to **Bradford et al. [25]** method via bovine serum albumin as standard.

2.7.2. Determination of oxidative stress biomarkers

The lipid peroxidation final product, Malondialdehyde (MDA), was estimated by **Ohkawa et al. [26]** method. The reduced glutathione (GSH) content was assayed by **Ellman [27]** method. Superoxide dismutase (SOD) activity was estimated by **Marklund and Marklund [28]** method. Catalase activity (CAT) was determined according to **Cohen et al. [29]** method. The glutathione S-transferase activity was estimated by **Habig et al. [30]** method.

2.8. Histopathological examination

The Lung specimens were fixed in formalin saline (10%) for 24 hours. Then the specimens were washed with tap water, dehydrated, cleared and then embedded in paraffin wax. Sections of 5-micron thickness were cut and stained with hematoxylin and eosin for investigation under a light microscope **[31].**

2.9. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows version 23 software was used to conduct the statistical analysis. For multiple comparisons between different groups, a one-way analysis of variance (ANOVA) was used, followed by Duncan's multiple range test post hoc analysis. The obtained results were represented as mean ± standard deviation (Mean ± SD). Values at $P< 0.05$ considered being significant.

3. Results and Discussion

Global toxicity of insecticides is becoming life-threatening, with an increased risk of compromised lung function. Pyrethroid accumulation in tissues has been related to the development of reactive oxygen species (ROS) and oxidative stress activation **[32].** Therefore, the present study was attempted to determine the toxicity of cypermethrin on rat's lung and to explore the possible ameliorative effect of *Moringa oleifera* leaves extract against cypermethrin toxicity.

Lipid peroxidation is a chemical reaction that occurs when free radicals attack lipids and disrupts the structure and function of biological membranes **[33].** The highly reactive Malondialdehyde (MDA) metabolite is the final lipid peroxidation by-product. MDA is used as a lipid peroxidation and oxidative stress indicator since it indirectly shows the degree to which free radicals invade the lipids of the cell membranes **[34].** The data shown in **Table 1** revealed that the administration of cypermethrin to normal rats (G3) has shown considerable increases in lung MDA level (51.63%) relative to that in control rats (G1). This MDA level elevation indicated CYP-induced lipid peroxidation reflecting the in vivo ROS accumulation which stimulates lung tissue injury.

MDA elevation may be attributable to the lungs susceptibility to oxidative stress because of their large surface area, their existence in a high oxygen environment and higher blood supply **[35].** Also, the observed MDA elevation may be due to the hydrophobic nature of CYP which may be accumulated in the cell membrane disturbing its structure. Moreover, this MDA elevation may be owing to CYP metabolism. CYP is metabolized through the cytochrome P450 microsomal system yielding reactive oxygen species that induce oxidative stress **[36].** During CYP metabolism, it forms cyanohydrins which are further decomposing to cyanides and aldehydes; substances that can induce reactive oxygen species production **[37]** and the excessive reactive oxygen species may attack the thiol group of cysteine residues and polyunsaturated fatty acids of biological membranes leading to cell damage **[38].** Our data are in accordance with **Hussien et al. [39]** who reported an increase in lipid peroxidation in rat brain tissue upon CYP administration. Additionally, **Mossa et al. [40]**

reported elevation in lipid peroxidation level in mice liver tissue upon CYP exposure.

Natural compound-based antioxidants act as protective agents against free radicals, making them one of the most important therapeutic agents for reducing illnesses caused by oxidative stress **[41].** As compared to the CYP-treated group(G3), the group given CYP + MOLE (G4) showed a substantial decrease (34.85%) in lung MDA levels **Table 1**. MOLE inhibited lipid peroxidation as it scavenged free radicals and reduced oxidative stress **[42]. Brilhante et al. [43]** reported that the presence of flavonoids and phenols in high concentrations in various parts of the *Moringa oleifera*, especially the leaves, favors the reduction of oxidative damage to the main biomolecules by inhibiting lipid peroxidation and preventing free radical generation. Furthermore, supplementation of α-tocopherol, one of the vitamins present in *Moringa oleifera*, correlated with lower levels of lipid peroxidation and improved lung function **[44].**

In addition to lipid peroxidation, CYP- induced lung oxidative stress is also evidenced by alteration of the antioxidant defense system. The lung antioxidant system disturbances observed in response to CYP administration characterized by changes in enzymatic and non-enzymatic antioxidant parameters. Reduced glutathione (GSH) is an intracellular, non-enzymatic antioxidant that detoxifies harmful chemicals like reactive oxygen species and xenobiotic compounds, as well as serving as a co-substrate for the antioxidant enzyme GST **[45].** As depicted in **Table 1**, GSH content was significantly decreased (29.5%) in rats treated with CYP alone (G3) when compared with that of the negative control group (G1). This GSH activity reduction could be due to the decreased expression of this non-enzymatic antioxidant during bronchial cellular damage together with probable damage to the structure of this protein molecule by ROS or aldehydes generated during CYP metabolism. Also, the decline in GSH can be attributed to increased GSH use to detoxify excess free radicals generated after CYP exposure **[46].** This was in agreement with **Afolabi et al. [47]** who demonstrated GSH reduction in rats' liver and kidney tissues following CYP exposure. **Anitha et al. [48]** reported that the reduction in GSH content was directly linked to oxidative stress progression. Furthermore, GSH level depletion augments tissues vulnerability to peroxidative injury and making them more susceptible to radical generation **[49].** In contrast, co-administration of MOLE (G4) showed a significant increase (31.6%) in the GSH content compared to that of the CYP-treated group (G3). *Moringa oleifera* contains a high concentration of essential sulphur-containing amino acids, which can stimulate GSH synthesis and thus restore GSH content. Also, MOLE raises GSH concentration because it contains many antioxidant components such as phenolic compounds, carotenoids, vitamin C, oleic acid and selenium **[22].**

Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) help organisms combat free radical formation. SOD converts superoxide radicals to hydrogen peroxide, whereas CAT catalyzes the hydrogen peroxide decomposition into water and oxygen **[37].** Glutathione S- transferase conjugates the electrophilic compounds to the GSH thiol group to form less toxic compounds thus secure the cell from xenobiotic detrimental consequences **[50].** These enzymes work together, and if their levels change, the cells are pushed into an oxidative stress state. As shown in Table 2, SOD, CAT and GST activities were increased (86.7%, 51.6% and 72.9% respectively) in rats exposed to CYP (G3) when compared to those of control rats (G1). The elevated levels of these enzymatic antioxidants may be a compensatory response to combat and mitigate the toxic effects of increased CYP superoxide radical and other ROS generation. Our findings were in agreement with **AlKahtane et al. [51]** who reported that SOD and CAT levels were increased in cultured human hepatocarcinoma (HepG2) cells following CYP exposure. Also, our results in consonance with **Khan et al. [32]** who reported an increase in GST activity in the brain tissue of rats exposed to deltamethrin (another synthetic type II pyrethroid).

As compared to the CYP-treated group (G3), co-administration of MOLE (G4) resulted in a significant reduction in the SOD, CAT and GST activities (63.4%, 34.9% and 18% respectively). These results may be attributed to the *Moringa oleifera* direct scavenging activity and the presence of a rich combination of antioxidants phytochemicals that have been reported to possess antioxidant and anti-inflammatory activities. Additionally, Plant products are known to exert their protective effects by scavenging free radicals and modulating antioxidant

defense system. Usually, natural compounds rich in polyphenols have strong antioxidant properties and can decrease oxidative damage in tissues by scavenging free radical **[52].**

Moringa oleifera extracts of mature and tender leaves show potent antioxidant activity against free

radicals and provide substantial oxidative damage defense due to polyphenol enrichment **[53].** Also, quercetin-3-O-glucoside and kaempferol-3-O-glucoside are in the leaves and are involved in

antioxidant protection as they scavenge free radicals and thus minimize oxidative stress **[11].** Some studies indicate that antioxidant like isoflavones, vitamin E and vitamin C, present in *Moringa oleifera* as well, avoid oxidative damage in rats triggered by cypermethrin **[54-56].** Several therapies have been taken, including herbal extracts in respiratory system diseases to reduce the phenomenon of oxidative stress **[57-59].**

Table 1: Effects of cypermethrin and Moringa oleifera leaves extract treatment on lung MDA level and

Groups	MDA level (nmol/L)	GSH content (µmol/g tissue)
G1	**24.6±2.4**	**1.39±0.04**
G2	**24.9±1.8**	**1.41±0.06**
G3	**37.3±2.5[a,b]**	**0.84±0.05[a,b]**
G4	**24.3±2.9[c]**	**1.21±0.31[c]**

GSH content.

Values are expressed as mean ± SD. (n =7). The (P value≤ 0.05) was set as statistically significant different while the (P value > 0.05) was set as statistically insignificant different. Symbols: (a) significant compared to G1, (b) significant compared to G2, (c) significant compared to G3.

Table 2: Effects of cypermethrin and *Moringa* leaves extract treatment on lung enzymatic oxidative stress biomarkers.

Groups	SOD activity (U/mg protein)	CAT activity (µmol/min/mg)	GST activity (µmol/min/mg protein)
G1	**3.23±0.7**	**2.97±0.31**	**1.22±0.31**
G2	**3.07±0.4**	**2.36±0.39**	**1.28±0.27**
G3	**6.03±1.8[a,b]**	**4.61±1.15[a,b]**	**2.11±0.28[a,b]**
G4	**2.21±0.4[c]**	**3.21±0.63[b,c]**	**1.73±0.21[a,b,c]**

Values are expressed as mean ± SD. (n =7). The (P value≤ 0.05) was set as statistically significant different while the (P value > 0.05) was set as statistically insignificant different. Symbols: (a) significant compared to G1, (b) significant compared to G2, (c) significant compared to G3.

In the present study the biochemical results supported by histopathological changes in the lung tissue of CYP-treated animals, which comprised interstitial pneumonia, marked perivasculitis, focal pulmonary hemorrhage and haemosidrosis and pulmonary edema **Fig. 1**. The histopathological changes could be attributed to CYP toxicity and ROS accumulation in the lung because of increased lipid peroxidation and oxidative stress. An imbalance in the production of reactive oxygen, such as free radicals and peroxides, causes oxidative stress, which damages cells and leads to collagen deposition, resulting in pulmonary fibrosis and edema **[60]**. The different histopathological changes in the lung that observed in this study are in agreement with other studies **[61-63].**

Environmental agents such as pesticides may cause or intensify airway inflammation that may be an indicator of chronic respiratory diseases, in addition to viruses and bacteria **[64].** Where repeated pesticide exposures can increase the risk of developing respiratory disorders **[65].** Also, toxicological studies indicate that pyrethroids may trigger lung inflammation creating oxidative stress, pulmonary fibrosis and edema which may impair the lung normal function **[66].** Moreover, cypermethrin toxicity induced inflammation and macrophage accumulation **[62].** Additionally, the presence of MDA in lung tissue may increase the chemotaxis of leucocytes. Conversely, the release of large quantities of superoxide anion and hydrogen peroxide by activated inflammatory cells in the pulmonary alveoli is the cause of MDA elevation, thus perpetuating inflammation **[60].**

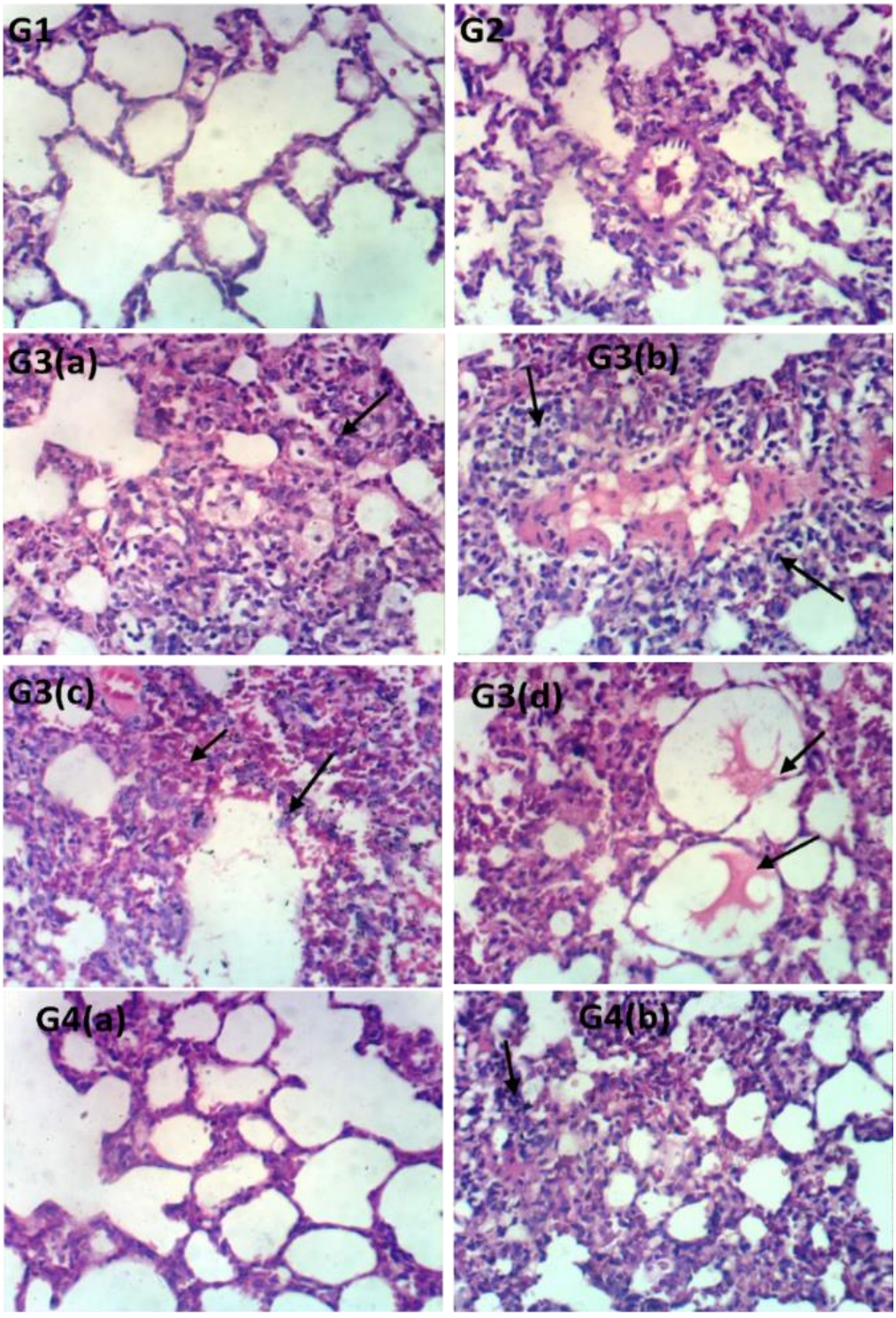

Fig.1: Photomicrograph of rat lung. G1 (negative control); G2 (positive control, 250 mg/kg bw MOLE) showing no histopathological changes; G3 (26.15 mg/kg bw CYP) showing (a) interstitial pneumonia; (b) marked perivasculitis; (c) focal pulmonary hemorrhage and haemosidrosis and (d) pulmonary edema and G4 (26.15 mg/kg bw CYP + 250 mg/kg bw MOLE) showing (a) no histopathological changes and (b) interstitial pneumonia (H & E X 400).

Animals fed MOLE with CYP (4) showed apparent normal pulmonary tissue with no histopathological changes Fig. 1 and this confirmed the protective role of MOLE and the improvement of biochemical parameters. Flavonoids significant effect in pulmonary diseases may be due to their antioxidant and anti-inflammatory effects **[67].** Quercetin and kaempferol were the flavonoid compounds in the *Moringa oleifera* leaves **[68].** Quercetin has a pneumoprotective effect via increasing the antioxidant status and reducing the production of inflammatory cytokines **[69].** Also, **Das et al. [70]** demonstrated that treatment with MOLE and its constituent quercetin inhibits high-fat diet mediated inflammation in mice. Ethanolic gradient solvent was found to be one of the strongest gradient solvents for extracting bioactive components from *Moringa oleifera* leaves and *Moringa oleifera* extracts at dosages of 150 to 300 mg/kg were found to have an important protective effect **[71]**. **Al-Omar et al. [63]** reported that ascorbic acid and α-tocopherol, vitamins found in *Moringa oleifera*, had a prophylactic effect against pyrethroid-induced damage in humans. Besides, one of several vitamins present in *Moringa oleifera* is vitamin D **[72]. Golden et al. [73]** reported that vitamin D reduces lung organic dust-induced inflammatory outcomes. So *Moringa oleifera* has bioactive and nutrient components which promote health that enhance its ability as a natural supplement to treating illness.

4. Conclusion

This study revealed that CYP oral administration produces oxidative stress, antioxidant defense system disturbance and histopathological changes in the lung tissue of rats. While, treatment of CYP- exposed rats with *Moringa oleifera* leaves extract ameliorated all the deleterious effects induced by CYP. Thus, *Moringa oleifera* may be a successful candidate for the treatment of toxicity due to CYP.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgment

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