CLOZAPINE-INDUCED MYOCARDITIS IN RATS: ROLE OF L-CARNITINE IN PROTECTION

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ABSTRACT

Clozapine (CLZ) is an atypical antipsychotic and often used in refractory schizophrenia. The present study was carried out to investigate clozapine-induced myocarditis in albio rats and evaluate if L-Carnitine can reduce myocarditis through cardio-protective effect. The rats were divided into four groups as follows; **group 1:** negative control; **group 2:** received. 25 mg/kg/day/4 weeks of CLZ intraperitoneal (I.P), **group 3:** received 500 mg/kg/day/4 weeks of L-Carnitine I.P and, **group 4:** received 25 mg/kg/day/4 weeks of CLZ I.P plus 500 mg/kg/day L-Carnitine I.P. The rats were sacrificed on the 29th day of the experiment. Measuring serum levels of lactate dehydrogenase (LDH) enzyme, creatine phosphokinase (CPK-MB), tissue malondialdehyde (MDA), and glutathione peroxidase (GSHPx) was done. Histopathological examination of heart was performed by light and electron microscopy. The current study revealed significant statistical elevation of serum cardiac enzymes; LDH and CPK-MB in CLZ-treated group in comparison to control and significant reduction of cardiac enzymes in rats co-treated with CLZ & L-Carnitine in comparison to CLZ-treated group. Significant statistical increase was observed in tissue MDA with significant reduction in tissue GSHPx in CLZ-treated group as compared to control group. Meanwhile, significant reduction in tissue MDA with significant increase in tissue GSHPx in rats co-treated with CLZ & L-Carnitine in comparison to CLZ-treated group. Histopathological changes in CLZ-treated group were showed by light microscopy and by electron microscopy. In group co-treated with CLZ & L-Carnitine, the histopathological changes were disappeared. Conclusion is that CLZ induced evident myocarditis which was partially relieved by L-Carnitine. So, L-Carnitine had cardio-protective effects in CLZ-induced myocarditis in rats.

Keywords: clozapine (CLZ), Lactate dehydrogenase (LDH); Glutathione peroxidase (GSHPx); cardiotoxicity; L-Carnitine, malondialdehyde (MDA)

INTRODUCTION

Clozapine (CLZ), a tricyclic dibenzodiazepine derivative, is commonly classified as an atypical antipsychotic. It is distinguished from typical antipsychotics by its greater efficacy and reduced tendency to cause extrapyramidal movement disorders **(Factor, 2002)** & **(Abidi and Bhaskara, 2003)**. Often used for refractory schizophrenia, it can be life-changing for affected patients and has been shown to reduce mortality rate, as it can reduce suicidal tendency **(Meltzer et al., 2003)**.

Clozapine has a strong affinity for D4-dopaminergic receptors and potent serotonergic, noradrenergic, histamine and cholinergic M2 receptor blocking abilities. It has relatively weak D2-receptor activity; in comparison with traditional antipsychotic drugs; and so it has few extrapyramidal side effects **(Basel et al., 2014)**.

Since its introduction in 1961, CLZ has been plagued by controversy because of its side-effects. Initial concerns were mainly related to agranulocytosis. Recently, the focus has shifted to potentially fatal cardiotoxicity **(Jamie et al., 2009)**. Myocarditis is one of the most publicized cardiotoxic side effects of clozapine. Also, dilated cardiomyopathy and pericarditis have been reported **(Newcomer, 2007)**.

L-carnitine is a γ-three methyl amino-β-hydroxyl fatty acid, which is an essential cofactor in mitochondrial respiration playing an important role in the transfer of long-chain fatty acids from cytosol to mitochondria. By combination with carnitine to form acylcarnitine, acyl groups could be transferred from cytosolic coenzyme-A on the outer surface of the mitochondrion membrane, then to the inner surface by exchange with free carnitine using an antiport mechanism. The acyl groups are then transferred from carnitine to coenzyme A within the mitochondrion **(Kelly, 1998)**.

The current study aims to investigate clozapine-induced myocarditis in rats and verify if L-Carnitine can reduce cardiotoxicity through cardio-protective effect.

MATERIALS & METHODS

1 Animals.

Forty adult male albino rats with average weight of 200-220 g were obtained from Minia University Animal Care Center.

2 Chemicals.

Clozapine was obtained by Novartis-Pharma Company (Switzerland).

L-Carnitine was obtained as L-Carnitine 1 gm/5ml ampoule produced by MEPACO Co., Egypt. Clozapine was dissolved in 0.1 M HCl and pH balanced in phosphate-buffered saline (PBS), was reconstituted in sterile water prior to use. MDA and GSHpx kits were obtained by Sigma Chemicals Co., USA.

3 Experimental protocol

Forty adult male albino rats of weighting 200-220 g were housed in plastic cages (six rats/cage) with wood shavings. Rats were kept in a temperature-controlled room at 24±1°C, 50% humidity and 12hrs /12 hrs. light/ dark cycle. Rats were adapted to the environment for one week prior to the start of experiment. Animals were fed on rat pellets and water was provided ad libitum during experimental period (28 days). The rats were randomly distributed into four equal groups of ten rats each as follows:

Group 1 (-ve Control): 1 ml sterile water was injected I.P daily for group rats.

Group 2: 25 mg/kg/day CLZ was injected I.P. on a daily basis. Rats were fed on the basal diet and received

Group 3: L-Carnitine in a dose of 500 mg/kg/day was injected I.P.

Group 4: 25 mg/kg/d CLZ I.P. plus 500 mg/kg/day L-Carnitine was injected I.P.

The rats were sacrificed at the end of study period (4 weeks) of the experiment. The choice of clozapine dose was based on previous reported study (Basel and Metwally, 2015).

The experiment was carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The Institutional Animal Care and Use Committee approved the experimental

protocol. All efforts were made to minimise animal suffering and to reduce the number of animals used.

4 Blood Collection and Separation

At the end of the experiment, the rats were fasted overnight. At the morning of the next day the rats were anesthetized by general volatile anesthesia using ether. After induction of mild anesthesia, the rat was rapidly pulled out. The animals were dissected under complete aseptic conditions. Blood samples were withdrawn by capillary microtubes (Micro Hematocrite Capillaries, Mucaps) from the retro-orbital plexuses of veins in inner canthus of the eye into plain tube with gel.

The blood samples were centrifuged at 10.000 rpm for 60 minutes. After centrifugation the upper layer was removed for determination of the protein concentration glutathione peroxidase (GSHPx) activity by the method of Lowry et al. (1951). The GSHPx was measured using the method of **Paglia and Valentine (1967)**. The level of tissue malondialdehyde (MDA) was measured as described by **Van Ye et al. (1993)**. MDA levels are represented as mmol/g tissue.

5 Serum Biochemical Analysis

Determination of serum LDH activity and serum CK-MB activity was carried out in laboratories of Minia University Hospital by using automated chemistry analyser using spectrophotometric techniques (Thermo Electron modelkone Lab 20i, Finland).

6 Histopathological study

After dissection of the rats at the end of period of administration, the ventricles were removed and kept on ice until homogenization on the same day. The samples were first washed with deionized water to separate blood and then homogenized (Braun homogenizer) at 1.000 U for about 3 min. The heart from the four groups were rapidly excised, cut into small pieces and dropped in formalin in which they were kept for appropriate time. After fixation, they were subjected to the normal procedure for paraffin embedding. Sections were cut at the thickness of 5 microns and stained with Haematoxyline-Eosin and Periodic acid Schiff reaction (PAS) and then tissue sections were investigated using light microscope (**Drury and Wallington, 1980).**

7 Electron Microscopic examination

Left ventricular tissue samples were excised and cut into ½ mm thickness then were fixed immediately in 3% glutaraldehyde in 200 mM sodium phosphate buffer, pH 7.4 for 3 hours at 4°C. Materials were washed with the same buffer and post fixed in 1% osmium tetroxide and in sodium phosphate buffer, pH 7.4, for 1 h. at 4°C. Tissue samples were washed with the same buffer, 3 times, for 3 h. at 4°C, dehydrated in a graded ethanol series and embedded in Epoxy Resin 812, overnight. Thin sections were cut with (a LKB U3) ultra-microtome which yields very thin sections (25-100 nm). Sections were finally stained with 2% uranyl acetate and lead citrate, covered with aglass coverslip using D.P.X. maintant. The sections were examined and photographed using 201 philips (transmission) electron microscope at 80 KV.

8 Statistical Analysis

The collected data was organized and tabulated. Data were expressed as means ± standard deviation (SD).

Statistical analysis of variance between mean values of different groups was performed using Kruskal-Wallis test followed by the Mann-Whitney test to determine the variance between all rat groups. Differences were considered significant at P<0.05. Statistical analysis was done using computerized SPSS program (Statistical Package for the Social Sciences, version 16).

RESULTS

1 Biochemical changes

- **Serum cardiac enzymes LDH and CPK-MB**

It was obvious from tables 1 & 2 and Figs. 1 & 2 that CLZ-treated rats afforded significant statistical (p<0.001) increase in serum levels of LDH and CPK-MB as compared to the control group. There were no significant changes in serum levels of LDH and CPK-MB enzymes in rats treated with L-Carnitine when compared to the control group. Group 4 rats (CLZ +L-Carnitine) afforded significant (p<0.001) statistical reduction in levels of serum LDH and CPK-MB enzymes as compared to CLZ-treated group and control group.

- **Myocardial oxidative stress parameters (MDA) and myocardial anti-oxidant (GSHPx)**

As shown from tables 3 & 4 and Figs. 3 & 4 that the amount of cardiac MDA significantly elevated in CLZ-treated rats compared with the amount observed in the controls (p < 0.001). In rats co-treated with L-Carnitine and CLZ, there was a significant reduction in the amount of cardiac MDA compared with rats treated with CLZ alone (p = 0.019). Myocardial GSH-Px activity was significantly reduced in CLZ-treated rats as compared to the GSH-Px activity observed in control rats (p = 0.03). Cardiac GSH-Px activity was significantly higher in rats co-treated with CLZ and L-Carnitine than in rats of CLZ-treated group (p < 0.001).

Table (1): Effect of Clozapine (CLZ) and L-Carnitine on rat 'cardiac enzyme (LDH) mean±SD in IU/L

Group	Mean LDH	P		
Group 1 (Control)	213.9±5.3	I vs II	I vs III	I vs IV
Group 2 (CLZ)	308.1±2.3	<0.001	0.449	<0.001
Group 3 (L-Carnitine)	215.7±8.6	II vs III	II vs IV	III vs IV
Group 4 (CLZ + L-Car)	261.3±1.8	<0.001	<0.001	<0.001

P is significant at P<0.05

Table (2): Effect of Clozapine (CLZ) and L-Carnitine on rat'cardiac enzyme (CPK-MB) in IU/L

Group	Mean CPK-MB	P		
Group 1 (Control)	18.3±2.3	I vs II	I vs III	I vs IV
Group 2 (CLZ)	36.1±2.3	<0.001	0.553	<0.001
Group 3 (L-Carnitine)	19.2 ±5.6	II vs III	II vs IV	III vs IV
Group 4 (CLZ + L-Car)	29.4±1.8	<0.001	<0.001	<0.001

P is significant at P<0.05

Table (3): Effect of Clozapine (CLZ) and L-Carnitine on malondialdehyde (MDA) mean ± SD in mmol/g activity in rats

Group	Mean MDA	P		
Group 1 (Control)	0.23±0.09	I vs II	I vs III	I vs IV
Group 2 (CLZ)	0.36±0.02	<0.001	0.684	0.007
Group 3 (L-Carnitine)	0.24±0.05	II vs III	II vs IV	III vs IV
Group 4 (CLZ + L-Car)	0.30±0.03	<0.001	0.019	0.019

P is significant at P<0.05

Table (4): Effect of Clozapine (CLZ) and L-Carnitine on glutathione peroxidase ($GSHP_X$) mean ± SD in IU/mg activity in rats

Group	Mean $GSHP_X$	P		
Group 1 (Control)	93.3±17.7	I vs II	I vs III	I vs IV
Group 2 (CLZ)	77.4±11.2	0.030	0.326	0.030
Group 3 (L-Carnitine)	100.3±23.2	II vs III	II vs IV	III vs IV
Group 4 (CLZ + L-Car)	109.2±3.3	0.002	<0.001	0.214

P is significant at P<0.05

2 Histopathological changes

Results of light microscopy

Light microscopic examination showed no differences in cardiac muscle cells morphology between L-Carnitine group compared to control group (Fig. 5 A & C). Micrographs of heart muscles of CLZ-treated group showed changes in the form of degenerated and widly separated myofibers with abnormal flat dense nuclei. In addition, extravasated blood in degenerated myocardial fibers with pyknotic nuclei was observed. Edema increase in between the myocardial fibers, areas of hemorrhage between the degenerated myofibers and mononuclear cellular infiltration (inflammatory cells) were observed also (Fig. 5 –B). No pathological changes were detected in the (L-Carnitine + CLZ), treated groups as shown in (Figs. 5- D).

Electron microscopy results

No differences in cardiac muscle cells morphology were observed between L-Carnitine treated group compared to control group (Fig. 6). The electron micrographs of heart muscles of CLZ-treated group showed changes in the form of degenerated nucleus, disorganized, fragmented myofibrils and some destructed mitochondria, wide spaces in the sarcoplasm of the cardiac myocytes. (Figs. 7 & 8). As shown in (Figs. 9) no pathological changes were detected in the (L-Carnitine + CLZ), treated groups.

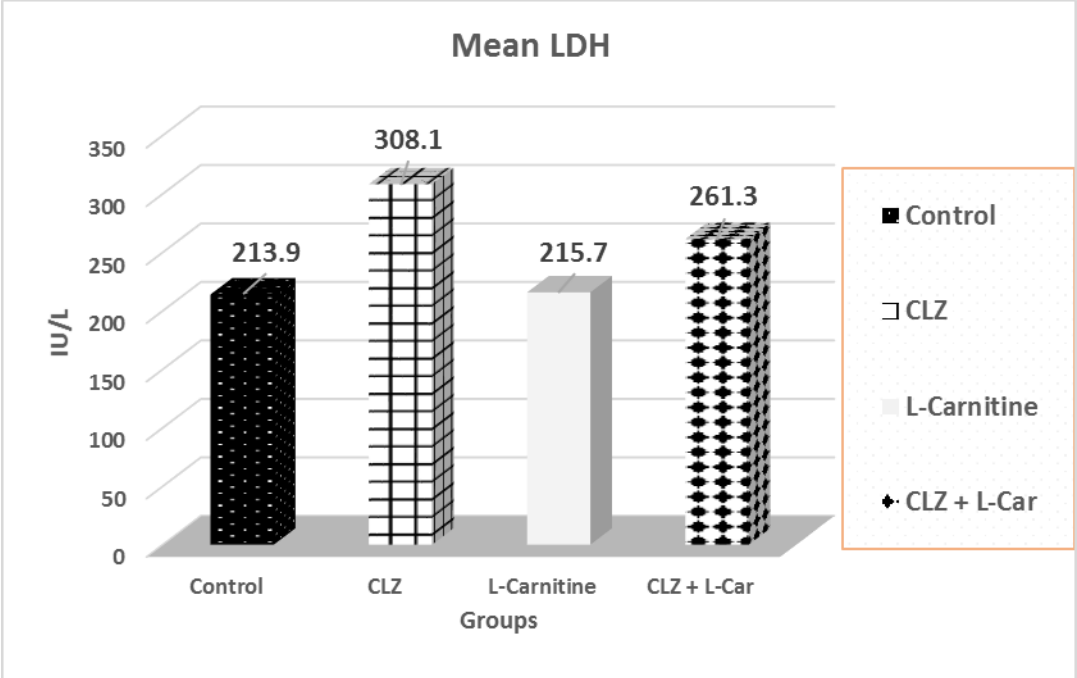

Figure (1): Mean values of LDH in different animals groups.

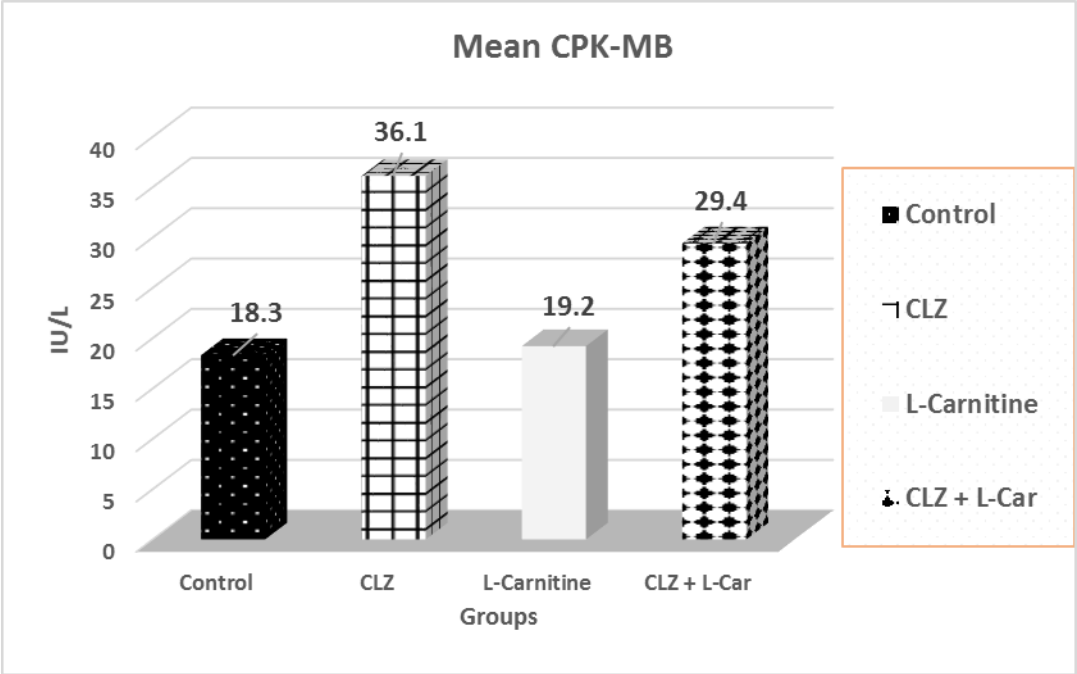

Figure (2): Mean values of CPK-MB in different animals groups.

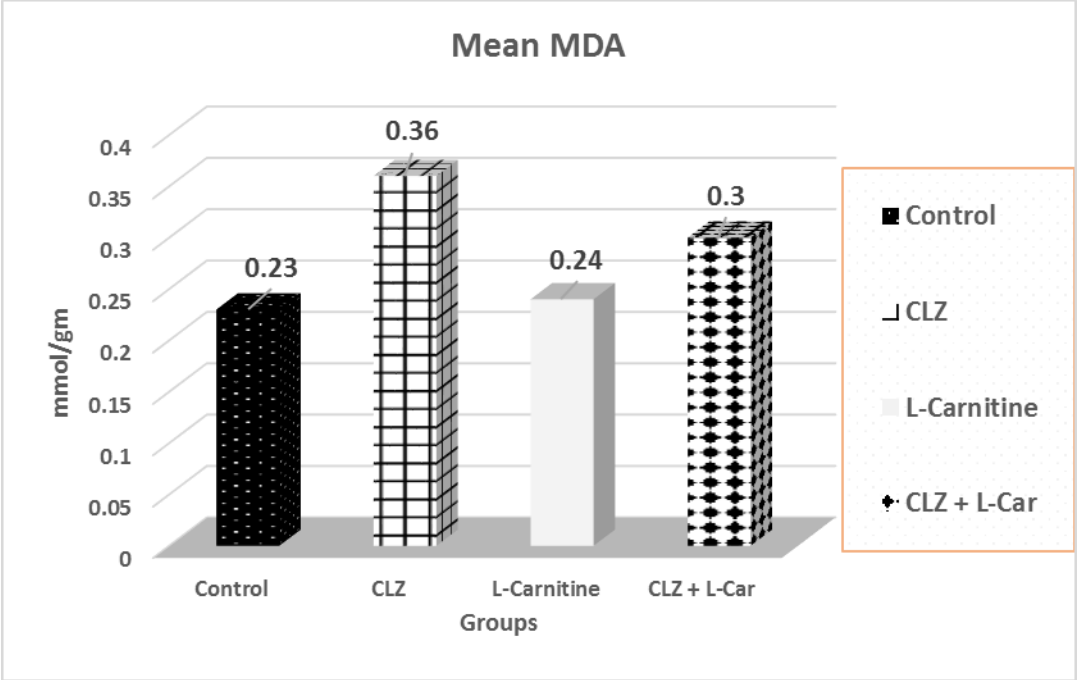

Figure (3): Mean values of MDA in different animals groups.

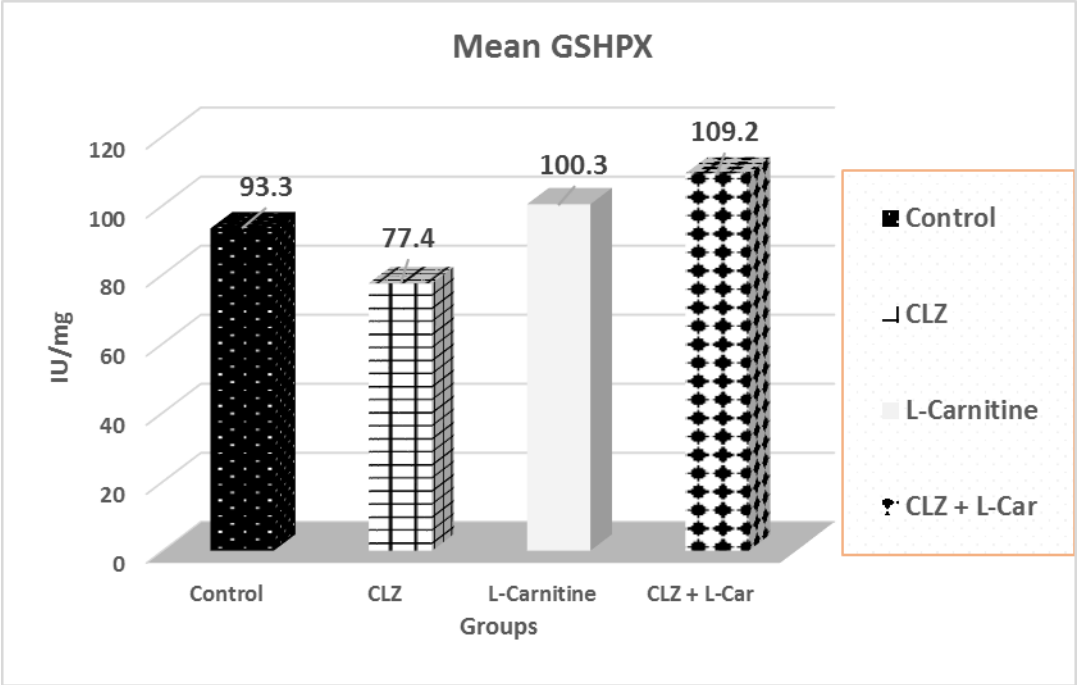

Figure (4): Mean values of GSHPx in different animals groups.

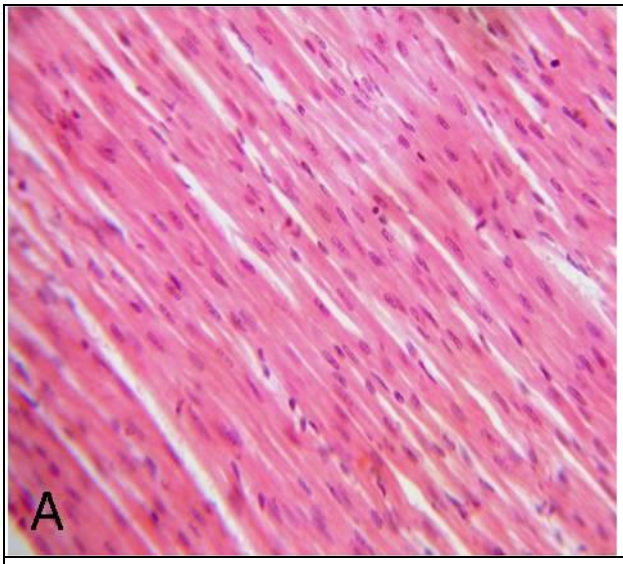

Figure (5- A):- A photomicrograph of a section of cardiac muscle control group and L-Carnitine showing branched striated cardiac muscle fibers cut longitudinally with acidophilic sarcoplasm and pale oval centrally located nuclei.

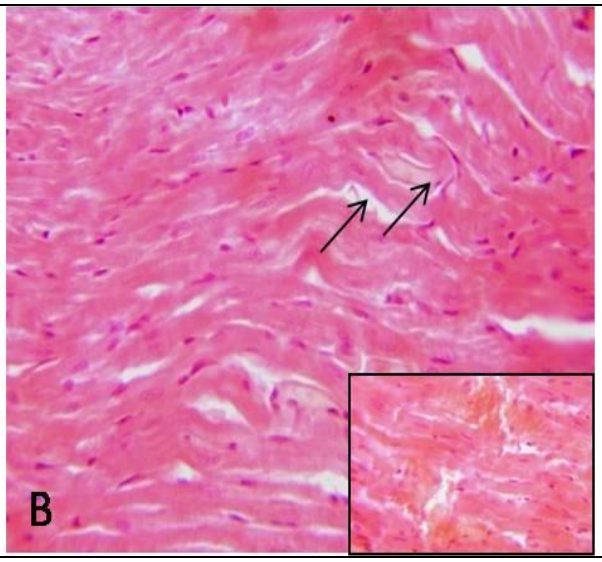

Figure (5-B):-. A photomicrograph of a section of cardiac muscle Clozapine group displaying degenerated and widly separated myofibers (arrow) with abnormal flat dense nuclei. Inset showing extravasated blood in degenerated myocardial fibers with pyknotic nuclei (arrow). Edema ↑↑ in between the myocardial fibers and areas of hemorrhage between the degenerated myofibers.

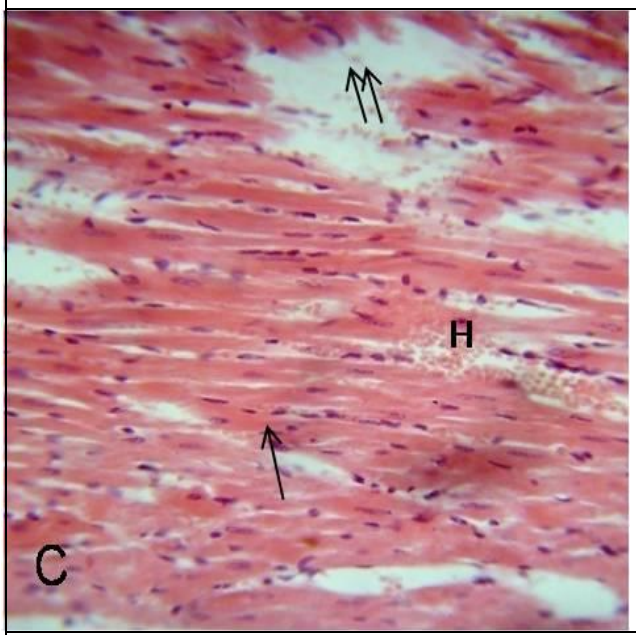

Figure (5-C): A photomicrograph of a section of cardiac muscle of some rats in clozapine group showed hemorrhage (H) and mononuclear cellular infiltration. (H&E X400)

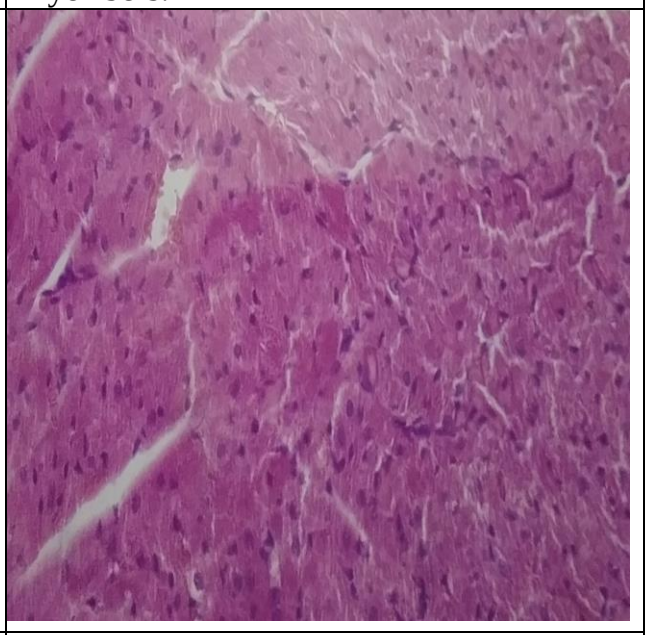

Figure (5-D): Photomicrograph of a section of heart of (CLZ+ L-Car) group showing normal histological structure of heart in which there are bundles of anastmosing cardiac muscle fibers.

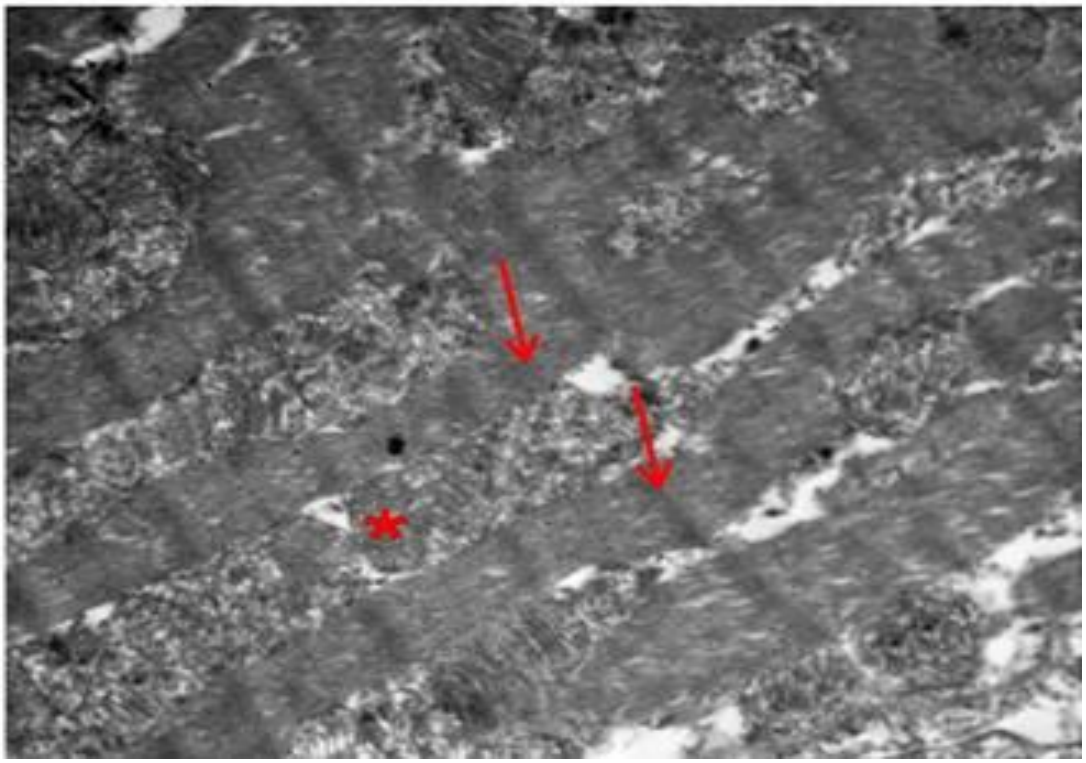

Figure (6):- A photomicrograph of TEM section of a heart from a rat of the control group showing regular arrangement of the myofibrils (arrows) and mitochondria (*)

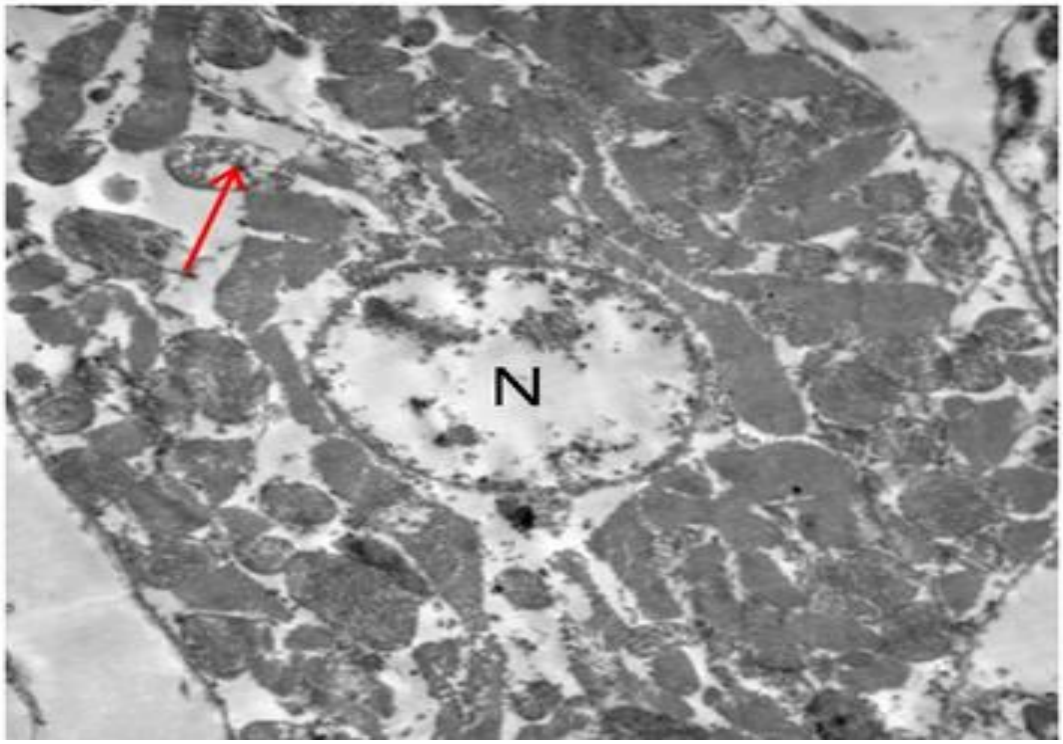

Figure (7):- A photomicrograph of TEM section of a heart from a rat of clozapine group showing degenerated nucleus (N), disorganized, fragmented myofibrils and some destructed mitochondria (arrow)

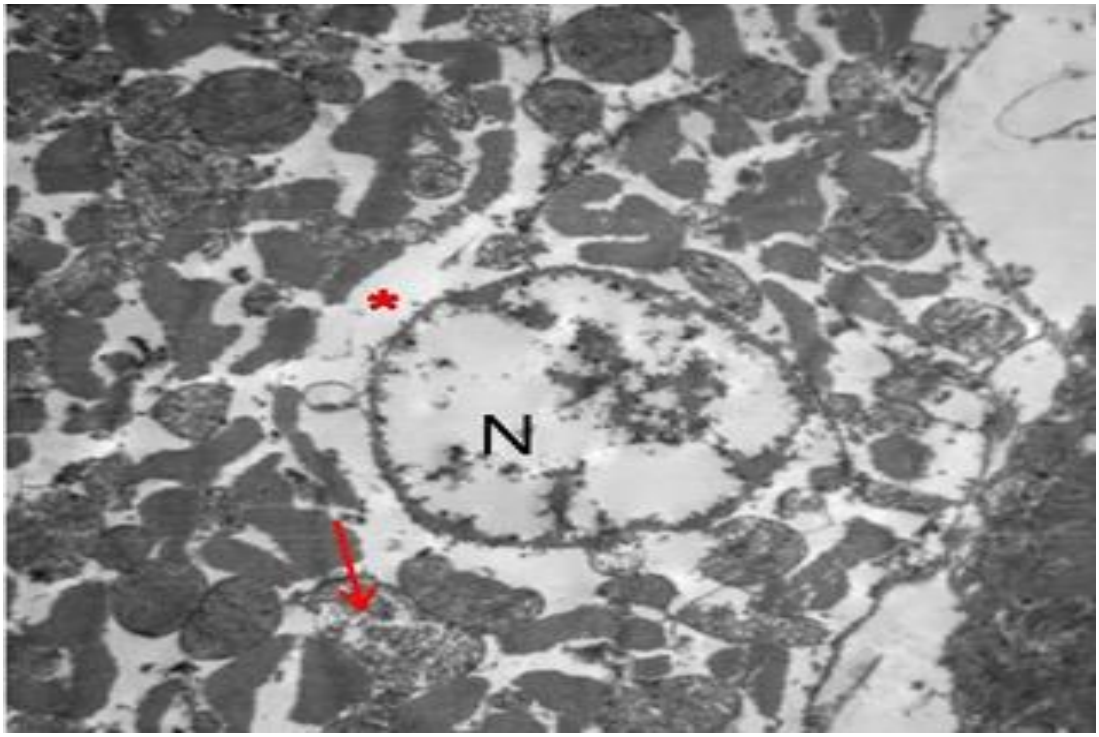

Figure (8): A photomicrograph of TEM section of a heart from a rat of clozapine group showing degenerated nucleus (N), marked disorganized, fragmented myofibrils and many destructed mitochondria (arrow). Notice wide spaces in the sarcoplasm of the cardiac myocytes (*)

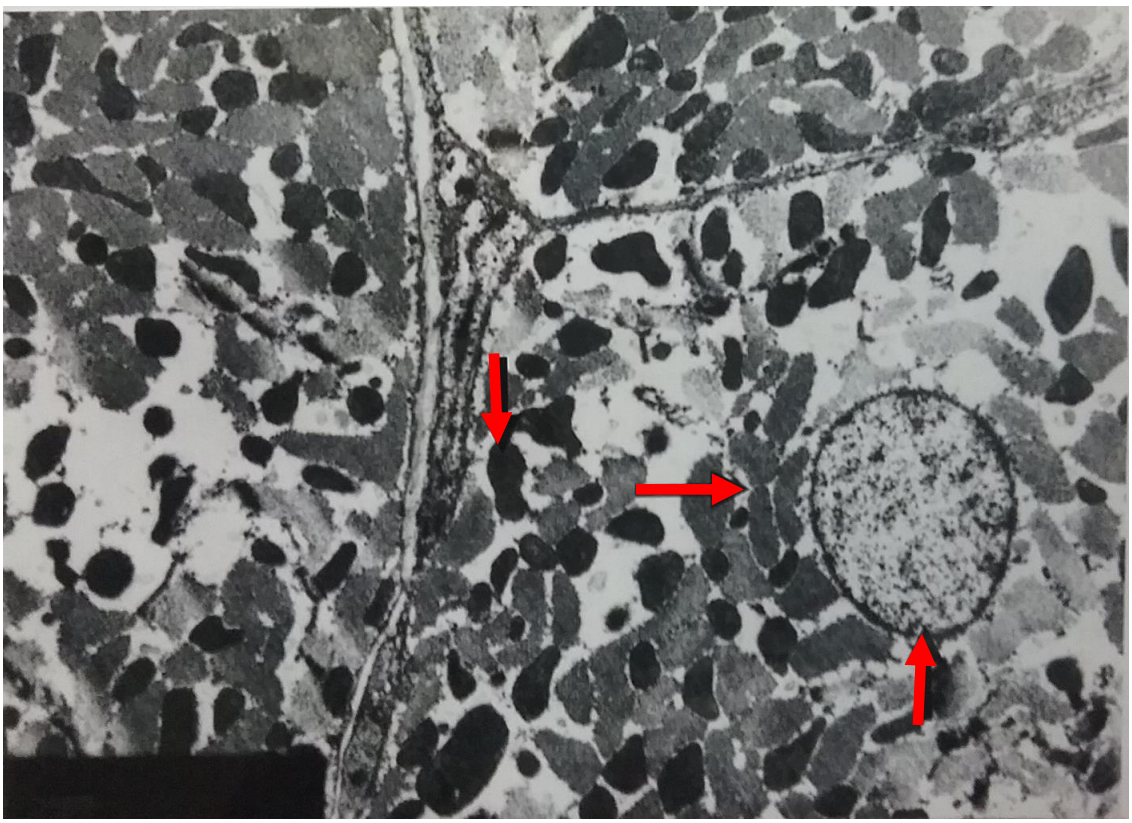

Figure (9): Electron micrograph of a heart of (L-carnitine+ CLZ) group showing normal structure of cardiac myofibrils (→) , normal mitochondria (↓), and normal nucleus (↑).

DISCUSSION

Clozapine, an atypical antipsychotic, is very effective in the treatment of resistant schizophrenia. However, cardiotoxicity of clozapine, particularly in young patients, has raised concerns about its safety **(wang et al., 2008).**

Myocarditis is one of the most publicized cardiotoxic side effects of clozapine. Also, dilated cardiomyopathy and pericarditis have been reported **(Newcomer, 2007).**

The mechanisms, by which clozapine causes cardiotoxicity, remain unclear. The current leading hypothesis is that of an IgE-mediated hypersensitivity reaction. This is supported by common observations of peripheral eosinophilia and eosinophilic inclusions within endomyocardial biopsy samples of affected patients, **(Kilian et al., 1999)** but these findings are inconsistent.

Elman et al., (1999) noted that patients treated with clozapine had higher noradrenaline levels than patients treated with other antipsychotics. While increased plasma noradrenaline levels may reflect existing left ventricular (LV) dysfunction, recent evidence suggests that catecholamines may actually cause cardiac dysfunction. For example, increased plasma catecholamine levels have recently been implicated in takotsubo cardiomyopathy, a reversible form of LV dysfunction **(Novak et al., 2007).**

Other unproven mechanisms include cytochrome 450 1A2/1A3 enzyme deficiencies, **(Devarajan et al., 2000)** blockade of calcium-dependent ion channels, increased production of inflammatory cytokines, and low serum selenium levels.

The present study revealed that, treatment with CLZ (25 mg/kg/d) for 28 days induced cardiotoxic effects in rats. CLZ-induced cardiotoxicity was evident from the laboratory results in the form of; elevated CK-MB and LDH levels in the serum of CLZ-treated rats. **Horacek et al. (2005)** demonstrated that biochemical markers of structural and functional myocardial damage have been gaining a ground in cardiotoxicity monitoring. Elevation in these enzymes level is considered as an important marker of cardiac injury **(Kemp et al., 2004).** The cardiotoxic effect was confirmed by histopathological changes in the heart as marked myocarditis had occurred.

The results of our study demonstrated that, treatment of rats with CLZ (25 mg/kg/d) for 4 weeks caused a significant increase in serum levels of both LDH and CK-MB compared with the control group. These results indicate occurrence of CLZ-induced cardiotoxicity. Co-administration of L-Carnitine with CLZ resulted in a significant decrease in the serum CK-MB level compared to treatment with CLZ alone. A decrease in the serum LDH level was also observed in serum of rats treated with L-Carnitine and CLZ when compared to serum of rats treated with CLZ alone

Disruption of cell membranes due to hypoxia or other injury releases CK from the cellular cytosol into the systemic circulation **(Daniel, 1990).** LDH-1 isozyme is normally found in the heart muscle and LDH-2 is found predominantly in blood serum. LDH levels are also high in tissue breakdown or hemolysis **(Rao et al., 1999).**

A marked significant elevation of myocardial lipid peroxidation product

level (MDA) and a significant reduction of antioxidant enzyme activity (GSH-Px) in CLZ-treated group in comparison with control group was obvious. These results confirmed the role of lipid peroxidation in CLZ-induced cardiac damage. Also, these findings support **Figueredo, (2011)** who reviewed that increased oxidative stress and weakening of antioxidant defenses play an important role in CLZ-induced myocarditis.

Malondialdehyde (MDA), the end product of lipid peroxidation, may be measured to indicate presence of radicals and lipid peroxidation-induced cardiotoxicity **(Bors et al., 1990).** Reduction of free radicals protects against lipid peroxidation, as reflected in this study by the decrease in the cardiac level of MDA.

Glutathione peroxidase (GSH-Px) is an important enzyme component of antioxidant defense for inactivation of peroxy radicals utilizing GSH. Myocardial GSH-Px activity was significantly reduced in rats treated with CLZ compared with the GSH-Px activity observed in control rats, reflecting the exhaustion of antioxidant enzymes by CLZ. CLZ depletes cardiac cells of selenium-dependant GSHPx an enzyme responsible for detoxifying oxygen-derived toxic species. Thus CLZ not only increase free radical production in the heart, but also decreases its ability to detoxify reactive oxygen species. Cardiac GSH-Px activity was significantly higher in rats co-administered CLZ with L-Carnitine than in rats treated with CLZ alone.

Many investigations had suggested that the increased oxidative stress associated with an impaired antioxidant defense status initiates a cascade of reactions responsible for CLZ-induced cardiotoxicity **(Heiser et al., 2010).** Free radical generation is induced by CLZ through direct and indirect mechanisms.

Directly, CLZ is bioactivated in the myocardial tissues to a chemically reactive nitrenium ion, which stimulates cellular injury, lipid peroxidation and free radical formation **(Williams et al., 2010).** Also, this nitrenium ion binds with proteins in the myocardium, leading to the formation of an antigenic complex that stimulates an immune response and macrophages **(Pirmohamed et al., 1995).** This triggers the release of proinflammatory cytokines such as TNF-α, which mediate cellular inflammation and myocarditis and generate additional free radicals. Increased oxidative stress may attenuate the release of anti-inflammatory cytokines such as IL-10, thereby enhancing inflammatory processes in cardiac tissues. This may represent a significant pathway for the development of myocarditis.

Indirectly, the above-mentioned CLZ-induced increase in catecholamines triggers tachycardia and increases myocardial oxygen demand by increasing work done and cardiac loads via catecholamines-induced vasoconstriction **(Braunwald, 2000).** In addition, catecholamines decrease myocardial oxygen perfusion via coronary vasoconstriction **(Simons & Downing, 1985)**

In response to long-term exposure to CLZ, these events can lead to myocardial ischemia and free radical generation. The released free radicals and reactive oxygen species (ROS) can attack membrane phospholipids, increasing lipid peroxidation and damage to the myocardial cell membrane. This process can explain

the elevated myocardial MDA level observed in this study.

As regards histopathological results, administration of CLZ for 4 weeks revealed degenerated and widly separated myofibers with pyknotic nuclei, odema in between the myocardial fibers, areas of hemorrhage between the degenerated myofibers and mononuclear cells infiltration (inflammatory cells). These findings were greatly similar to **Basel et al., (2014)** who observed focal subendocardial fibrosis, marked interstitial edema and perinuclear vacuolation, inflammatory lesions in both the ventricles, mainly in the myocardium below the endocardium of the left ventricle. Similar histopathological changes have been reported by **wang et al., (2008).**

Electron microscopic studies showed that CLZ damages myocardial cells (in the form of degenerated nucleus, disorganized, fragmented myofibrils and some destructed mitochondria). These damages disappeared on using L-carnitine with CLZ.

Co-administration of L-Carnitine with CLZ in this study attenuated CLZ-induced myocarditis. This was evidenced by significant reduction in the activities of serum cardiac enzymes and the improvement of the histological changes in cardiac tissues associated with cardiotoxicity in rats co-administered with CLZ and L-Carnitine compared with rats treated with CLZ alone.

In addition, co-administration of L-Carnitine with CLZ remarkably attenuated the effect of CLZ on biochemical markers of oxidative stress and lipid peroxidation, reflecting a great antioxidant role of L-Carnitine. A study by **Gülçin (2006),** L-Carnitine was found to be an effective antioxidant agent in different in vitro assays, especially due to its capacity to scavenge hydrogen peroxide and superoxide radical and also chelate transition metal ions. L- Carnitine may also protect the endogenous antioxidant defense system, including GSHPx, Catalase and superoxide dismutase activities from peroxidative damage **(Augustyniak and Skrzydlewska, 2010, Binienda and Ali, 2001 and Li et al., 2012).** In another study, treatment with L- Carnitine provoked an upregulation of antioxidant enzymes, an increase in the plasma total antioxidant capacity and a reduction of lipid peroxidation and superoxide radical production in the heart of spontaneously hypertensive rats **(Miguel-Carrasco et al., 2010)**.

Recent studies have reported that L-Carnitine may protect cells against oxidative damage in important neurodegenerative disorders, such as in Parkinson's and Alzheimer's diseases **(Abdul and Butterfield, 2007 and Beal, 2003).** L- Carnitine treatment also was shown to prevent oxidative injury in renal and cardiovascular disease models, in vascular dysfunction and in diabetes mellitus **(Rajasekar and Anuradha, (2007) & Uysal et al., (2005). Zambrano, (2013)** showed that LC improved the oxidative stress in renal cortex of hypertensive rats

CONCLUSION

L-Carnitine can protect against myocarditis induced by CLZ in rats. The cardio-protective effect of L-Carnitine was accompanied with a significant attenuation of CLZ's effect on serum cardiac enzymes, oxidative stress parameter (MDA) and activation

of antioxidant defenses (GSH-Px). The previous findings in this study, reflect the multiple mechanisms in the cardioprotective effect of L-Carnitine against CLZ-induced myocarditis may be involved. It can be suggested that using L-Carnitine in the protection against CLZ-induced myocarditis in patients with psychiatric illnesses may be beneficial.

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الملخص العربي

التهاب عضلة القلب المستحث بواسطة الكلوزابين في الفئران: دور ال-كارنيتين في الوقاية

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الكلوزابين هو أحد مضادات الذهان غير النمطية وغالبا ما تستخدم في الفصام العنيد. أجريت هذه الدراسة للتحقق من التهاب عضلة القلب الناجم عن استخدام عقارالكلوزابين في الفئران البيضاء وتحديد ما إذا كان ال-كارنيتين يمكن أن يقلل من التهاب عضلة القلب وعرض تأثيراته الواقية على القلب. قسمت الفئران إلى أربع مجموعات على النحو التالي؛ المجموعة الأولى: الضابطة؛ المجموعة الثانية: حقنت بالكلوزابين بالحقن البريتوني بجرعة ٢٥ ملج / كج / يوميا لمدة ٤ اسابيع ، المجموعة ٣: حقنت ال-كارنيتين بالحقن البريتوني بجرعة ٥٠٠ ملج / كج / يوميا لمدة ٤ اسابيع ، والمجموعة الرابعة: حقنت الكلوزابين بالحقن البريتوني ٢٥ ملج / كج / يوم بالاضافة الى ال-كارنيتين بالحقن البريتوني ٥٠٠ ملج / كج / يوميا لمدة ٤ اسابيع. تم قياس مستويات انزيم لاكتات ديهيدروجينيز و انزيم الكرياتين فسفوكيناز ام-بي (CPK-MB)، وقياس المالون داي الدهيد والجلوتاثيون البيروكسيديز في انسجة البطين الأيسر للقلب. تم إجراء فحص الأنسجة من القلب بواسطة المجهر الضوئي والإلكتروني. وقد كشفت الدراسة الحالية ارتفاع إحصائي كبير لإنزيمات القلب في الدم لاكتات ديهيدروجينيز و الكرياتين فسفوكيناز في المجموعة التي تلقت العلاج بالكلوزابين بالمقارنة بالمجموعة الضابطة. كما لوحظ انخفاض بدرجة كبيرة من إنزيمات القلب في الفئران التي تناولت ال-كارنيتين مع الكلوزابين بالمقارنة مع المجموعة التي تلقت العلاج بالكلوزابين. هناك زيادة ذات دلالة إحصائية في مستوى المالون داي الدهيد في الأنسجة مع انخفاض كبير في مستوى الجلوتاثيون البيروكسيديز بالأنسجة وذلك في المجموعة التي عولجت بالكلوزابين مقارنة بالمجموعة الضابطة. وفي الوقت نفسه، لوحظ انخفاض كبير في مستوى المالون داي الدهيد فى الأنسجة مع زيادة كبيرة في مستوى أنسجة الجلوتاثيون البيروكسيديز في الفئران التي تناولت الكلوزابين &ال-كارنيتين بالمقارنة مع المجموعة التي تلقت الكلوزابين. باستخدام المجهر الضوئي، أظهرت المجموعة التي تلقت العلاج بالكلوزابين بعض التغيرات التشريحية المرضية في صورة تدهور و فصل كبير للالياف العضلية، ارتشاح بين الليفات العضلية، ومناطق نزفية، وتجمع الوحيدات الخلايا الالتهابية. باستخدام المجهر الإلكتروني وجد ان الميتوكوندريا دمرت، ومساحات واسعة في sarcoplasmالخلايا القلبية، تحولت النواة، ، الليفات العضلية مفتتة وغير منتظمة في المجموعة التي تلقت عقار الكلوزابين. اختفت التغيرات الهستولوجية المرضية في المجموعة التي تناولت ل-كارنيتين & CLZ. خلصت الدراسة إلى أن الكلوزابين احدث التهاب بعضلة القلب وقد تحسن جزئيا باستخدام ال-كارنيتين الذي كان له دور وقائي في الحماية من التهاب عضلة القلب الناتج من استخدام الكلوزابين في الفئران.