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Research Article

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Chronic Abuse of Tramadol Induces Traumatic Pulmonary Histopathological Lesions in Rats – An Ultrastructural & Immunocytochemical Exploration

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Abstract

Tramadol is an opioid extensively used to treat moderate to severe pain; however, prolonged therapy is associated with several tissues damage. Chronic use of tramadol was linked to increased hospitalizations due to pulmonary and respiratory complications. Limited literature has described the effects of tramadol on the respiratory system, so we tried to investigate these actions and elucidate the underlying mechanisms. Rats received tramadol hydrochloride (20, 40 & 80 mg/kg body weight) orally (Or) and injected intraperitonealy (ip) for 3 successive months and results were studied on the ultrastructural and immunohistochemical levels. In spite of 20 mg/kg of tramadol did not exhibit marked neither histopathological nor immunohistochemicsl lesions in rat lung tissues, both 40 and 80 mg/kg of tramadol reveales moderate to severe extensive cellular damages either adminidtered orally or ip injected. Endothelial cells appeared swollen, cells tend to be apoptotic with mitochondrial disruption. In addition, r-ER & s-ER showed markable damage, other results demonstrated marked histopathological alterations in pulmonary tissues after exposure to chronic tramadol abuse. Tramadol caused alveolar epithelial edeme, granular cytoplasm and degenerated picnotic nuclei. Atipia cells were also observed, with lysosomal disruption and lymphocytes and monocytes infiltration Finally, these results highlight the risks of tramadol abuse on the respiratory system either abused as an opioid or for medical uses.

Keywords: Tramadol - Chronic dose - Rats Lungs – Ultrastructure – Immunocytochemical

I. Introduction

Tramadol is a synthetic opioid analgesic commonly prescribed for moderate to severe pain, usual doses being up to 200 mg/day (Gana *et al*., 2006; McKeon *et al*., 2011). The maximum allowed daily dose is 400 mg. Tramadol provides analgesia through three mechanisms: 1. mu-opioid binding (through its metabolite O-desmethyltramadol), 2- serotonin reuptake inhibition (through (+) -tramadol) and, 3. nor-epinephrine reuptake inhibition (through (-)-tramadol).O-desmethyltramadol (which is formed from tramadol through O-demethylation catalyzed by CYP2D6) which is responsible for the opiate-type effects of tramadol (Reeves & Burke, 2008; Sansone & Sansone, 2009).

Tramadol abuse is becoming more and more popular among teens in most countries. Being an opioid, tramadol carries all possible risks known from other opiates (Cicero *et al*., 2005; Adams *et al*., 2006). Side effects include dizziness, headache, somnolence, nausea, constipation, sweating, pruritus, and central nervous system stimulation (Reig, 2002; Kabel & van Puijenbroek, 2005).

Tramadol causes respiratory depression, although usually weaker than that seen with other opiates and opioids (Senay *et al*., 2003). Tramadol can cause psychological and physical addiction similar to that of other opiates and the analgesic efficacy of tramadol can further be improved by combination with a non-opioid analgesic (Ripamontic *et al*., 2004; Lanier *et al*., 2010).

On the otherhand, Tramadol is a centrally acting analgesic with a multimode of action. It acts on serotonergic and noradrenergic nociception, while its metabolite (O-desmethyltramadol) acts on the μ-opioid receptor. Its analgesic potency is claimed to be about one tenth that of morphine. Tramadol is used to treat both acute and chronic pain of moderate to severe intensity Barbera *et al*., (2013).

Tramadol is used worldwide and is listed in many medical guidelines for pain treatment. It is mentioned as a step-2 analgesic in the WHO guidelines for cancer pain relief. Tramadol is also listed on several national essential medicines lists. It is, however, not listed on the WHO Model List of Essential Medicines (April 2013).

Tramadol is used to treat moderate to severe pain (most countries) or moderate to moderately severe pain. It has a wide range of applications in both acute (e.g., postoperative trauma), chronic (cancer and non-cancer) pain and is worldwide available as a medicine (Barkin, 2008, Grond, 2004, Keating, 2006, Leppert, 2005 and Scott, 2000). Tramadol is listed in many medical guidelines for pain treatment. It is mentioned as a step-2 analgesic in the WHO guidelines for cancer pain relief. In chronic non-cancer pain, tramadol may be appropriate when non-opioid analgesics are ineffective or contraindicated.

In general, the analgesic effect of tramadol immunotherapy is modest. In meta-analyses, tramadol showed no significant effect on pain relief in chronic nonspecific low back pain (Chung, 2013), some effect (low quality evidence) in chronic low back pain , and a modest effect (fair evidence) in chronic osteoarthritis (Cepeda, 2006), (Manchikanti, 2011) and (McCarberg, 2013).

Tramadol immunotherapy does not usually provide adequate analgesia. In chronic non-cancer pain, there is little evidence for the use of tramadol for more than three months. Tramadol is considered to be a relatively safe analgesic. The main adverse reactions to tramadol therapy are nausea, dizziness, and vomiting, particularly at the start of the therapy .At therapeutic doses, tramadol does not cause clinically relevant respiratory depression. Tramadol is contra-indicated, however, in patients with diminished respiratory function. Tramadol is generally considered as a medicinal drug with a low potential for dependence relative to morphine. Nevertheless, tramadol dependence may occur when used for prolonged periods of time (more than several weeks to months) Chung *et al*., (2013).

There are growing evidences of abuse of tramadol in some African and West Asian countries considering large seizures of such preparations in North and West Africa. Abuse of tramadol is reported by Egypt, Gaza, Jordan, Lebanon, Libya, Mauritius, Saudi Arabia and Togo. Expert Committee on Drug Dependence (2014). The nature of the immune-modulatory activity of opioids has been the subject of a great deal of research for the last years. There is increasing evidence that effects of opioids on the immune response are mediated at several levels. Modulation of the inflammatory response appears to be a target of these compounds, including effects on phagocytic activity, as well as, the response of cells to various chemo-attractant molecules. Moreover, the findings from several laboratories have demonstrated the impact of opioid treatment on antibody responses, and the molecular basis for this effect is likely due, at least in part, to the modulation of both cytokine and cytokine receptor expression (McCarthy *et al*., 2001; Liu *et al*., 2008).

II. Aim of the work:

In this study, we are aiming to investigate the impacts of tramadol abuse in a chronic administration modality of rats pulmonary functions. In addition, we tried to check all possible traumatic histopathological effects of tramadol toxicity on lungs of rats. This was to notify the severity of side effects of the chronic therapeutic doses of tramadol and its abuse effects as a narcotic drug among drug-addicts in Egypt as well.

III. Materials and Methods

III. 1. Drugs:

a. Tramadol (Tramadol Hydrochloride, 100mg/2ml /ampoules) was obtained as a solution from ADWIA Co.S.A.E; 10th of Ramadan City - Egypt.

b. Tramadol Hydrochloride Bp-225mg was used as tablet.

c. All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

III. 2. Experimental Animals:

A total of 40 male adult rats (*Rattus norvegicus*) weighing approximately 180-200g were used in the present study (Animal house of the Faculty of Agriculture Suppliers, Minia University, Minia, Egypt). Animals were housed five per cage under normal room temprature and a 12: 12 light/dark cycle. Water and food were available *ad libitum*. All procedures were in accordance with the approval of Institutional Animal Ethics and with the recommendations of the proper care and use of experimental animals that followed the guidelines of the national law for the use of laboratory animals in research.

Table [1]: Tramadol Administration

Ser.	Group	Intra-peritoneal (Ip) Injection		Oral (Or) Administration via Gastric Gavage		Duration
		No. of rats	Dose Tramadol HCL 100 mg/2 ml Ampoules in Saline Solution	No. of rats	Dose Tramadol HCL 100 mg/Tablet Suspended in Saline Solution	
1	Group I	5	20 mg (T HCL 1 ml/ twice/week)	5	20 mg (T HCL 1 ml/ twice/week)	3 Mons
2	Group II	5	40 mg (T HCL 1 ml/ twice/week)	5	40 mg (T HCL 1 ml/ twice/week)	3 Mons
3	Group III	5	80 mg (T HCL 1 ml/ twice/week)	5	80 mg (T HCL 1 ml/ twice/week)	3 Mons
4	Group IV	5	Control (1 ml/twice/week Sal.Sol.)	5	Control (1 ml/twice/week Sal. Sol.)	3 Mons

Experimental Design: All experimental animals received the calculated doses for 3 months.

A) Intra-peritoneal administration:

1. **Group I Ip:** Five rats were injected intra-peritoneally with 20 mg/kg.b.wt. of tramadol Hcl twice a week.
2. **Group II Ip**: Five rats were injected intra-peritoneally with 40 mg/kg.b.wt. of tramadol Hcl twice a week.
3. **Group III Ip**: Five rats were injected intra-peritoneally with 80 mg/kg.b.wt. of tramadol Hcl twice a week.
4. **Group IV Ip:** Five rats were served as control group and injected intra-peritoneally with saline solution twice a week.

B) Oral aministration:

1. **Group I Or:** Five rats were administered 20 mg/kg.b.wt. oral dose of tramadol Hcl suspended in saline solution twice a week.
2. **Group II Or**: Five rats were administered 40 mg/kg.b.wt. oral dose of tramadol Hcl suspended in saline solution twice a week.
3. **Group III Or**: Five rats were administered 80 mg/kg.b.wt. oral dose of tramadol Hcl suspended in saline solution twice a week.
4. **Group IV Or:** Five rats were served as control group and administered oral dose of saline solution twice week using a gastric gavage.

III. 4. Ultrastructural & Immuno-histochemical Studies:

A- Ultra-structural Studies:

1-Primary fixation:

For transmission electron microscopy, small pieces of lungs were fixed in 2.5% glutaraldehyde buffered with .1M cacodylate (pH7.2) at room temperature for 2 hours. Fixation continued at 4°C for slows down autolysis processes and reduce tissue shrinkage.

2-Post fixation:

Specimens were washed three times with the same buffer and post fixed in phosphate-buffered 1% osmium tetroxide for 2 hours at room temperature then dehydrated in ascending grades of ethanol.

B- Immuno-histochemical Studies:

Sections were taken on positive slides and immunostained using avidin-biotin technique Zarnescu & Brehar 2008

IV. Results

A- Electron microscopic structure of lungs

1) Control group:

Lung consists of an alveolar region (parenchyma) and non-parenchyma conducting airways with larger vessels. The airways branch in irregular dichotomy into the lung together with the arteries, thus defining broncho-arterial units “inside-out” (from the hilum to the periphery). In the most distal branching generations, alveoli are connected to the airways. Clusters of alveoli are arranged in functional units termed acini. An acinus is a blind-ending parenchymal unit beginning with a transitional (i.e. the first generation of an alveolated) bronchiole. Within an acinus, all airways (alveolar ducts and respiratory bronchioles) have alveoli attached to their walls and. Actually, the “wall” of alveolar ducts consists of a network of alveolar openings. It separates the air compartment (alveolar airspace) from the blood compartment (capillary lumen). (Fig. 1).

2) Treated groups:

Lungs of rats that were treated with 20 mg/kg. of tramadol orally (**group I Or**) showed most likely ultrastructural achitectures. Rats in **group II Or**, that were treated with 40 mg/kg. of tramadol orally, lungs exhibited swollen endothelial cells and the extensions of the swollen cells are sheltering the red blood cell. The red blood cells appeared flattend and irregular in shape. Apoptotic pulmonary cells with apoptotic nucleus scattered in the lungs septa and increased vaculated cytoplasm were also noticed. Lungs septa appeared proliferated and lined with accumulation of collagenous fibrous (Fig 2).

Other electron microscopic findings were noticed in rat lungs of **group II Or**, at which animals were treated with 40 mg/kg of tramadol orally as an infiltration of natural killer cells with granular cytoplas, necrotic lungs cells with nuclear disappear, apoptotic cells with picnotic nuclei & proliferation of smooth endoplasmic reticulum and pus cells (Fig.6)

In **group III Or**, rats were treated with 80 mg/kg. of tramadol orally, most lungs cells appeared to be apoptotic. Nuclear membrane ruptured, cells contents exudation and nucleus appeared with irregular shape. Necrosis appeared with degeneration of nucleus and cells content. Some mitochondria were swollen and disrupted, other mitochondria appeard with disintegrated cristae and the osmiophilic lamellar bodies were fused or disappeared, while others damaged. The alveolar walls appeared thickened and proliferated with marked fibrosis (Fig. 3).

Moreover, in **group III Or**, rat lungs cells exhibited an atibia appearance together with with autophagosomes & autolysosomes as well. Fibroblasts, apoptotic type I cells with proliferation of the rough endoplasmic reticulum were noticed and macrophages appeared with pseudopodia and necrosis (Fig .7).

Intraperiteanally njected rats with 20 mg/kg of tramadol (**Group I Ip**) showed almost normal lungs appearance, while those of **group II Ip** that were injected with 40 mg/kg of tramadol revealed degenerated lungs cell membrane with exudation of cellular content. The nucleus appeared with pores (beginning of degenerated). Necrosis appeared at degenerated cells. Proliferationss and degeneration of rough endoplasmic reticulum were notices as well. Moreover, smooth endoplasmic reticulum appeared scattered together with degenerated mitochondria. More increase of the vacuolated cytoplasm due to enhanced cell degeneration (Fig. 4) was mostly common.

Also in **group III Ip,** rats lungs that were treated with 40 mg/kg of tramadol ip injected showed activated macrophages with lysosomes and plasma cells with autolysosomes. Some cytoplasmic electron-dense deposits and several multi-vesicular bodies with disorganized phospholipid membranes and amorphous contents appeared with infiltration of lymphocytes & monocytes and heterochromatin on the peripheral nuclear membrane, collagen fibrous and elastic fibrous increased (Fig. 8).

However, lungs of rats treated with 80 mg/kg of tramadol (**group III Ip**) revealed clear thickened alveolar septa by replacement of residual cells with fibrosis. There was substantial alveolar epithelial edema and the alveolar epithelial cells showed slight swelling and degeneration. Apoptotic nucleus appeared with vacuolated cytoplasm and increased necrotic cells. (Fig. 5).

Also, rats of **group III Ip** showed also activated macrophages with lysosomes in their pulmonary cells. Mitochondria in macrophage lungs cells appeared swollen with disrupted or disintegrated cristae and the osmiophilic lamellar bodies were fused or disappeared. Few multi vesicular bodies insertions & some cytoplasmic electron-dense deposits were noticed. Several multi-vesicular bodies with disorganized phospholipid membranes and amorphous content, necrosis and apoptotic cells were investigated.(Fig. 9).

3). Immunohistochemistry staining of lungs:

A. Control group

Immunohistochemical (IHC) method was applied to visualize the distribution and amount of molecules in lung tissues using specific antigen-antibody reaction. Lungs section of control animals revealed normal lungs cells alvuoli with no detectable immunostaining for caspase-3 (Fig. 10).

B. Treated groups

Lungs of rats in either **group ! Or** or **group I Ip** (that were treated with 20 mg/kg of tramadol orally and ip injected) did not exhibit detectable immunostaining for caspase-3. Animals of group II Or and group II Ip (that were treated with 40 mg/kg of tramadol orally and ip injected) showed a moderate to sever intense caspase-3 immunostaining in their lungs cells (Figs. 11 & 13). Type-1 and type-2 alveolar cells revealed strong to sever intensive cytoplasmic and nuclear caspase-3 immune-positive reaction product scattered in rats lungs cells (group III Or and group III Ip) that were treated with 80 mg/kg of tramadol orally and ip injected (Figs. 12 & 14).

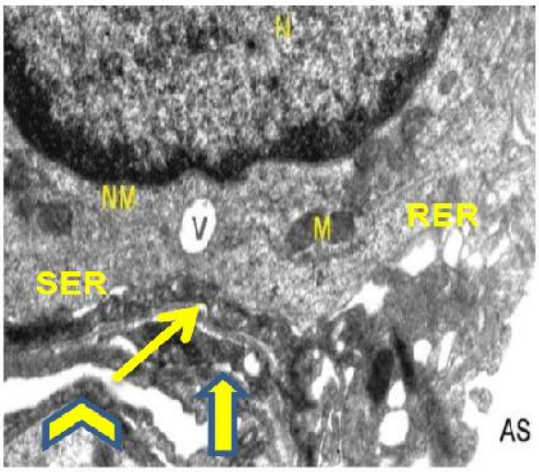

Fig (1): An electron micrograph of lungs cells: alvuolia type II of a control rat group vi showing the nucleus (N), nuclear membrane (NM), mitochondria (M), rough endoplasmic reticulum (rER), smooth endoplasmic reticulum (sER), alveoli endothelium (thin arrows), alveolar epithelium (head arrows), alveoli type I (thick arrows) and alveolar sac (AS).

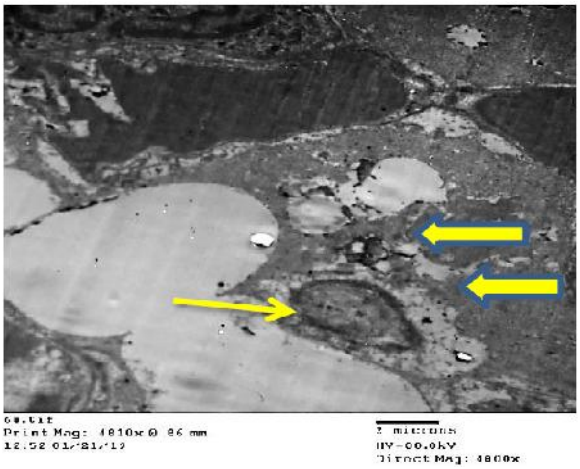

Fig (2): An electron micrograph of rat lungs section (group II, rats were treated with 40 mg/kg. of tramadol orally) showing: endothelial cells appeared swollen and extensions of the swollen cells are sheltering the red blood cell (thick arrows) and apoptotic cells with small nucleus (thin arrows).

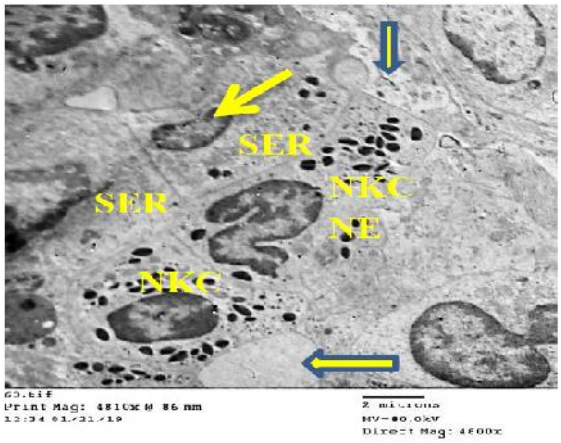

Fig (3): An electron micrograph of rat lungs section (group III, rats were treated with 40 mg/kg of tramadol orally) showing: infiltration of natural killer cells with granular cytoplasm (NKC) , necrotic lungs cells (NE) with disappeared nucleus, apoptotic cells (thin arrows) with picnotic nuclei & proliferation of smooth endoplasmic reticulum (sER) and pus cells (thick arrows).

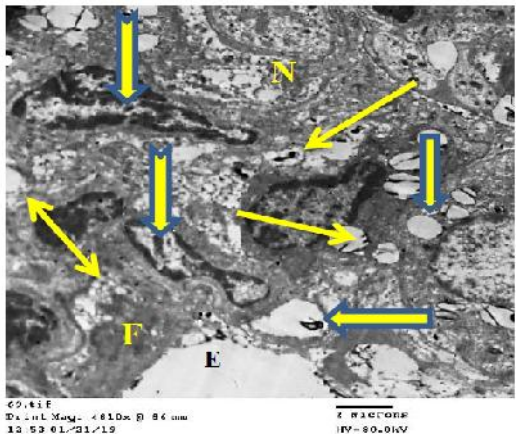

Fig (4): An electron micrograph of rat lungs section (group III, rats were treated with 80 mg/kg. of tramadol orally) showing: Apoptotic cells, apoptotic nucleus with rupture of cells membranes and exudation of cells matrix out of cells (tail arrows). Mitochondria swollen & disrupted, other mitochondria appear with disintegrated cristae and the osmiophilic lamellar bodies either fused or disappeared (thin arrows), other mitochondrial damage (thick arrows), the alveolar walls were thickened (double head arrows), there was substantial alveolar epithelial edema (E) and the alveolar epithelial cells showed slight swelling and degeneration, vacuolated cytoplasm (V), necrotic cells with execration of cells content (N), elastic fibres (F).

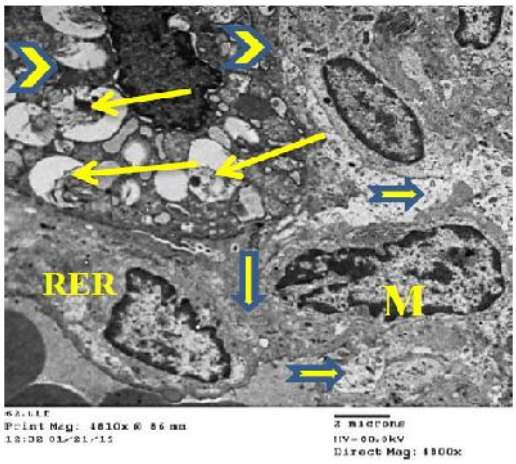

Fig (5): An electron micrograph of rat lungs section (group III, rats were treated with 80 mg/kg of tramadol orally) showing: Atibia cells with autophagosomes (head arrows) & autolysosomes (thin arrows). Fibroblast (thick arrows), apoptotic type I lungs cells with proliferation rough endoplasmic reticulum cells (rER), macrophage appear with pseudopodia (MA) and necrosis (tail arrows).

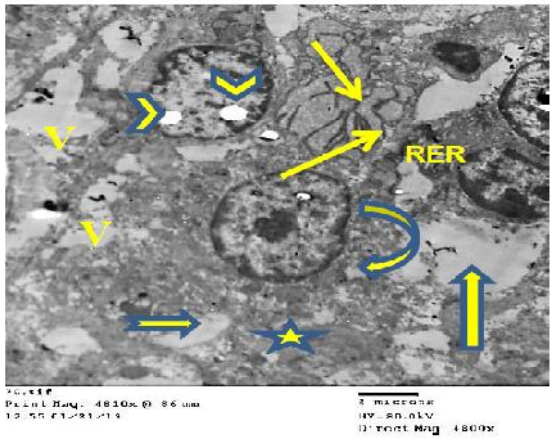

Fig (6): An electron micrograph of rat lungs section (group II, rats were treated with 40 mg/kg. of tramadol injected) showing : elastic fibres (thin arrows), vacuolated cytoplasm (V), necrotic cells with execrated of cells content (thick arrows), mitochondrial damage (tail arrows) , apoptotic vacuolated nucleus (head arrows), collagen fibres (star), hyper trough of rough endoplasmic reticulum (rER), basement membrane degeneration and exudation of cells substance out of cells (curved arrows).

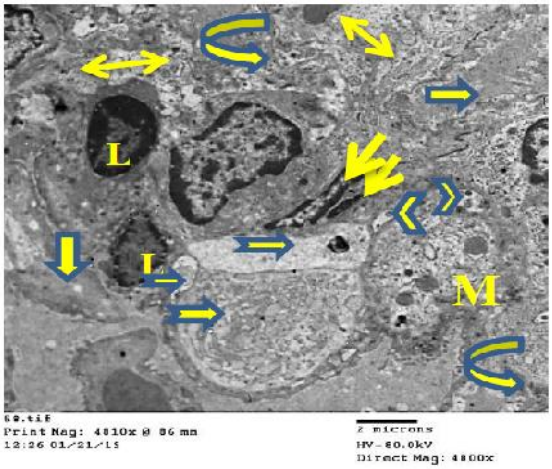

Fig (7): An electron micrograph of rat lungs section (group III, rats were treated with 40 mg/kg of tramadol injected) showing: activated macrophages (M) with lysosomes (head arrows), plasma cells with autolysosomes (tail arrows). Some cytoplasmic electron-dense deposits (thin arrows) and several multi vesicular bodies (thick arrows) with disorganized phospholipid membranes and amorphous content inserted, infiltration of lymphocytes (L) & monocytes (MO) with heterochromatin on the peripheral nuclear membrane, collagen fibres (double head arrows) and elastic fibres(curved arrows) increased .

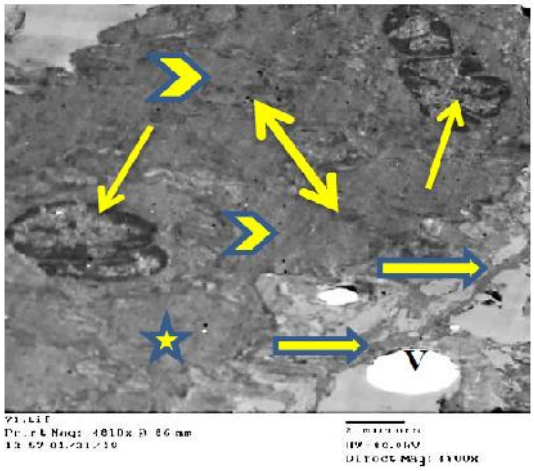

Fig (8): An electron micrograph of rat lungs section (group III, rats were treated with 80 mg/kg. of tramadol injected) showing: the alveolar septa were thickened (double head arrows), there was substantial alveolar epithelial edema (E) and the alveolar epithelial cells showed slight swelling and degeneration, apoptotic nucleus (thin arrows), vacuolated cytoplasm (V), necrotic cells with excreted cells contents (stars), fibrosis (thick arrows) and secretory substance found between alveolar cells (head arrows).

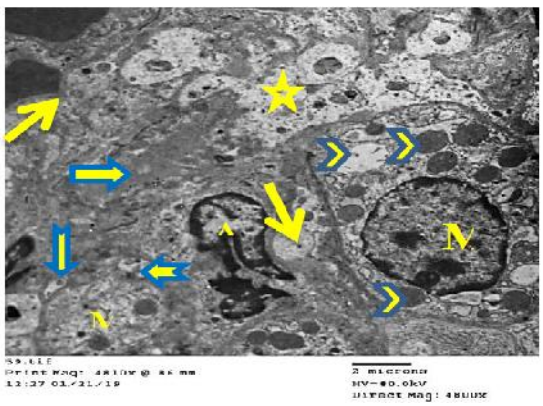

Fig (9): An electron micrograph of rat lungs section (group Ill, rats were treated with 80 mg/kg of tramadol injected) showing: Activated macrophage with lysosomes (tail arrows), the mitochondria in macrophage lungs cells were swollen with disrupted with disintegrated cristae and the osmiophilic lamellar bodies were fused or disappeared (head arrows), granular accumulation can be visualized in the alveolar space (thin arrows), few multi vesicular bodies inserted & some cytoplasmic electron-dense deposits and several multi vesicular bodies (thick arrows) with disorganized phospholipid membranes and amorphous content inserted, necrotic (stars) and apoptotic cells (AP).

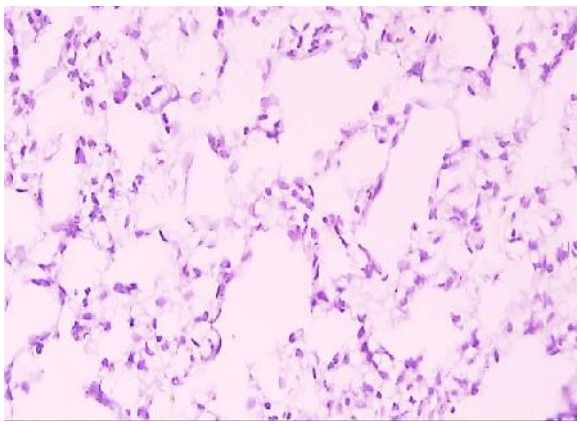

Fig (10): A photomicrograph of rat lungs section (control group) showing: Caspase 3-negative reaction in lungs cells. (Caspase 3 immune staining X 400).

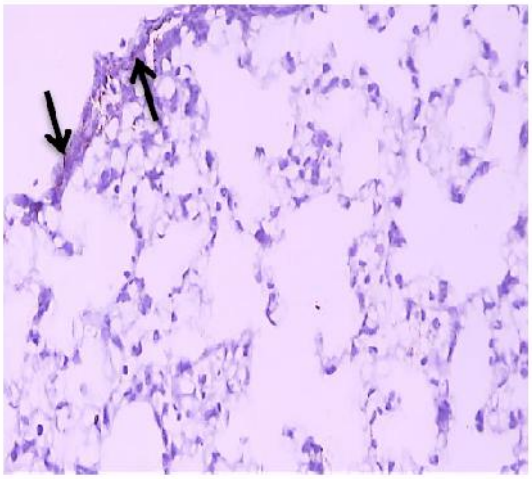

Fig (11): A photomicrograph of rat lungs section (group II, rats were treated with 40 mg/kg of tramadol orally) showing: moderate Caspase 3-positive reaction in lungs cells. (Caspase 3 immune staining X 400).

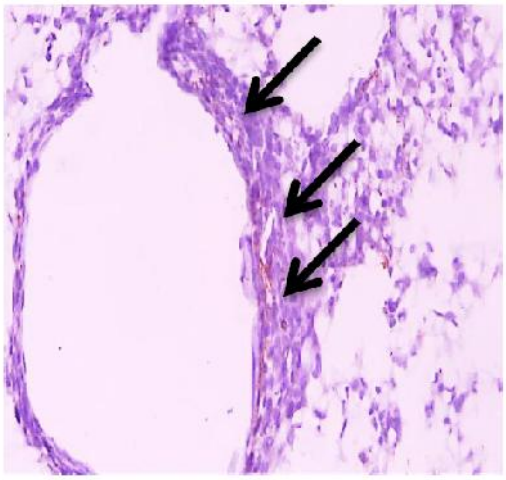

Fig (12): A photomicrograph of rat lungs section (group III, rats were treated with 80 mg/kg of tramadol orally) showing: intensive Caspase 3-positive reaction in lungs cells. (arrows) (Caspase 3 immune staining X 400).

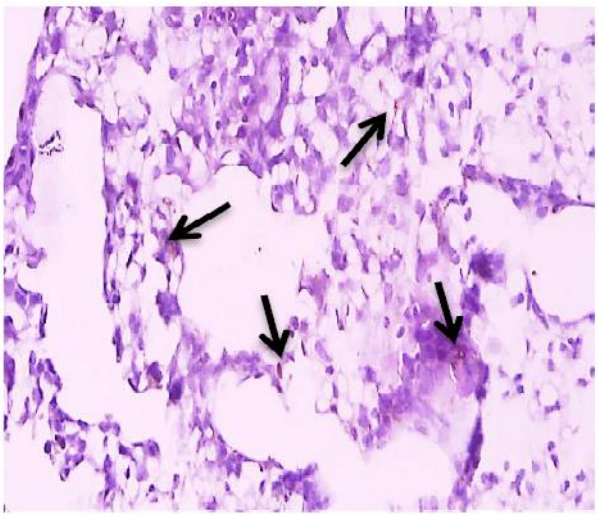

Fig (13): A photomicrograph of rat lungs section (group II, rats were treated with 40 mg/kg of tramadol injected) showing: moderate Caspase 3-positive reaction in lungs cells. (Caspase 3 immune staining X 400).

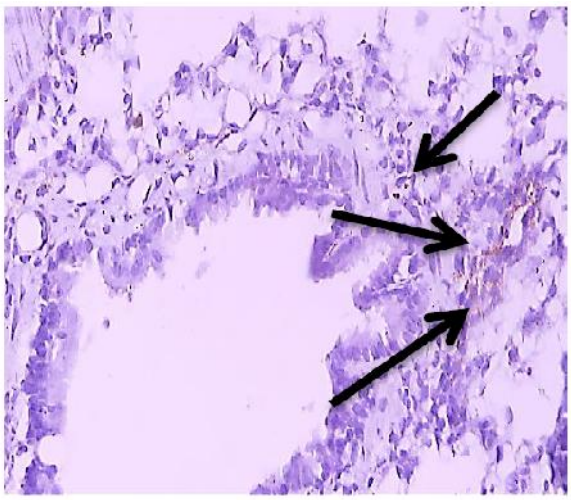

Fig (14): A photomicrograph of rat lungs section (group III, rats were treated with 80 mg/kg of tramadol injected) showing: intensive Caspase 3-positive reaction in lungs cells. (Caspase 3 immune staining X 400).

V. Discussion

1) Ultrastructural observations:

Due to that lung is the essential organ of respiration and the organ that receives the entire cardiac output and it plays an important role in host defense and regulation of circulating levels of biologically active materials by extensive surface of pulmonary vascular bed, study of the relation between tramadol toxic substance and respiratory health needs to take into account its anatomical, histological and the toxicological effects on the respiratory system. .

Acute lung injury is a common clinical illness. The current acute lung injury mortality rate is as high as 35% - 40% and reaches to 50% in acute respiratory distress syndrome Wheeler *et al*.,(2007). Acute lung injury pathogenesis is complex, and there is still much controversy to be had before we reach a definitive conclusion Villar *et al*., (2011). Increased oxidative stress has been implicated in its pathogenesis Choi *et al*., (2012). Tramadol induced acute lung injury in rat model is a classic animal model.

Recently, some researchers found that mitochondrial dysfunction plays an important role in the course of acute lung injury. Mitochondria utilize approximately 98% of total body oxygen consumption. This would maintain tissue oxygen levels by decreasing demand and protect against cell death.So mitochondrial dysfunction is the key factor to cell damage. In sepsis, mitochondrial dysfunction in vital organs can make cell organisms lack energy, causing multiple organ failure. The lung is a special organ relatively susceptible to injury. The mitochondrion is a complex and sensitive organelle. Acute lung injury can lead to abnormal mitochondrial structure and function and tends to cause abnormal mitochondria organelles or other changes in the entire cell, thereby increasing the degree of acute lung injury meanwhile, mitochondrial dysfunction is also prone to result in acute lung injury Singer (2014).

In lung tissue, treatment with tramadol can produce large radical NO, O^{-2}, and $ONOO^{-}$. The mitochondrial film, which is rich in unsaturated fatty acids, is a major free radical attack target. These lead to mitochondrial membrane swelling expansion, lipid oxidation increase, and decrease membrane fluidity. Mitochondrial ATP enzyme activity and mitochondrial ATP production were also decreased because of these. Mitochondria have intrinsic defense mechanisms to protect against ROS-induced damage through its large array of antioxidants (e.g., superoxide dismutase, glutathione, thioredoxin) Yin *et al*., (2012).

The formation of various density-graded granules in the cytoplasm of lung tissues and an increased number of vacuoles were observed both in group IIIc. These results agreed with Ji-Young *et al*., (2012) which explain the autophagy pathway is a catabolic intracellular process that activates the lysosomal degradation pathway.During autophagy, cytoplasmic structures are sequestered into double-membraned or multi-layered autophagosomes and fused with lysosomes to form secondary lysosomes or autophagolysosomes for degradation. In addition to the formation of various density-graded granules in the cytoplasm of lung tissues, an increased number of vacuoles were observed in treated groups with tramadol and combination control groups. Moreover, the autophagy pathway is a catabolic intracellular process that activates the lysosomal degradation pathway.

Most of the published articles focused on a fact which shows that generation of free radicals inducing oxidative stress leads to molecular and cellular damage which are considered the cause of tramadol toxic effects on the different body organs Argani *et al*., (2011). The effects of released reactive oxygen species (ROS) by normal respiratory system are counteracted by glutathione and antioxidants enzymes such as catalase and peroxidase; therefore more generation of ROS via tramadol toxic substance leads to the balance disturbance with antioxidants defense mechanism inducing toxic cellular substances which lead to histopathological changes Lee, (2010).

The present study showed that tramadol increases fibrous tissues formation in the lung interstitial tissues and around alveoli depending on its dose in consistency with Katrin *et al*., (1986) who referred to the fact that tramadol can be trigger to stimulate fibroblast proliferation via mediators which are induced by the epithelial cells of airway passages. According to Esposito *et al*., (2000) there is a correlation between tramadol cytotoxicity and mitochondrial enzyme activity disturbance and its ability to react with the nucleus receptors to prevent genetic transcription of proteins that are secreted by fibroblasts, macrophages, monocytes, and endothelial cells.

Our results revealed that tramadol induced a remarkable histomorphological and severe histopathological changes in rats' lung when compared to that of control. On molecular level, the expression of the pro-apoptotic and Caspase-3 showed a significant moderate to severe Caspase-3 reaction in groups that treated with 40 mg/kg of tramadol. However, positive Caspase-3 reaction product that markedly increased indicating that tramadol is harmful at cellular level and can induce apoptotic changes in pulmonary tissues. Our data confirmed the risk of increased oxidative stress, neuronal and pulmonary damage due to tramadol abuse. Although tramadol is reported to be effective in pain management, its toxicity should be kept in mind (Awadalla et al., 2016).

The *in vitro* experiments showed that tramadol treatment significantly inhibited cell proliferation, migration and invasion in a time-dependent manner. Moreover, administration of tramadol suppressed tumor growth in vivo. The data also revealed that tramadol could up-regulate the protein expression level of PTEN and consistently inhibit the phosphorylation level of PI3K and Akt, whereas the total level of PI3K and Akt remain unchanged (Xia M et al., 2016). These findings indicated that tramadol inhibited proliferation, migration and invasion of human lung adenocarcinoma cells.

VI. Conclusion

In conclusion, these results may highlight the risks of tramadol abuse on the respiratory system either abused and taken in very large doses (much higher than what would be prescribed) can stop breathing altogether and may experience a fatal overdose or as a prescription Opioid Painkiller for moderate pain and if used for pain after surgery or for chronic pain from conditions as well. Finally, we can strongly support those studies that recommended the safe tramadol dose as 25 mg once a day and doctors may increase th dose as needed, but not more than 400 mg per day.

Authors' contributions:

Both authors confirm contribution to the paper as follows: study conception and design: **G. El-Sherif**. Author, **H.K. Abdel-Zaher**. Author; data collection: **G. El-Sherif & H.K. Abdel-Zaher** Authors; analysis and interpretation of results: **G. El-Sherif & H.K. Abdel-Zaher**; draft manuscript preparation: **G. El-Sherif** reviewed the results and approved the final version of the manuscript.

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