

<b>Original Article</b>	<b>Cardiotoxicity and Hepatotoxicity Induced by Clozapine in Adult Male Albino Rats and Possible Protection by Selenium: A Histological Study.</b>
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### ABSTRACT

**Background:** Clozapine is an efficacious antipsychotic drug particularly in schizophrenic patients refractory to other agents. Treatment with clozapine was reported to be associated with sudden death, myocarditis and hepatotoxicity in some patients.

**Aim of the Work:** This study was conducted to investigate the toxic effect of clozapine on cardiac muscle and liver of adult male albino rats and to assess the potential protective role of selenium.

**Materials and Methods:** This study employed fifty adult male albino rats randomly divided into five equal groups. Group I: Control group, Group II received daily 1 ml of 0.1 M HCl, balanced in phosphate buffered saline intraperitoneally (i.p.), Group III received oral selenium (1.5 mg/kg), Group IV received Clozapine (25 mg/kg) i.p. daily and Group V received both clozapine and selenium. After 14 days, the liver and ventricular myocardium of each animal were dissected and processed for light and electron microscopic studies.

**Results:** After clozapine administration, the ventricular myocardium showed fragmentation and separation of cardiac muscles with diffuse mixed inflammatory cellular infiltrate. The Z-lines were irregularly oriented with rupture of mitochondrial membranes and cristae. The liver exhibited extensive inflammatory cellular infiltration around the portal areas and vacuolated hepatocyte cytoplasm with rupture of membranes and cristae of some mitochondria. Concomitant administration of selenium with clozapine displayed an observable protection against these changes.

**Conclusion:** Selenium may have a protective role against cardiotoxicity and hepatotoxicity induced by clozapine therapy.

**Key Words:** Heart, liver, clozapine, selenium, histology, ultrastructure.

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### INTRODUCTION

Schizophrenia is a common disorder with a lifetime prevalence of about 0.55% and an annual incidence of about 10–15 cases per 100 000 population (Layland *et al.*, 2009). Clozapine, a tricyclic dibenzodiazepine derivative, is commonly classified as an atypical antipsychotic characterized by its great efficacy in treating patients with schizophrenia that do not respond to conventional therapy (Green *et al.*, 2000). Meltzer *et al.* (2003) considered clozapine to be life-changing for affected patients as it was shown to reduce overall mortality, largely through a reduction in suicide.

However, since its introduction in 1961, clozapine had been plagued by controversy because of its side-effect profile. Initial concerns were mainly related to agranulocytosis, but in recent years the focus had shifted to fatal cardiotoxicity (Fischer *et al.*, 1992; Layland *et al.*, 2009).

The *Committee on Safety of Medicines in the United Kingdom* (1993) reported four cases of myocarditis in patients exposed to clozapine, three of them were fatal. In the same respect, La Grenade *et al.*, (2001) reported 28 cases of myocarditis while on clozapine therapy, includ-

ing 18 deaths, in whom myocarditis was confirmed at autopsy in 13 of them. They also reported 41 cases of cardiomyopathy, including 10 deaths, associated with the use of clozapine.

*Pieroni et al. (2004)* considered myocarditis to be a rare, but frequently fatal side effect of clozapine. Moreover, the medical literature suggested a strong association between clozapine and cardiomyopathy as well as hypersensitivity myocarditis. Other cardiac effects such as heart failure and electrocardiographic abnormalities were also described (*Hägg et al., 2001*). However, only few cases have been sufficiently studied and confirmed by autopsy (*Kilian et al., 1999*).

Clozapine-induced hepatotoxicity with asymptomatic increased serum transaminase was frequent, being encountered in about 37.3% of cases (*Hummer et al., 1997; Erdogan et al., 2004*). Moreover, liver failure was reported in 0.06% of patients (*Macfarlane et al., 1997*).

For its efficiency, clozapine is still used in therapy despite its adverse effects (*Masi et al., 2006*). However, *Haas et al. (2007)* believed that these adverse effects have recently limited its clinical use.

Selenium is an essential trace element, present as a constituent of selenoproteins, that plays an important antioxidant role (*Rayman, 2000*). Low selenium levels were causally linked to an endemic form of cardiomyopathy (Kashan disease) that found in high prevalence in Northeast China. Dietary selenium supplementation had greatly reduced the incidence of this fatal condition (*Ge & Yang, 1993*).

*Vaddadi et al. (2003)* found that selenium levels in plasma and red blood corpuscles of schizophrenics treated with clozapine were significantly lowered. They concluded that low selenium levels in clozapine-treated patients might be important in the pathogenesis of life threatening cardiac side effects associated with clozapine. Daily selenium supplementation to these patients was recommended.

This recommendation of selenium supplementation needed to be sufficiently studied and confirmed by histology. So, the aim of this work was to investigate the toxic effect of clozapine on cardiac muscle and liver of adult male albino rat and to assess any potential protective role of se-

lenium by using light and electron microscopic techniques.

## MATERIALS AND METHODS

### Drugs :

- Clozapine (8-chloro-11-(4-methyl-1-piperazinyl) -5H-dibenzo [b,e] [1,4] diazepine) was purchased as a yellow crystalline powder from Sigma Aldrich (St. Louis, MO and USA).
- Selenium (sodium selenite) was used in this study in the form of white crystalline powder obtained from (Sigma Aldrich, St. Louis, MO).

### Animals:

This study was carried on fifty adult male albino rats weighing 180–200 g. The rats were obtained from the Animal House of the Faculty of Medicine, Cairo University. Animals were housed in clean stainless steel cages under standard conditions of humidity, temperature and fed with standard diet and allowed water ad libitum. All our methods conformed to the guidelines for the care and use of laboratory animals.

### Experimental Design:

Animals were randomly divided into five groups of 10 animals each and treated in the following way:

- **Group I – Control Group:** Received no treatment.
- **Group II–Positive control group:** Received daily 1 ml 0.1 M HCl, pH balanced in Phosphate Buffered Saline (PBS) intraperitoneally (i.p.) for 14 days.
- **Group III – Selenium treated group:** Received daily 1.5 mg/kg bw selenium dissolved in 1ml distilled water orally via gastric gavage for 14 days (*Tanguy et al., 2003*).
- **Group IV–Clozapine treated group:** Received daily 25 mg/kg bw Clozapine dissolved in 1 ml 0.1 M HCl and pH balanced in Phosphate Buffered Saline (PBS) i.p. for 14 days (*Ip & Utrecht, 2008*).

- **Group V–Clozapine and Selenium treated group:** Received both Clozapine (25 mg/kg bw/day) i.p. in addition to oral Selenium (1.5 mg/kg bw/day) for 14 days.

Twenty four hours after the last dose, rats were sacrificed by decapitation under light isoflurane anesthesia. The liver and heart were dissected out and small specimens were selected for EM while the rest of the organ specimens were immediately fixed in 10% formol saline. Paraffin sections of 5µm thickness were prepared from the liver and myocardium from both right and left ventricles and stained with hematoxylin and eosin [Hx.&E.] (*Bancroft & Gamble, 2002*). For E.M., the chosen specimens from the liver and myocardium of both ventricles were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) and post-fixed in 1% osmium tetroxide. Ultra-thin sections (60–70 nm) were mounted on copper grids and contrast-stained with uranyl acetate and lead citrate (*Hayat, 1970*). Transmission electron microscopic analysis was carried out by a JEM-1400A transmission electron microscope (JEOL, Tokyo, Japan) operated at 80 kV at the Faculty of Agriculture Research Park, Cairo University.

## RESULTS

### The Heart:

Light microscopic examination of the myocardium of both ventricles of control rats belonging to group I showed branching chains of cardiac muscle cells joined end to end by intercalated discs appearing as darkly stained irregular lines, some of which extending across the fiber in a step-like pattern. The nucleus appeared oval, relatively large and pale-staining in shape and occupying a central position in the muscle cell (Figs.1, 2).

Light microscopic examination of the myocardial sections in both ventricles from rats of Groups II and III revealed a similar histological structure to that of the control group.

Myocardial sections from clozapine-treated rats (Group IV) showed inflammatory lesions in both the left and right ventricular myocardium, but were encountered much more in the left ventricle. There were hem-

orrhage, inflammatory exudate, fragmentation and separation of cardiac muscle fibers as well as inflammatory cellular infiltration (Figs. 3, 4). Some cardiac muscle fibers exhibited increased eosinophilia particularly in the left ventricle and eosinophils were frequently spotted inside some blood vessels (Fig. 5). There was diffuse mixed inflammatory cell infiltration of the myocardium of both ventricles but more in the left ventricle (Fig. 6). The infiltrate consisted of eosinophils, polymorphonuclear leucocytes, lymphocytes, monocytes and macrophages (Fig. 7). Inflammatory cellular infiltrates were also seen under the pericardium as well as between the cardiac muscle fibers (Fig. 8).

Heart sections from Group V rats treated with both clozapine and selenium showed almost normal appearance of cardiac muscle fibers apart from mild congestion of blood vessels and limited perivascular inflammatory cellular infiltration (Figs. 9, 10).

Ultrastructurally, the fine structure of cardiac muscle in both right and left ventricles showed regularly arranged myofibrils, varying in diameter (Fig. 11). The myofibrils exhibited alternating A (dark) and I (light) bands, with regular Z lines in the middle of I bands. Between the myofibrils lie numerous mitochondria exhibiting regular cristae. The nuclear chromatin appeared evenly dispersed inside the Nucleus (N) and the nuclear membrane was well defined.

Ultrathin sections of the myocardium from rats of Groups II and III had the same ultrastructure as the control rats (Group I).

Regarding the ultrathin sections of the heart of rats treated with clozapine (group IV), there was fragmentation of the myofibrils of both ventricles which appeared widely separated. The Z lines were irregularly oriented while the nucleus exhibited slight reduction in the amount of chromatin compared to the control (Fig. 12). The mitochondria appeared more electron dense and showed rupture of their membranes and cristae.

Ultrathin sections from the heart of rats of treated with both clozapine and selenium (Group V) revealed an almost similar ultrastructure to the control (Fig. 13). The myofi-

brils were regular in architecture and the orientation of the Z lines. Mitochondria exhibited minimal affection in the form of ill-distinct membranes in limited areas but the cristae were preserved.

### The Liver:

Light microscopic examination of liver sections from the control rats (Group I) showed the characteristic architecture of the classic hepatic lobules with hepatocytes radiating outwards from the central vein towards the periphery of the lobules that enclose the portal areas (Fig. 14). The hepatocytes displayed an eosinophilic granular cytoplasm with vesicular nuclei and several binucleated cells were spotted. Narrow blood sinusoids were seen between the cords of hepatocytes and displayed Von Kupffer cells in their walls (Fig. 15).

The histological picture of liver sections from rats of Groups II and III were similar to those of control rats.

Concerning clozapine-treated rats (Group IV), liver sections presented extensive inflammatory cellular infiltration in the portal areas with congestion of the central veins and blood vessels of the portal areas (Figs. 16–18). Mild dilatation of the blood sinusoids was noticed (Fig. 17). Many hepatocytes displayed vacuolations in their cytoplasm (Figs. 16, 17), others exhibited pyknotic nuclei while some hepatocytes were anucleated; showing cell outlines only without nuclei (Figs. 17, 18). The inflammatory cellular infiltration in the portal areas consisted of eosinophils, neutrophils, macrophages and lymphocytes (Fig. 19).

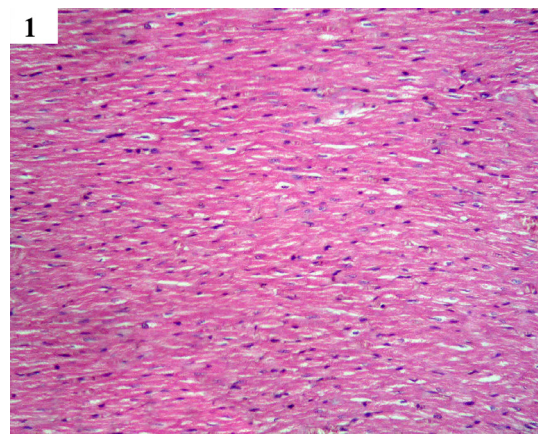
Light microscopic examination of liver sections from rats treated with both clozapine and selenium (Group V) showed milder affection. There was minimal congestion of blood vessels and mild inflammatory cellular infiltration around the portal area (Fig. 20). Although the blood sinusoids were slightly dilated, only few hepatocytes displayed pyknotic nuclei, while many others exhibited vesicular nuclei and binucleated forms, with minimal vacuolation detected in the peripheral hepatocytes (Figs. 20, 21).

Electron microscopic examination of ultrathin sections of the liver of control rats (Group I) revealed normal ultrastructure of hepatocytes with euchromatic nuclei and cytoplasm rich in cell organelles in the form of abundant mitochondria, smooth and rough endoplasmic reticulum. The mitochondria exhibited regular cristae and intact membranes. The bile canaliculi and blood sinusoids could be seen between the hepatocytes (Fig. 22).

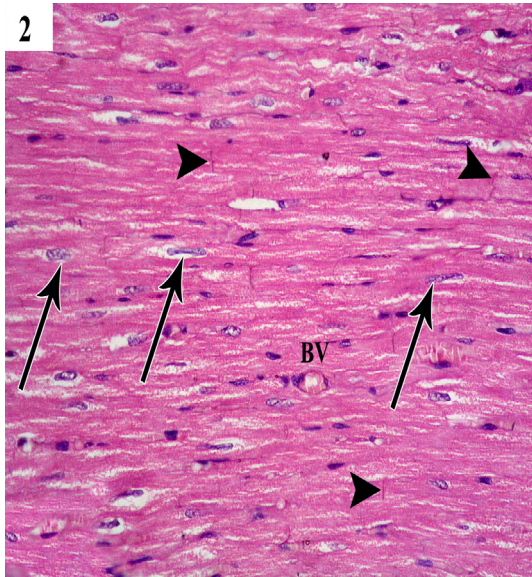
T. E. M. examination of ultrathin liver sections of rats belonging to Groups II and III disclosed the same findings as the control rats.

The ultrastructure of the hepatocytes of clozapine-treated rats (Group IV) showed increased peripheral condensation of their nuclear chromatin with accumulation of many cytoplasmic vacuoles having a smooth outline. Mitochondria were reduced in number and appeared variable in size and shape. Some mitochondria exhibited irregular outlines, while others showed rupture of their membranes and cristae. Two lymphocytes were spotted inside a blood sinusoid (Fig. 23).

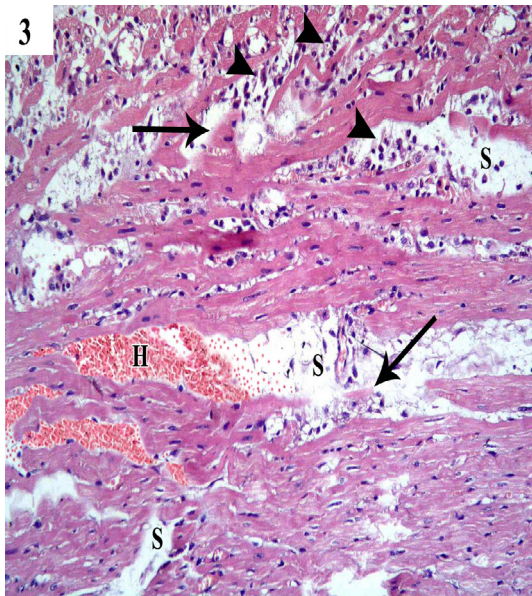
Ultrathin liver sections from rats treated with both clozapine and selenium (group V) showed a very similar ultrastructure to the control. Hepatocytes displayed euchromatic nuclei with prominent nucleoli and intact cytoplasmic organelles. The mitochondria were uniform in size and shape with preserved membranes and cristae (Fig. 24).



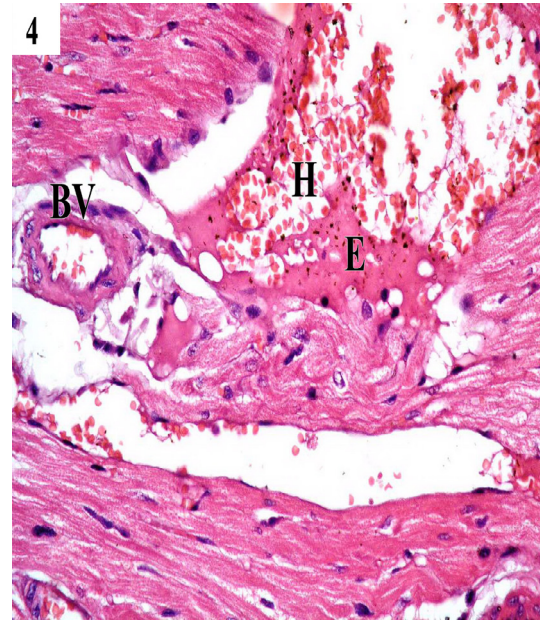
**Fig. 1:** A photomicrograph of a section in the ventricular myocardium of a control rat (Group I) showing the characteristic branching chains of cardiac muscle fibers. Hx.&E.; x200



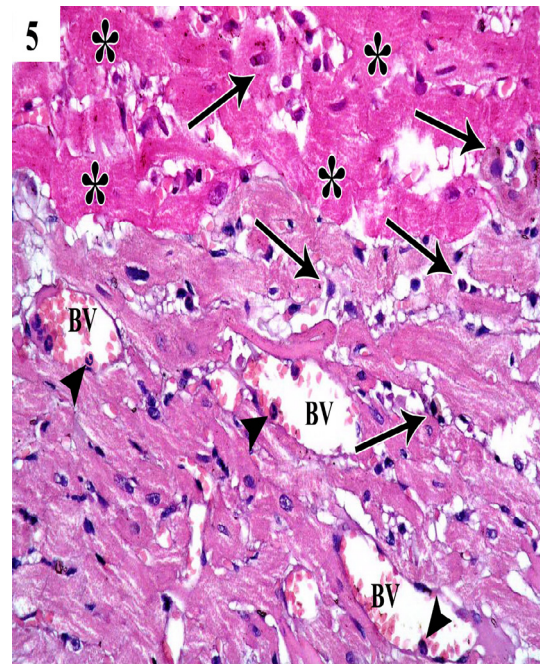
**Fig. 2:** A photomicrograph of a section in the ventricular myocardium of a control rat (Group I) showing that the relatively large oval nuclei of cardiac muscle fibers (arrow) occupying a central position and pale in staining. The cardiomyocytes are joined end to end by intercalated discs (arrowhead) that appear as darkly stained irregular lines. Blood Vessels (BV) are seen between the cardiac muscle fibers.  
Hx. & E.; x400



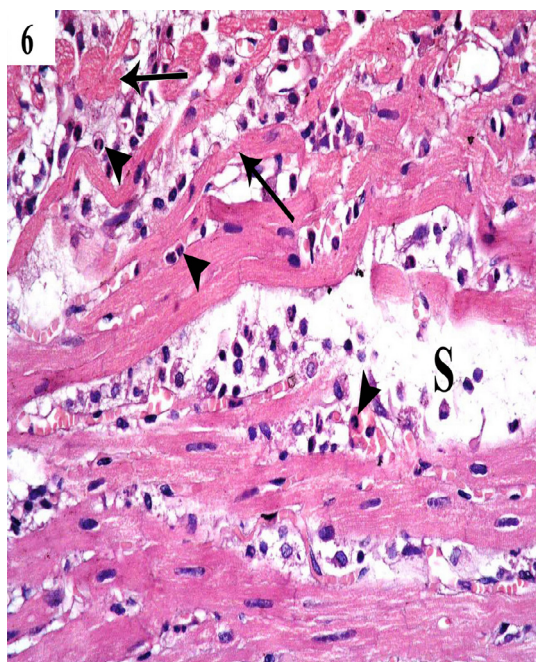
**Fig. 3:** A photomicrograph of a section in the ventricular myocardium of a clozapine treated rat (Group IV) showing Hemorrhage (H) between cardiac muscle fibers together with Separation (S) of cardiac muscle fibers. In some areas, fragmentation of cardiac muscle fibers (arrow) and cellular infiltration between cardiomyocytes are observed (arrowhead).  
Hx.&E.; x200



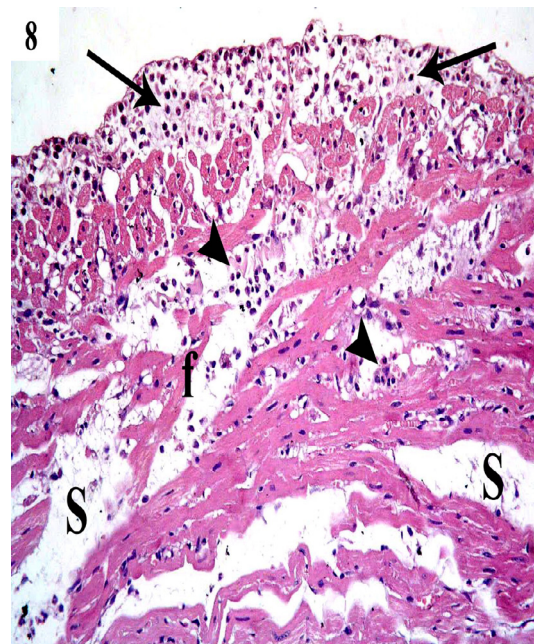
**Fig. 4:** A photomicrograph of a section in the ventricular myocardium of a clozapine treated rat (Group IV) showing Hemorrhage (H) and inflammatory Exudate (E) between the cardiac muscle fibers beside a Blood Vessel (BV).  
Hx.&E.; x400



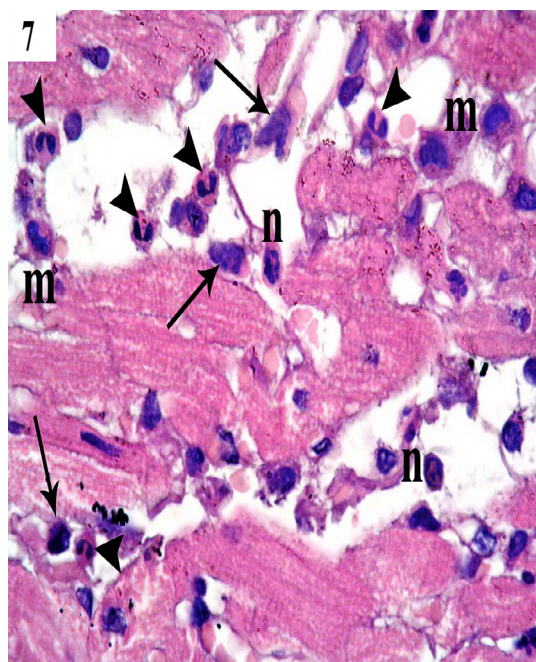
**Fig. 5:** A photomicrograph of a section in the ventricular myocardium of a clozapine treated rat (Group IV) showing increased eosinophilia of some cardiac muscle fibers (\*) as well as inflammatory cellular infiltrates (arrows). Inflammatory cells are also seen inside Blood Vessels (BV) especially eosinophils (arrowheads).  
Hx. & E.; x400



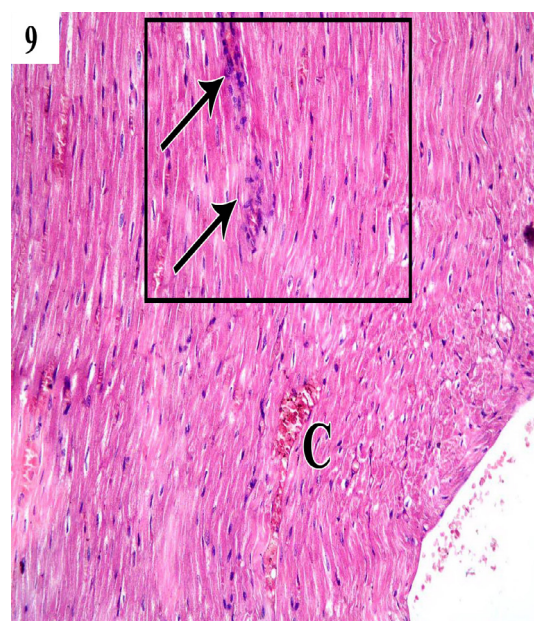
**Fig. 6:** A photomicrograph of a section in the ventricular myocardium of a clozapine treated rat (Group IV) showing fragmentation of cardiac muscle fibers (arrow) as well as inflammatory cellular infiltration containing eosinophils (arrowhead). Hx.&E.; x400



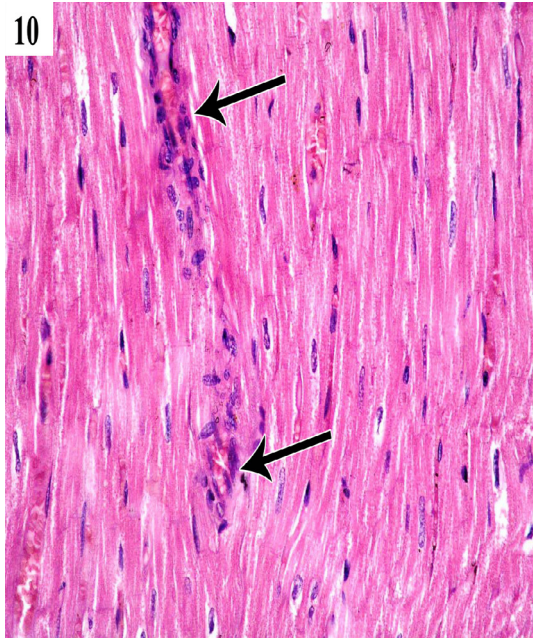
**Fig. 8:** A photomicrograph of a section in the ventricular myocardium of a clozapine treated rat (Group IV) showing inflammatory cellular infiltration both under the pericardium (arrow) as well as between the cardiac muscle fibers (arrowhead). Fragmentation (f) and Separation (S) of cardiac muscle fibers is also observed. Hx.&E.; x200



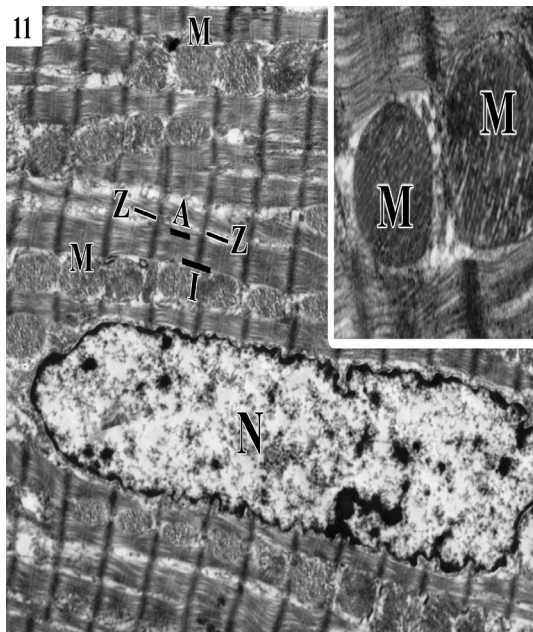
**Fig. 7:** A photomicrograph of a section in the ventricular myocardium of a clozapine treated rat (Group IV) showing inflammatory cellular infiltration containing eosinophils (arrowhead), neutrophils (n), monocytes (m), and macrophages (arrow) detected between the cardiac muscle fibers. Hx.&E.; x1000



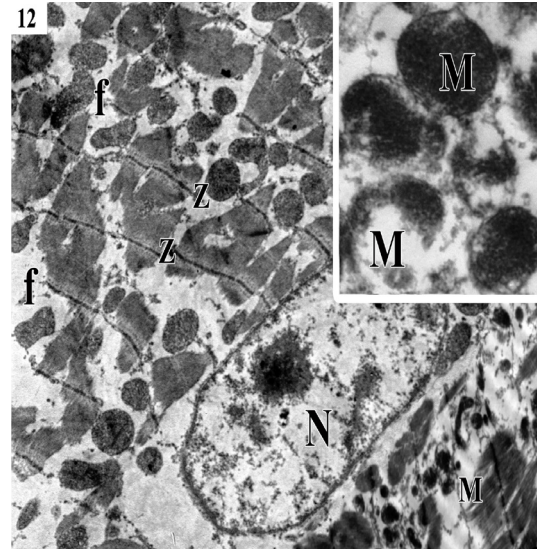
**Fig. 9:** A photomicrograph of a section in the ventricular myocardium of a rat treated with both clozapine and selenium (Group V) showing almost normal appearance of cardiac muscle fibers apart from mild Congestion (C) of blood vessel and limited inflammatory cellular infiltration of the myocardium (arrow). Hx.&E.; x200



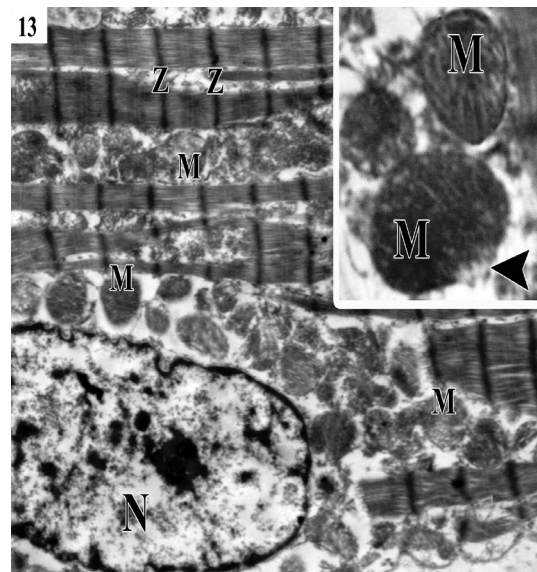
**Fig. 10:** Higher magnification of the boxed area in the previous photomicrograph showing that the inflammatory cellular infiltration was mainly perivascular. Hx. & E.; x400



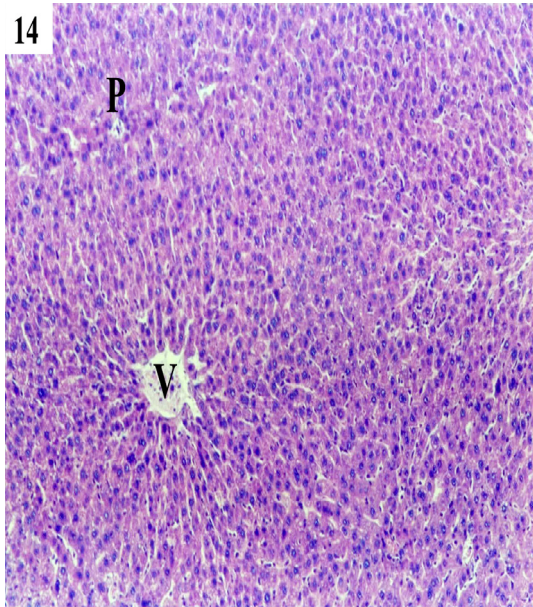
**Fig. 11:** Electron micrograph of the ventricular myocardium of a control rat (Group I) showing regularly arranged myofibrils with the alternating dark (A) and light (I) bands and regular Z lines (Z). mitochondria (M) are seen between the myofibrils. The nuclear chromatin is evenly dispersed inside the nucleus (N) and the nuclear membrane is well defined. Inset: shows higher magnification of mitochondria exhibiting regular cristae and intact membranes. x5,000; inset: x15,000



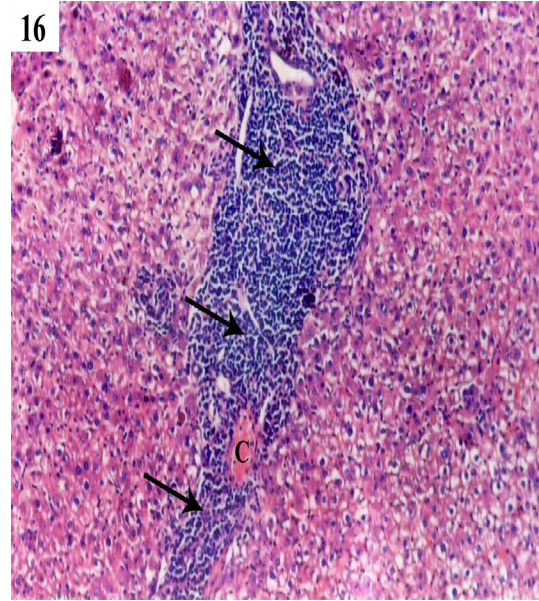
**Fig. 12:** Electron micrograph of the ventricular myocardium of a clozapine treated rat (group IV) shows fragmentation of the myofibrils (f) which look widely separated from each other with irregular orientation of the Z lines (Z). The amount of nuclear chromatin is slightly less than in the control but the nuclear membrane is well defined. Inset: mitochondria (M) appear more electron dense and showed rupture of their membranes and cristae. x5,000; inset: x15,000



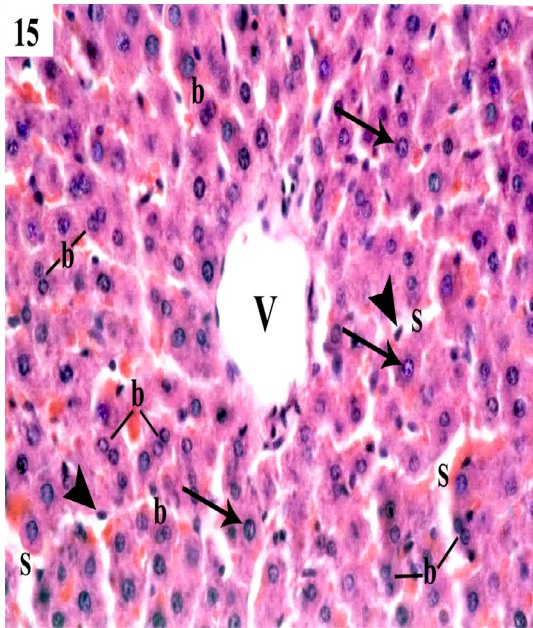
**Fig. 13:** Electron micrograph of the ventricular myocardium of a rat treated with both clozapine and selenium (group V) showing an almost similar ultrastructure to the control. The myofibrils were regular in architecture and Z lines' orientation (Z). The mitochondria (M) exhibit almost normal appearance. Inset: minimal affection of mitochondria (M) in the form ill-distinct membranes in limited areas (arrowhead) but with preserved cristae. x5,000; inset: x15,000



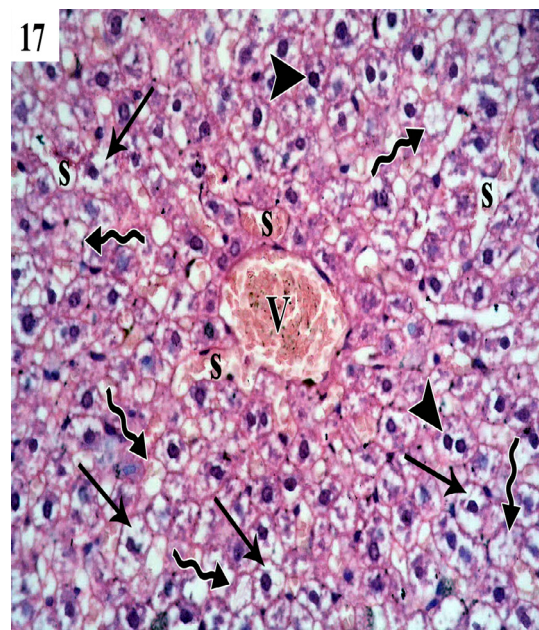
**Fig. 14:** Photomicrograph of a section in the liver of a control rat (group I) shows the characteristic architecture of the classic hepatic lobule with the hepatocytes radiating outwards from the central vein (V). Note the presence of a portal tract (P) with its vessels at the periphery of the lobule.  
Hx.&E.; x100



**Fig. 16:** Photomicrograph of a section in the liver of a clozapine treated rat (group IV) showing extensive inflammatory cellular infiltration (arrow) in the portal areas which display a congested blood vessel (c). Note that many hepatocytes exhibit vacuolations especially in the lower right corner of the picture.  
Hx.&E.; x100

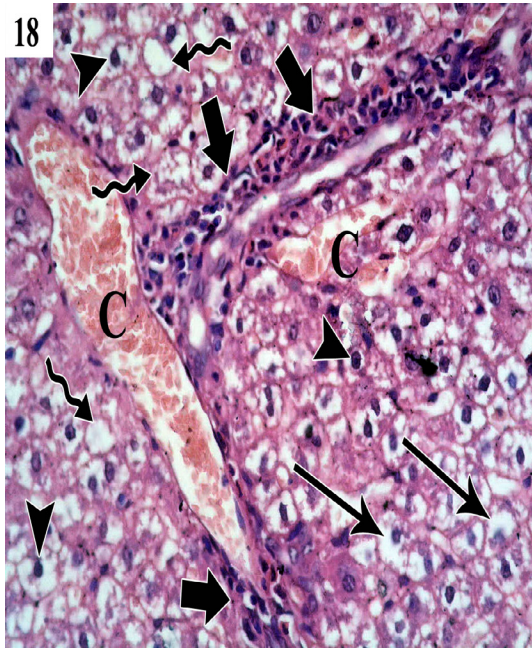


**Fig. 15:** Photomicrograph of a section in the liver of a control rat (group I) showing hepatocytes having eosinophilic granular cytoplasm and vesicular nuclei. Several binucleated cells (B) are spotted. Narrow blood sinusoids (S) are present between the cords of hepatocytes with Von Kupffer cells (arrowhead) in their walls. Note the presence of the central vein (V).  
Hx.&E.; x400

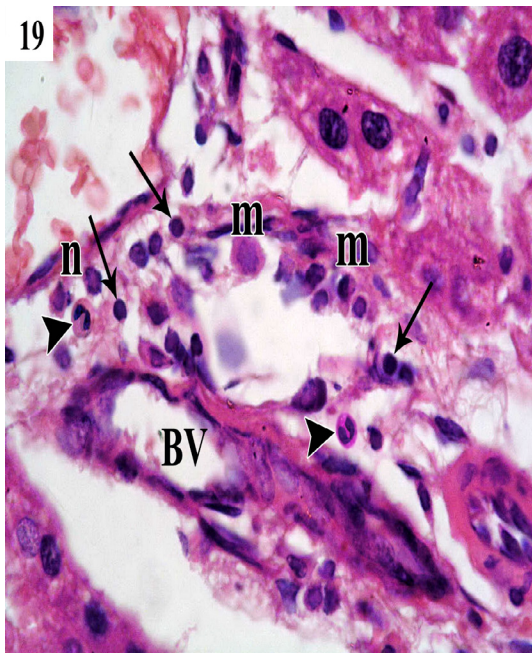


**Fig. 17:** Photomicrograph of a section in the liver of a clozapine treated rat (group IV) showing congestion of the central vein (V) and mild dilatation of the blood sinusoids (S). Many hepatocytes display vacuolations in their cytoplasm (arrow), others exhibit pyknotic nuclei (arrowhead) while some hepatocytes show cell outlines only (curved arrow) without nuclei (anucleated cells).  
Hx.&E.; x400

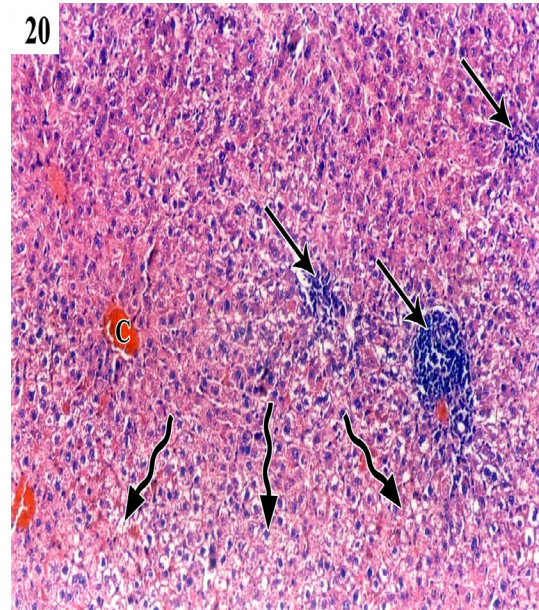




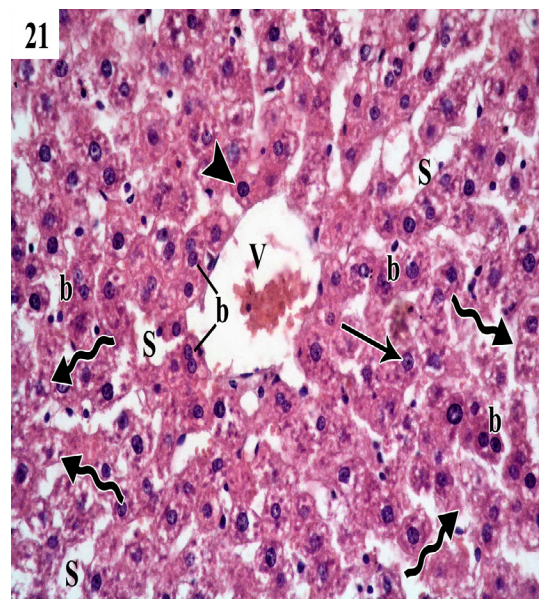
**Fig. 18:** Photomicrograph of a section in the liver of a clozapine treated rat (group IV) showing inflammatory cellular infiltration (thick arrow) in the portal areas with congestion of blood vessels (c). Note the presence of vacuolations (arrow) in many hepatocytes, pyknotic nuclei in others (arrowhead) together with some anuclear hepatocytes (curved arrow). Hx.&E.; x400



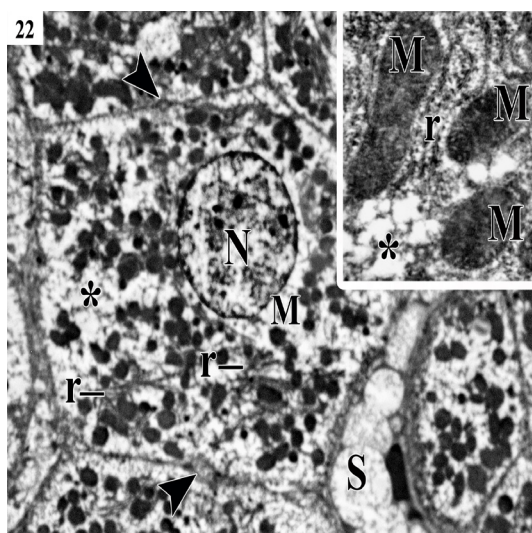
**Fig. 19:** Photomicrograph of a section in the liver of a clozapine treated rat (group IV) showing inflammatory cellular infiltration in the form of eosinophils (arrowhead), neutrophils (n), macrophages (m) and lymphocytes (arrow) around a blood vessel (BV) in the portal area. Hx.&E.; x1000



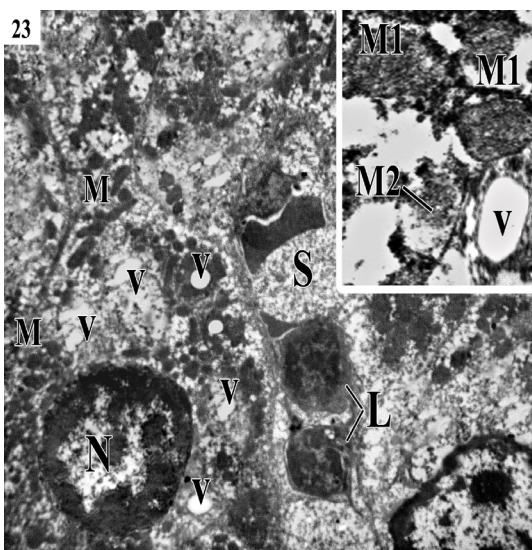
**Fig. 20:** Photomicrograph of a section in the liver of a rat treated with both clozapine and selenium (group V) showing minimal congestion (C) of blood vessels (V) and mild inflammatory cellular infiltration around the portal area (arrow). Note the presence of minimal vacuolations in the peripheral hepatocytes (curved arrows). Hx.&E.; x100



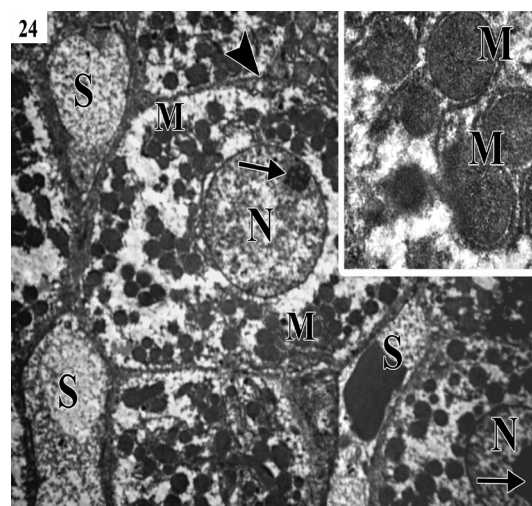
**Fig. 21:** Photomicrograph of a section in the liver of a rat treated with both clozapine and selenium (group V) demonstrating mild affection than in group IV. The hepatocytes reveal a nearly normal appearance with minimal vacuolations in peripheral hepatocytes (curved arrows). Only few hepatocytes show pyknosis (arrowhead), while many others display vesicular nuclei (arrow) and are binucleated (B). Note the blood sinusoids (S) are slightly dilated. Hx.&E.; x400



**Fig. 22:** Electron micrograph of the liver of a control rat (group I) showing the normal ultrastructure. The hepatocyte displayed an euchromatic nucleus (N) and a cytoplasm rich in cell organelles such as abundant mitochondria (M), smooth (\*) and Rough (r) endoplasmic reticulum. Note the bile canaliculi (arrowhead) and blood sinusoid (S). Inset: shows higher magnification of mitochondria exhibiting regular cristae and intact membranes beside smooth (\*) and rough (r) endoplasmic reticulum. x15,000



**Fig. 23:** Electron micrograph of the liver of a clozapine treated rat (group IV) shows increased peripheral condensation of nuclear chromatin (N) and accumulation of many Vacuoles (V) with smooth outlines in the cytoplasm. Mitochondria (M) are reduced in number and reveal variability in size and shape. Two lymphocytes (L) are seen inside a blood sinusoid (S). Inset: Some Mitochondria exhibit irregular outline (M1), while others show rupture of their Membranes and cristae (M2). Note the presence of cytoplasmic vacuole having a smooth outline. x2,000; inset: x15,000



**Fig. 24:** Electron micrograph of the liver of a rat treated with both clozapine and selenium (group V) showing a very similar ultrastructure to Group I with the hepatocytes displaying euchromatic nuclei (N) with prominent nucleoli (arrow) and intact cytoplasmic organelles. The mitochondria (M) are uniform in size and shape. Note the bile canaliculus (arrowhead) and blood sinusoids (S). Inset: preserved outline of mitochondria with regular membranes and cristae. x2,000; inset: x15,000

## Discussion

Although clozapine remains the “gold standard” treatment for resistant schizophrenia, its use has been limited by the risks of sudden death, cardiovascular side effects and agranulocytosis (Kilian *et al.*, 1999 & La Grenade *et al.*, 2001; Bitter *et al.*, 2004). Hägg *et al.*, (2001) was able to demonstrate a quantitative association between clozapine and myocarditis.

The results of this study revealed that clozapine administration led to cardiac damage as evidenced by fragmentation and separation of cardiac muscle fibers, inflammatory cellular infiltration of the myocardium of both ventricles in the form of predominant eosinophils, together with neutrophils, monocytes and macrophages. Ultrastructurally, fragmentation of myofibrils and irregular orientation of the Z lines was evident and the mitochondria exhibited rupture of their membranes and cristae. These results are consistent with the several published reports.

The *Australian Adverse Drug Reactions Bulletin* (1994) had reported a case of myocarditis nine days after starting clozapine therapy.

Two days later, the patient died and autopsy revealed considerable infiltration of the myocardium with eosinophilic granulocytes, suggestive of allergic myocarditis.

*Kilian et al. (1999)* reported two cases of sudden cardiac death on clozapine therapy. Necropsy revealed acute myocarditis with eosinophilic infiltrate and myocytolysis. *Pieroni et al. (2004)* also reported a case of hypersensitivity myocarditis secondary to clozapine administration that was diagnosed for the first time by endomyocardial biopsy and successfully treated with corticosteroids. Histological findings in all samples showed extensive inflammatory infiltrates, mainly represented by degranulated eosinophils and lymphocytes which were often adherent to the injured cardiomyocytes. Even recently, *Ronaldson et al. (2010)* reported that three patients died while on clozapine therapy and cardiac histology confirmed the diagnosis of myocarditis. Clozapine-induced hypersensitivity myocarditis was considered to be the most likely cause of death. The time of onset of myocarditis was found to be between the 14<sup>th</sup> and 22<sup>nd</sup> day after the start of clozapine therapy with an average time of about 15 days (*Kamphuis et al., 2010* & *Ronaldson et al., 2010*).

It was reported in the literature that the causes of clozapine-induced myocarditis included hypercatecholaminergic states (*Haack et al., 2003; Wang et al., 2005*), Type I IgE-mediated acute hypersensitivity reaction as well as a direct toxic effect on the heart (*Kilian et al., 1999*). Moreover, *Chan et al. (2000)* suggested the lack of metabolic enzymes (CYP450-1A2 and CYP450-1A3) resulted in extreme clozapine concentrations and direct cardiotoxic effects of eosinophils through blockage of cholinergic M2-receptors or high concentrations of atmospheric ozone resulting in cholinergic receptor dysfunction. Additionally, *Haack et al. (2003)* and *Burian et al. (2005)* proposed an immune mechanism for clozapine related myocarditis where the release of cytokines such as the Tumour Necrosis Factor-alpha (TNF- $\alpha$ ) and soluble cytokine receptors was found to mediate autoimmune myocarditis. This finding justified treatment of clozapine-related myocarditis with corticosteroids (*Hägg et al. 2001*).

Considering the predominant eosinophil infiltration in the myocardium of clozapine-treated rats noticed in the current study, it could be suggested that clozapine induced an acute drug reaction in the heart or a hypersensitivity myocarditis.

The results of the present study revealed that oral selenium administration with clozapine therapy protected the heart against the clozapine-induced damage. This agrees with *Vaddadi et al. (2003)* who concluded that low selenium levels in clozapine-treated patients might be important in the pathogenesis of life threatening cardiac side effects associated with clozapine.

Selenium had important antioxidant properties which helped to regenerate antioxidant systems and maintained the intracellular redox status (*Rayman, 2000*). It might protect endothelial cells against lipid peroxidation (*Burk & Hill, 2005*). In addition, selenium might reduce the production of inflammatory prostaglandins and leukotrienes (*Rayman, 2000*).

In animal studies, selenium consumption had many beneficial effects on the heart. It was found to increase the cardiomyocyte glutathione peroxidase activity (*Tanguy et al., 1998*), improve cardiac recovery from ischemia-reperfusion injury, limit ischemia-induced structural alterations of mitochondria and sarcomeres (*Tanguy et al., 2003*) and reduce myocardial infarct size (*Tanguy et al., 2004*). Selenium-intake also markedly minimized the incidence of ischemia-induced ventricular arrhythmias in rats (*Tanguy et al., 1998*).

The present study also revealed that clozapine produced hepatotoxicity evidenced by vacuolations in cytoplasm of many hepatocytes, pyknotic nuclei and anucleated cells. Extensive inflammatory cellular infiltrates in the form of eosinophils, neutrophils, macrophages and lymphocytes were demonstrated especially in the portal areas. Ultrastructurally, there were vacuolations of liver cells and reduced number of mitochondria which were irregular and displayed ruptured membranes and cristae. These results are in agreement with several previous reports. (*Worrall et al., 1995; Macfarlane et al., 1997; Luo et al., 2007; Chang et al., 2009*).

*Marinkovic et al. (1994)* and *Worrall et al. (1995)* reported dose-dependent elevation

of hepatic enzymes with clozapine therapy. However, they suggested that continuing clozapine treatment might be possible in some patients.

*Macfarlane et al. (1997)* described a case of fatal acute fulminant liver failure caused by clozapine. On the other hand, *Kilian et al. (1999)* reported mild inflammation in the portal tracts of the liver associated with eosinophilic infiltrates in a case that died 18 days after starting clozapine treatment. Additionally, *Luo et al. (2007)* presented a case of clozapine-induced hepatitis that developed acute onset of ascites.

*Chang et al. (2009)* described the results of a liver needle biopsy in a case of clozapine-induced fatal fulminant hepatic failure. It revealed massive zonal necrosis with approximately two-thirds of the tissue being necrotic, periportal sparing, collapse of the reticulin framework, mild chronic inflammation, marked bile stasis and immature fibrosis.

However, *Erdogan et al. (2004)* advised that clozapine could be cautiously continued in spite of liver enzyme elevation in selected patients in whom marked psychiatric improvement had been imposed. Moreover, *Franke (2007)* pointed out that asymptomatic elevation in liver enzymes with the use of clozapine was likely to be a biochemical abnormality rather than an indication of necrotic liver injury.

*Lu et al. (2008)* suggested that the hepatotoxicity of clozapine was induced by the reactive metabolites of cytochrome P450 (CYP) particularly CYP 3A and CYP 2E1. They further added that Reactive Oxygen Species (ROS) were possibly the subsequent attributor for cell damage after clozapine metabolism and suggested that they could be suppressed by addition of a scavenger of ROS.

The present results revealed that selenium also protected the liver against the toxicity induced by clozapine as evidenced by milder histological changes in the animals which received selenium together with clozapine. This finding is in correspondence with previous reports.

*Uslu et al. (2010)* found that serum selenium concentration was low in cirrhotic children and

recommended supportive selenium administration. Moreover, *Martínez-Peinado et al. (2010)* pointed out that the selenium component of the antioxidant system was severely impaired in cirrhosis.

*Messarah et al. (2010)* confirmed an evident protective effect of selenium against liver injury in rats by its strong antioxidant capacity, but they used arsenic to induce hepatotoxicity in their study.

In conclusion, selenium proved to have a protective role against cardiotoxicity and hepatotoxicity induced by clozapine therapy. So, it may be highly beneficial to supplement clozapine therapy with oral selenium administration. Moreover, patients on clozapine should be informed of the potential alarming cardiac symptoms. At the same time, physicians must be aware of the strong association between clozapine use and the cardiac and hepatic complications. Consequently, they have to maintain a high degree of clinical suspicion throughout the duration of treatment. Further studies are needed to evaluate the capacity of selenium to protect against other serious side effects of clozapine therapy especially agranulocytosis.

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## تأثير عقار الكلوزابين على قلب وكبد الجرذان البيضاء الذكور واحتمال دور وقائي للسلينيوم: دراسة هستولوجية

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### ملخص البحث

يعتبر عقار الكلوزابين من الأدوية المضادة للذهان والتي تستخدم بفعالية شديدة في علاج مرض الذهان حيث يؤدي العلاج بهذا العقار لحدوث التهاب بعضلات القلب والوفاة المفاجئة كذلك حدوث تسمم بالكبد. كما يعتبر عنصر السلينيوم من العناصر القليلة المكونة من بروتين السلينيوم التي تلعب دورا هاما كمضاد للتأكسد.

تهدف هذه الدراسة الى بحث تأثير عقار الكلوزابين على عضلة القلب وخلايا الكبد ودراسة التأثير الواقي المحتمل لعنصر السلينيوم بواسطة كل من المجهر الضوئي والالكتروني.

تمت الدراسة على عدد خمسين من ذكور الجرذان البالغين تم تقسيمهم الى خمس مجموعات تكونت كل منها من عشرة جرذان. اعتبرت المجموعة الأولى كمجموعة ضابطة والمجموعة الثانية تم إعطاؤها محلول ملح متوازن بتركيز 0.1% لمدة 14 يوما والمجموعة الثالثة تم إعطاؤها سلينيوم بتركيز 1.5 مجم لكل كجم بالفم يوميا لمدة 14 يوما والمجموعة الرابعة تم إعطاؤها عقار الكلوزابين بتركيز 25 مجم لكل كجم بالحقن داخل تجويف الغشاء البريتوني بينما أعطيت المجموعة الخامسة عقار الكلوزابين بتركيز 25 مجم لكل كجم بالحقن داخل تجويف الغشاء البريتوني بالإضافة الى سلينيوم بتركيز 1.5 مجم لكل كجم بالفم عن طريق الأنبوبة المعدية يوميا لمدة 14 يوما.

عقب انتهاء التجربة تم تخدير الجرذان قبل إزالة رؤوسها ثم تم تشريحها واختيار عينات من بطني القلب الأيمن و الأيسر وكذلك من الكبد لفحصها بكل من المجهر الضوئي والالكتروني.

وقد أظهرت النتائج في المجموعة الرابعة حدوث تكسير وأنفصال لألياف عضلة القلب مع حدوث أنتشار للخلايا الانتهابية كذلك أظهرت نتائج المجهر الكتروني عدم أنتظام الخطوط الليفية مع حدوث تكسير لجدران الحبيبات الخيطية.

أما بالنسبة لخلايا الكبد فقد أظهرت النتائج أيضا حدوث أنتشار خلوي كثيف بالمنطقة البوابية للكبد كما أظهرت القطاعات الرقيقة بالمجهر الألكتروني حدوث تكسد لتجاويف داخل السيتوبلازم مع حدوث تكسر في جدران الحبيبات الخيطية.

وعلى الجانب الآخر فقد تبين وجود تحسن ملحوظ في كل التغيرات السابقة في المجموعة الخامسة عند استعمال السلينيوم مع الكلوزابين ونستنتج من ذلك أن السلينيوم له دور وقائي مضاد للتسمم الناتج من عقار الكلوزابين في عضلات القلب وخلايا الكبد.