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THE POSSIBLE PROTECTIVE EFFECT OF *MORINGA OLEIFERA* LEAF ON AMIDARONE INDUCED PULMONARY ALVEOLAR TOXICITY IN RATS; HISTOLOGICAL STUDY

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Abstract:

Background: Moringa oleifera (Lam) has gained popularity especially in recent times due to several publications reporting various nutritional and health benefits of the plant. It has many antioxidant and anti-inflammatory properties. Conversely, Amiodarone is a chemotherapeutic drug, which has been associated with many side effects involving different organs, particularly pulmonary fibrosis. Aim of the work: The aim of this study was to investigate the protective role of Moringa oleifera *against the toxic effect of amiodarone on rat pulmonary alveoli by histopathological examination. Materials and Methods: A total of 40 adult male Sprague-Dawley rats were used for the study and were randomly distributed into four groups. Group I was given normal saline (5 ml/day) for one month, group II was given Moringa oleifera leaf powder (50 mg/rat) via oral gavage daily with laboratory diet and water for one month, group III was given amidarone (30 mg/rat) via oral gavage daily for one month, and group IV was given amidarone concomitant with moringa oleifera. Results: administration of amidarone induced alteration in the pulmonary alveoli as evident by proliferation and abnormal vacuolation of type II pneumocyte, numerous foamy macrophages, intra-alveolar cellular debris and exfoliated cells, thickening of inter-alveolar septa due to mononuclear cellular infiltration, congestion of the blood vessels associated with increased collagen deposition. Concomitant administration of Moringa oleifera leaves ameliorate most of these changes. Conclusions: It could be concluded from our study that Moringa oleifera (Lam) administration could ameliorate the alveolar – toxic effect of amiodarone to the lung.*

Keywords: *Moringa oleifera, Amidarone, Pulmonary alveoli, Lung, Histopathology*

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INTRODUCTION:

Pulmonary fibrosis is a fatal lung disorder with a progressive nature and high mortality rate [1, 2]. A large number of drugs are associated with chronic pulmonary fibrosis, with or without a preceding as acute pneumonitis [3]. Amiodarone (AM) is commonly used chemotherapeutic agents used for treating heart dysrhythmias. However, it was associated with many side effects involving different organs, with the most severe adverse effect is pulmonary fibrosis [4, 5]. Interstitial pneumonitis was the most common presentation of amiodarone-induced pulmonary toxicity (AIPT) [6]. The exact mechanism by which amiodarone produced its side effects is not well elucidated, although some studies presumed the possibility of cytotoxicity and hypersensitivity [7]. Amiodarone-induced pulmonary toxicity was presented microscopically, as increased phospholipids within the lung cells, accumulation of fluids, increased thickness of alveoli wall with inflammatory cells infiltrates, and pulmonary fibrosis. Therefore, amiodarone pulmonary induced fibrosis models were introduced in scientific research [8-10].

Moringa oleifera (Lam) is an umbrella shaped tree, with many medical and nutritional uses [11-13]. All parts of the tree are useful but leaves are the most commonly used part due to its strong antioxidant value [12]. Moringa oleifera (Lam) considered as a rich source of many compounds such as β-carotene, vitamins A, B, C, and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals, flavonoids, ascorbic acid, iron, calcium and potassium and various phenolic compounds [13, 14]. The rich chemical composition of this plant compared to other plants makes it a miracle plant that is widely used in many food industries [13, 15]. Chemical extracts of this plant were reported for its beneficial effects, such as, radioprotective, cardioprotective, antioxidant, antitumor, antiepileptic, antidiuretic, and anti-inflammatory effects [16-20].

The pathogenesis of pulmonary injury and lung toxicity has been reported to be caused by oxidative stresses [21-23]. Amiodarone intake has been correlated with lung toxicity and injury due to many reasons. For example, its interstitial alveolar inflammation [24, 25], direct toxic effect of on pneumocytes cellular membranes [26], increased free radicals generation or might be through its metabolism into to an aryl radical (26). Furthermore, an immune mediated effect was suggested through increasing macrophages present in lungs to produce more free radicals [27, 28]. Interestingly, amiodarone and diclofenac treatments for induced edema in rats resulted in modulation of inflammatory and oxidation status in favor of healing by altering proinflammatory and antioxidant markers such as, increasing glutathione, decreasing peroxidases and increased myeloperoxidase [29]. In a rat model of amiodarone induced lung fibrosis, it has been reported that amiodarone interferes with cellular mechanisms that lead to free radical production which increased certain markers in lungs such as; lactate dehydrogenase (LDH), total protein and myeloperoxidase (MPO) [30].

The aim of this study was to investigate the protective role of Moringa *oleifera* against the toxic effect of amiodarone on rat pulmonary alveoli.

MATERIALS AND METHODS:

Chemicals and Plant Material: Amiodarone (in the form of cordarone 200 mg tablets, Sanofi Pharmaceuticals Company, France). Dried Moringa Oleifera (Lam) leaves were obtained from a local herbal market.

Study Design: This study was approved by the Scientific and Ethics Committee of the Faculty of Medicine in Mutah University. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Forty adult Sprague-Dawley rats (150-200 gm) were housed in cages under standard laboratory conditions with dark and light cycle. All rats were permitted free access to standard diet and water *ad libitum*. Rats were randomly divided into four groups, 10 animals each. Group I: rats served as a control group, received standard diet, free access to water with no treatment throughout the study period. Group II: rat served as a positive control group, rats received Moringa oleifera leaves 50 mg/rat administered via oral gavage daily via gastric tube for a one month. Group III: rats served as experimental group, rats received Amiodarone in a daily dose of 30 mg/kg body weight dissolved in 3 ml of saline for one month based on the previously identified toxic dose *in vivo* via oral gavages [31]. Group IV: rats served as protected group were rats received amiodarone concomitant with Moringa oleifera leaves by gavage for one month. At the end of the experiment (one month), all rats were anesthetized using ether anesthesia before being sacrificed by cervical dislocation. Lungs were removed for histopathological examinations.

Histopathology: The left lobe of the lung from all animals were excised and cut into two specimens. The specimens were subjected to light and electron microscopic studies: The first specimen was immediately removed after scarification and immediately fixed in 10% formalin and processed to get 5 µm thick paraffin sections. These sections were stained with Haematoxylin & Eosin and trichrome

stains for routine histological examination. The second specimen fixed in 3% phosphate buffered glutaraldehyde for 2 hours at 4ºC, and further processed for examination and photography by transmission electron microscope.

RESULTS:

Light Microscopic findings**:** H&E and Trichrome stained sections of the control group (group I and II) revealed a normal histology of the lung tissue; alveoli are lined by pneumocytes type I and type II. Type I are squamous shape while type II appears rounded shape (Figure 1 a). Interalveolar septum appears thin with normal organization of collagen fibers (Figure 1 b). Lung tissues of rats that administered amiodarone for one month (group III) showed distorted alveoli with proliferation and vacuolation of its lining epithelium, intra-alveolar cellular debris and thickening of inter-alveolar septa with cellular infiltration and acidophilic vacuolated material (Figure 2) and increased collagen fibers (Figure 2). Concomitant administration of Moringa olifera leaves with amiodarone (group IV) revealed marked decrease in histological changes in alveoli seen in amiodarone treated group, however, mild increase in the inter-alveolar septum was seen due to slight cellular infiltration associated, slight congestion of blood vessels and slight increase in collagen fibers (Figure 3).

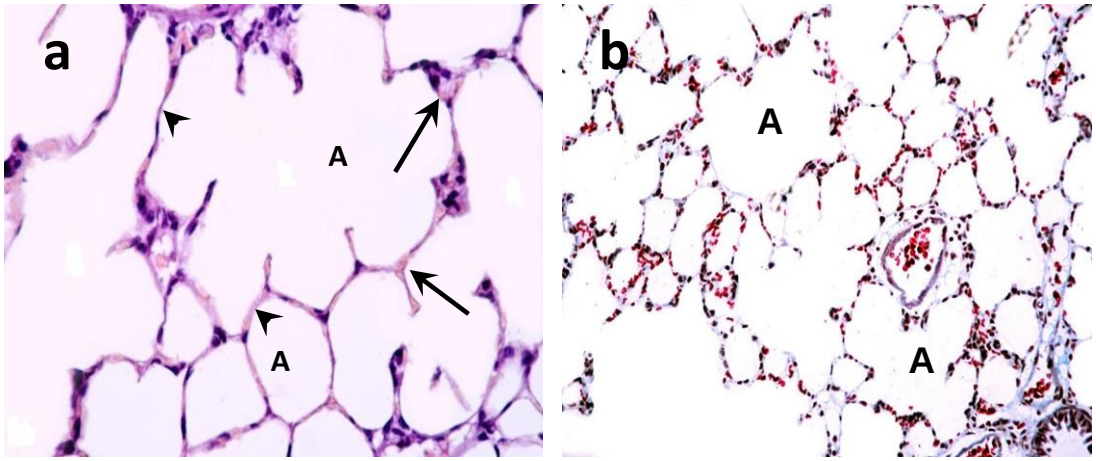

Figure 1: Photomicrograph of groups I and II (controls). (a) Showing normal structure of lung alveoli (A) separated by very thin interalveolar septa. The alveoli are lined by squamous type I pneumocyte (arrowheads) and cuboidal type II pneumocyte (arrowed). (b) Showing, normal distribution of collagen fibers in the control group. (a: H&E stain, X400; b: Gomori's trichrome stain, X200).

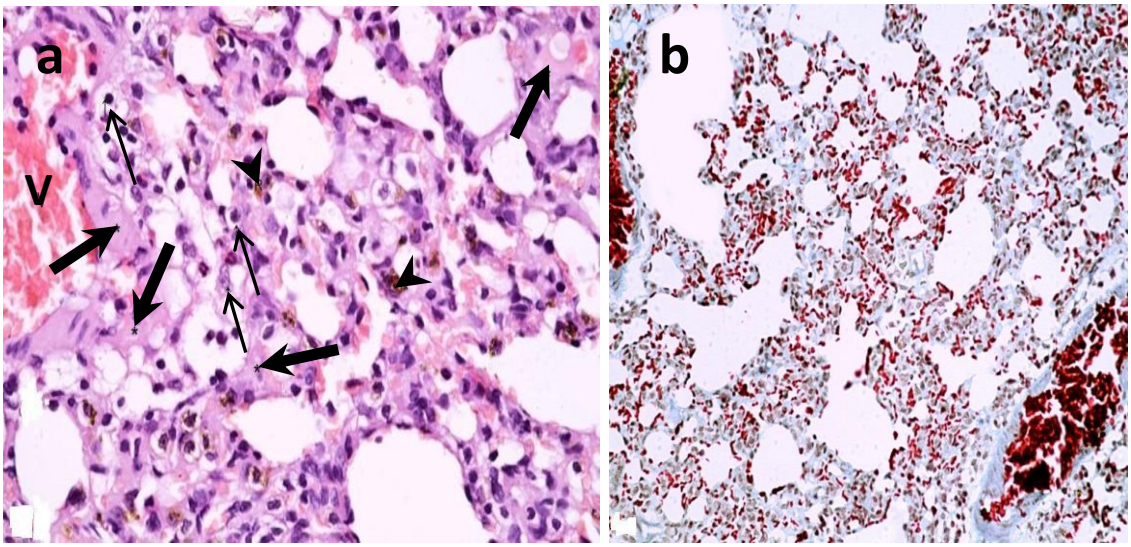

Figure 2: Photomicrograph of group III (received amiodarone). (a) Showing collapsed alveoli separated by very thick interalveolar septa. Note mononuclear cellular infiltration, congestion of the blood vessels (V), acidophilic hyaline material (wide arrow) numerous foamy macrophages (arrowed) and extravasated RBCs (arrowheads). (b) Showing thick interalveolar septa, congestion of the blood vessels and increase in collagen fibers. (a: H&E stain, X100; b: Gomori's trichrome stain, X200).

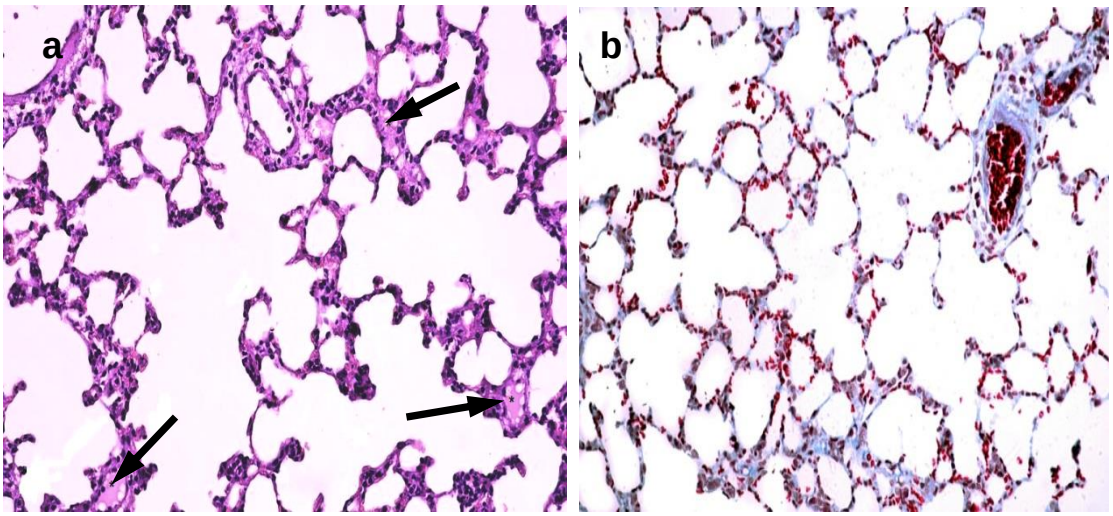

Figure 3: Photomicrograph of group IV (received amiodarone and Moringa olifera leaves). (a) Showing, preserved lung structure except very mild increase in thickness of interalveolar septa, some acidophilic hyaline material (arrowed). (b) Showing, very mild increase in interalveolar septa associated with mild increase in collagen fibers. (a: H&E stain, X100; b: Gomori's Trichrome stain, X200)

Ultrastructural findings: Transmission electron microscope for lung tissues of the control groups (group I, II) showed normal morphology of alveoli with thin walls (septa), contained few cells and fibers and lined by a squamous-shape pneumocyte cells (type I) and cuboidal-shape pneumocyte cells (type II) with its lamellar bodies and microvillous border (Figure 4). Amiodarone treated rats (group III) showed marked changes in the alveoli morphology. Alveoli appear distorted and lined mainly by vacuolated type II pneumocyte and inflammatory infiltrate predominantly vacuolated cytoplasm macrophages (foam cells). The septal wall of the alveoli was thickened due to increased collagen fibers deposition, blood vessels congestion and inflammatory cellular infiltration. Alveoli lumen contains sloughed or shed cells and cellular debris (Figure 5). On the other hand, electron microscopy for lung tissues of the protected group (group IV) showed mild changes in alveoli histological structure. Most of the alveoli were lined with almost normal type I and II pneumocytes and the septal wall was mildly thickened due to few collagen fibers and cellular infiltrations. Macrophages and septal cells count were less than group III (Figure 6).

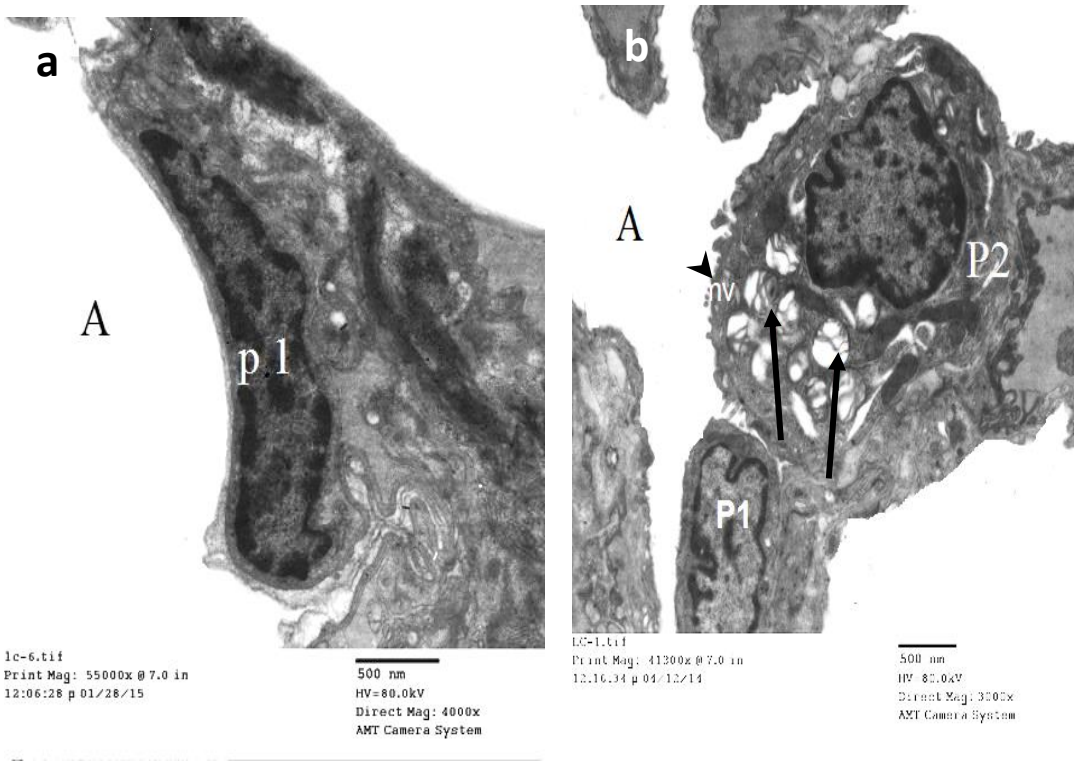

Figure 4: Electromicrograph groups I and II (controls). (a and b) Showing normal and intact alveoli (A) lined by squamous type I pneumocytes (P1) and cuboidal type II pneumocytes (P2) with characteristic lamellated structure (arrowed) and apical microvilli (arrowheads).

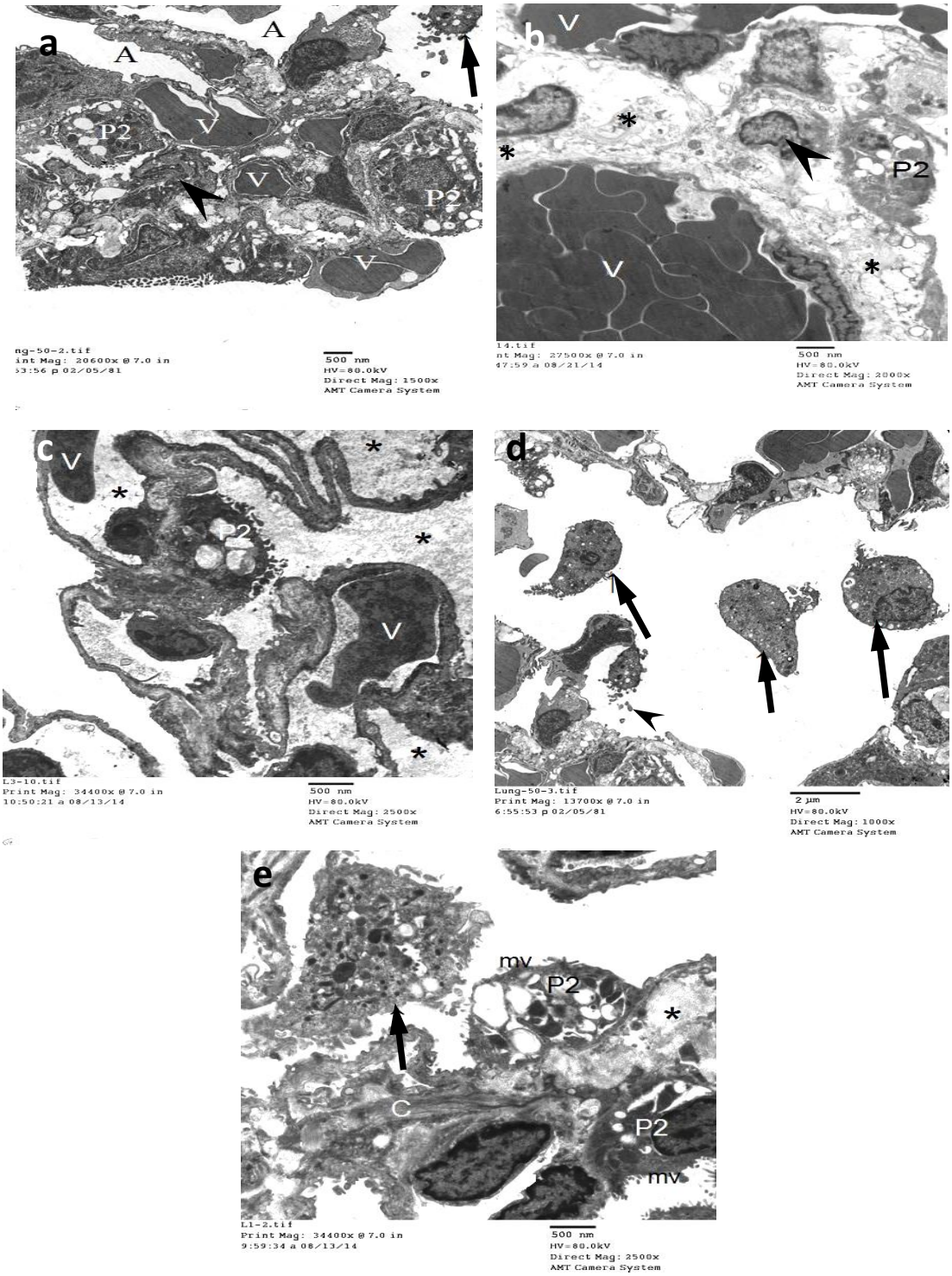

Figure 5: Electromicrograph of group III (received amiodarone). (a and b) Showing thick interalveolar septa due to mononuclear cellular infiltration (arowheads), hyaline material (*) and congestion of the blood vessels (V). Note type II pneumocyte filled with empty lamellated structure (P2) and cellular debris (arrowed). (c and d) Showing

intra-alveolar sloughed or shed cells and cellular debris (arrowheads), alveolar macrophages (arrowed). Note type II pneumocyte contain empty lamellated structure (P2), congested blood vessels (V) and hyaline material (*). (e) Showing thick interalveolar septa, hyaline material (*), alveolar macrophages (arrowed) and increased collagen fiber (C).

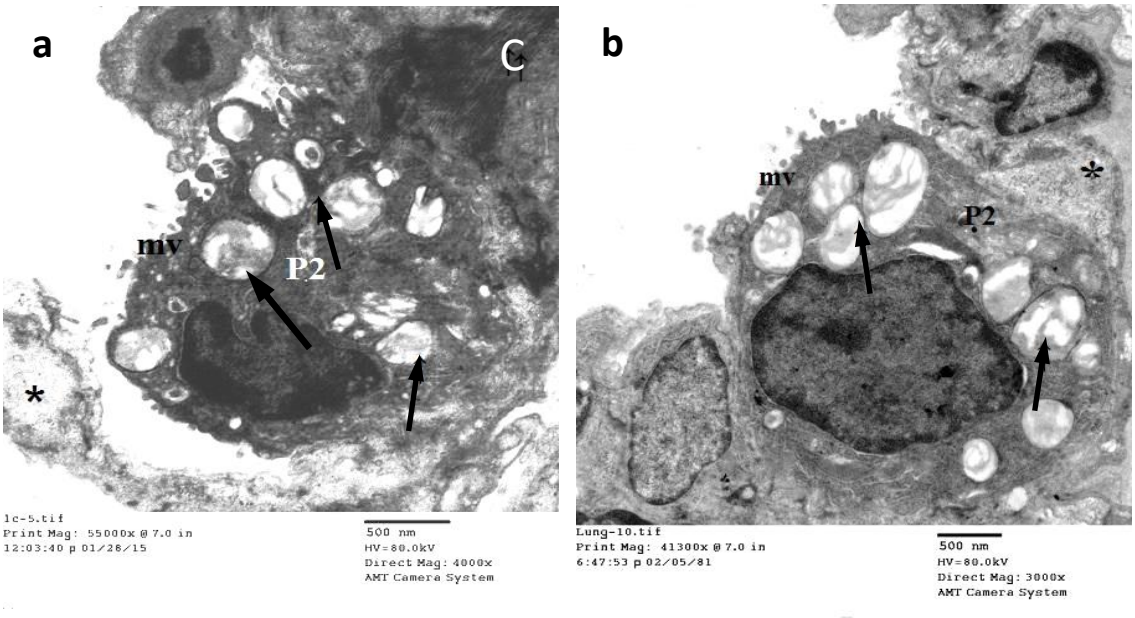

Figure 6: Electromicrograph of group IV (received amiodarone and Moringa olifera leaves). (a and b) Showing, type II pneumocyte (P2). with apical microvilli (mv) and lamellated structure (arrowed) Note mild increase in collagen fibers (C) and hyaline material (*****).

DISCUSSION:

Microscopic examination of histological sections of lung tissue revealed detrimental changes in lung tissues sections of the experimental group (III) (amiodarone-treated rats) in the form of interstitial pneumonia including abnormal alveoli, fibrosis and hemorrhage inflammatory infiltrate. These results are comparable with other studies [3, 25, 32]. These histological changes were ameliorated in rat's lung tissues harvested from protected group (IV) were rats received amiodarone concomitant with Moringa oleifera, which support a protective effect of intaking Moringa. This protective effect could be attributed to amiodarone modification of oxidative stress on rats lung cells, as explained by previous studies [16-20].

The antioxidant properties of Moringa oleifera support its use in minimizing drug toxicity by modulating the redox reactions in lung tissue [30] and liver tissue [33] by decreasing free radical release, which means increased antioxidative activity and improve the antitoxic capability of tissues in rats treated with amiodarone. The mechanism by which Moringa exerted its beneficial effect is by interfering the release of high concentrations of certain toxicity serum markers such as; serum tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β) and lactate dehydrogenase (LDH) activity, total protein and myeloperoxidase decrease in (MPO) [30, 34]. The anti-inflammatory effects (slight septal wall thickness and slight inflammatory cell infiltrates) of Moringa that were revealed histologically in lung tissue, are in agreement with a previous study reported that Moringa extracts intake improve lung tissue by decreasing lead toxicity effects through the increase in LDH enzyme level, and improving tissue histology of lung cells and alveoli [35]. Furthermore, anti-inflammatory effect of Moringa is mediated by inhibition of certain proinflammatory cytokines, which is supported by a previous *in vitro* study that reported anti-inflammatory effect of Moringa by modulating TNF-α and TGF-β factors [36]. Anti-fibrosis effect of Moringa, shown in lung tissue sections above, is supported by a previous studies that reported that Moringa modulates the stimulation of a marker of liver stellate cell, called alpha smooth muscle actin, which is important in liver fibrosis [37]. On the other hand, this anti-fibrosis effect could be via modulating

TGF-β activity [38], which affects the components of fibrosis process, such as collagen and fibronectin.

Conclusion: Amiodarone cause observable damage to lung tissue, amiodarone-induced intoxicated histological changes support the use as a model of lung fibrosis. Moringa oleifera (Lam) administration could ameliorate the alveolar – toxic effect of amiodarone to the lung. Moringa oleifera has less adverse effects with lower economic burden than commercial drugs. Hence, it could be used as adjuvant therapy in amiodarone induced alveolar - toxicity.

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