Original Article

Evaluation of Azithromycin Induced Cardiotoxicity in Male Albino Rats and the Possible Protective Role of Nigella Sativa Oil

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ABSTRACT

Background: Azithromycin (AZ) is a broad spectrum macrolide antibiotic frequently used in treatment of bacterial infections. There are many cardiac adverse effects asso¬ciated with AZ treatment according to case reports and cohort studies. However, there are only few published experimental studies reported its cardiotoxicity in rats.

Aim of the Work: The present work aimed to study the histopathological changes in the heart of male albino rat induced by AZ treatment. Also, to clarify the underlying mechanisms regarding oxidative stress, inflammatory marker release, apoptotic cell-death and myocardial fibrosis. Finally, the supposed protective effects of nigella sativa oil (NSO) co-treatment were assessed.

Material and Methods: Twenty four adult male albino rats were divided into equal 4 groups; Control, AZ (30 mg/ kg/ day), AZ (30 mg/ kg/ day) +NSO (4 ml/ kg/ day), and NSO (4 ml/ kg/ day). The drugs were administrated intragastrically once daily for 2 weeks. Rats were sacrificed, blood and tissue samples were collected and processed for biochemical, histolopathological and immunohistochemical studies.

Results: AZ treated group showed marked elevation in creatine phosphokinase (CPK), lactate dehydrogenase (LDH), Malondialdehyde (MDA), and tumor necrosis factor alpha (TNFα) levels. It induced significant myocardial necrosis, fibrosis, and apoptosis. It was the first time to demonstrate the effect of AZ on myofibroblasts proliferation by evaluating alpha smooth muscle actin (α-SMA) immunohitochemical expression which revealed significant increase after AZ treatment. Co-treatment with NSO significantly lowered CPK, LDH, MDA, and TNFα levels, preserved the cardiac morphology, and decreased Caspase-3 and αSMA immunoreactivity as compared to AZ group.

Conclusion: Concluded that AZ induced cardiac adverse side effect in rats. NSO could prevent AZ-induced cardiotoxicity, and its mechanism may be related to antioxidant, antiinflammatory, antiapoptotic and antifibrotic properties. Further studies are required to confirm the efficacy of NSO as a protective agent in human AZ intoxication.

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Key Words: Azithromycin, biochemical, histological, immunohistochemical, nigella sativa oil.

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INTRODUCTION

Azithromycin (AZ) is a macrolide antibiotic used as first line therapy for prophylaxis and treatment of Mycobacterium avium intracellulare disseminated infection in acquired immunodeficiency syndrome (AIDS) patients and for the treatment of pulmonary disease in AIDS patients[1,2]. Twenty cases of Torsades de pointes associated with AZ have been reported according to the data from the adverse effect notification system of FDA (Food and Drug Administration)[3]. Furthermore, the results of cohort studies conducted on patients who received AZ treat¬ment reported sud¬den deaths due to ventricular arrhythmia as a result of AZ use[4].

Although these adverse effects asso¬ciated with AZ treatment, there is only two recently published experimental studies reported its cardiotoxicity in rats[5,6]. However, none of them studied the effect of AZ on myofibroblasts proliferation which is the main cause of myocardial fibrosis that leads to increased myocardial wall stiffness, cardiac dysfunction, and arrhythmias[7].

Nigella sativa (NS) seeds were documented to have antioxidant[8], anti-inflammatory[9], and antihistaminic effects[10]. It has been reported that NS attenuates isoproterenol induced myocardial infarction (MI)[11]. Also, Nigella sativa oil (NSO) supplementation reduced lead-induced cardiotoxicity by mechanisms related to its ability to decrease the pro-inflammatory cytokines, oxidative stress and cardiac tissue damage, and preserve the activity of antioxidant enzymes[12].

Therefore, the present work aimed to clarify the histopathological changes in the left ventricular myocardium of male albino rats treated with AZ. Also, the underlying mechanisms regarding oxidative stress, inflammatory marker release, apoptosis, myofibroblasts proliferation and fibrosis were studied. Finally, the supposed protective effects of nigella sativa oil (NSO) co-treatment were assessed.

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MATERIALS AND METHODS

Experimental Animals

Twenty four adult male albino rats weighting 200-250 gm and aging 10-12 weeks were obtained from AL-Nile Experimental Animal Center, Mansoura, Egypt. The animals were housed in separate stainless steel mesh cages (three rats/cage) at a constant temperature (20±2°C), humidity (50±10%), and illumination (12 h light/12 h darkness). All rats were maintained under specific pathogen-free conditions with ad libitum access to food and water. All the experiments were carried out according to the rules and regulations laid down by the committee on animals' experimentation of Mansoura University.

Chemicals

1. Nigella sativa oil was purchased from local pharmacy. It was manufactured by Pharco-Company, Alameria, Alexandria.
2. Azithromycin powder was purchased from local pharmacy. It was manufactured by Hikma Pharma S.A.E, 6TH OF October City, Egypt.

Experimental protocol

The animals were divided randomly into 4 groups 6 rats each.

Group 1 (CN): Control non-treated group. Rats were received equivalent amount of distilled water intra-gastrically.

Group 2 (AZ): Azythromycin (AZ) treated group. Rats were received AZ (30 mg/ kg/ day) intra-gastrically for 2 weeks[5]. The solution was prepared at concentration of 20mg/1ml by dissolving 300 mg Azithromycin powder in 15 ml distilled water.

Group 3 (AZ+NSO): Azythromycin and Nigella sativa oil (NSO) co-treated group. Rats were received both AZ (30 mg/ kg/ day) and NSO (4 ml/ kg/ day) intra-gastrically for 2 weeks[12].

Group 4 (NSO): rats of this group were received only NSO (4 ml/ kg/ day) intra-gastrically for 2w.

Sacrifice of Rats and Specimens Collection

Rats of each group were anaesthetized with Ketamine (60 mg/kg i.p.), thoracotomy was done, blood samples were collected, and heart tissues were dissected, immediately and fixed in 10% formol saline for one day.

Biochemical Study

Assessment of Cardiac Enzymes

Serum creatine phosphokinase (CPK) and serum lactate dehydrogenase (LDH) were measured using the colorimetric kit (Stanbio Laboratory, Boerne, Texas 78006, USA)[13,14].

Assessment of oxidative stress state

Lipid peroxide Malondialdehyde (MDA) was measured in the plasma using a commercially available kit (Biovision, cat. No. K739-100, San Francisco, USA). Briefly, plasma was mixed with trichloroacetic acid and heated in a boiling water bath for 30 min. MDA was determined by the absorbance at 532 nm in a spectrophotometer[15].

Assessment of Inflammatory Marker

Plasma concentrations of Tumor Necrosis Factor Alpha (TNF-α) were measured using ELISA assay kits (Assaypro, USA)[6].

Histolopathological Study

Formalin fixed heart tissues were used for paraffin sections (5µm). The slides were stained with hematoxylin-eosin (H&E)[16] and Masson's trichrome[17] and observed under a light microscope, then photomicrographs were taken to assess the histopathological changes.

Immunohistochemical Study

For immunohistochemical staining, the heart sections (5 µm) were deparaffinized and rehydrated. Nonspecific endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide. Then, antigen retrieval was done by heating the sections in citrate buffer (0.01 M, pH 6.0), in a water bath at 95°C for 30 min. Sections were incubated with primary antibodies; anti-Caspase-3 (ab2302, Abcam, Cambridge, UK, 1:1000)[18] and Anti-alpha-smooth muscle actin (α-SMA) (abcam,ab5694, 1:100)[19] at 4oC overnight. These sections were incubated with biotinylated horse antimouse IgG for 30 min and thereafter with the avidin-biotin peroxidase complex. The reaction was visualized with DAB solution after counterstaining with hematoxylin. The positive control of Caspase-3 was done in tissue of tonsil, and that of α-SMA was done in tissue of colon according to the manufacturer instructions. Omission of the primary antibody served as a negative control for each sample.

Morphometric study

Ten fields from each slide were examined and photographed using Olympus® CX41light microscope (X400) with Olympus® SC100 digital camera installed on it. Morphometric study was done using program NIH Image J program (National Institutes of Health, Bethesda, MD, USA), according to the program instruction.

The following parameters were measured

The mean area percentage of collagen fibers in Masson's trichrome stained sections were measured in ten non overlapping fields per slide[20].

The mean area percentage occupied by brown pixels for Caspase-3[18] and α-SMA[21] were measured in ten non-overlapping fields per slide.

Statistical Analysis

Statistical analyses of data were carried out using SPSS v22 program. Data were expressed as the mean ± SD. Comparisons between groups were made with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. Statistical significance occurred at $P \leq 0.05$.

RESULTS

Biochemical results

Assessment of Cardiac Enzymes

Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were measured in different groups (Table 1). The AZ group showed significant increase in both CPK and LDH compared to CN group. The levels of these enzymes were significantly reduced in AZ+NSO co-treated group compared to AZ group. Rats administered with NSO alone did not show significant changes compared to CN group (Histogram 1).

Assessment of Plasma MDA

Plasma MDA levels were measured in different groups to assess the oxidative stress state (Table 1). There was significant increase in MDA level of AZ group compared to CN group. On the other hand, AZ+NSO co-treated group showed significant decrease in MDA level to reach the CN group level (Histogram 2).

Assessment of Plasma TNFα

Plasma TNFα levels were measured in different groups to assess the inflammatory state (Table 1). Significant elevation in the TNFα level was observed in AZ group when compared to CN group. The level of TNFα was significantly decreased in AZ+NSO co-treated group compared to AZ group. Treatment with NSO per se did not show any significant change in the level of TNFα compared to CN group (Histogram 3).

Histopathological Results

Histological examination of the CN group by routine H&E stain showed regular arrangement of cardiac muscle fibers with apparent normal striations and branching. The cardiac myocytes showed normal histological architecture with acidophilic sarcoplasm and central oval nuclei. Few small blood capillaries were evident in the intercellular spaces (Figure 1).

Histological examination of the AZ group revealed marked distortion, fragmentation, and loss of cardiac muscle striation. Signs of myocardial necrosis in the form of hypereosinophilia, cytoplasmic vacuolation, and peripheral pyknotic nuclei were evident. There was marked increase in the tissue spaces with interstitial edema, inflammatory cellular infiltration. Dilated, congested, and even ruptured blood vessels were noticed (Figure 2,3).

Co-treatment of AZ-treated rats with NSO resulted in marked preservation of cardiomyocyte morphology and tissue space with no evidence of focal necrosis. Normal myocardial cells with minimal vacuolization of the cytoplasm were observed. There were little cellular infiltration and absent interfibrillar hemorrhage (Figure 4). Histological examination of the NSO group showed the same finding as the CN group (Figure 5).

The myocardial fibrosis was evaluated in all studied groups using Masson's-staining. The CN group showed minimal amount of blue stained fibers between the cardiac muscles (Figure 6). The AZ treated group illustrated significant increase in the percentage area of collagen fibers which was deposited mainly around engorged dilated blood vessel (Figure 7, 8, 9). The AZ+NSO co-treated group revealed minimal collagen fiber deposition between cardiac myocytes (Figure 10) with significant decrease in its percentage area as compared with AZ group. The NSO group showed few amount of blue stained fibers with non-significant difference as compared to the CN group (Figure 11). Differences in the mean Masson's stained surface areas between all studied groups were shown in (Table 2) (Histogram 4).

Immunohistochemistry for Caspase-3

Examination of the Caspase-3 immunostained sections in CN group showed minimal Caspase-3 expression in the sarcoplasm of the cardiac muscle fibers (Figure 12). Sections of AZ group revealed significant increase in Caspase-3 immunoreactivity compared to CN group (Figure 13). Co-treatment of AZ-treated rats with NSO resulted in marked decrease in Caspase-3 expression compared to AZ group (Figure 14). The NSO group demonstrated a non-significant difference as compared to the CN group (Figure 15). Differences in the mean Caspase-3 immunnostained surface areas between all studied groups were shown in (Table 2) (Histogram 5).

Immunohistochemistry for αSMA

Examination of the αSMA immunostained sections in CN group showed negative αSMA expression in the myocardium and positive immunoreactivity in the smooth muscle fibers of the blood vessels (Figure 16). Myocardial sections of AZ group revealed significant increase in αSMA surface area compared to CN group. There were intensely stained elongated cells among the cardiomyocytes (Figure 17). These elongated cells were characterized as myofibroblasts. Sections from AZ+NSO group exhibited minimal positive αSMA immunoreactivity in the myocardium with significant decrease in its surface area compared to AZ group (Figure 18). The NSO group revealed the same CN pattern of αSMA expression (Figure

19). Differences in the mean αSMA immunnostained surface areas between all studied groups were shown in (Table 2) (Histogram 6).

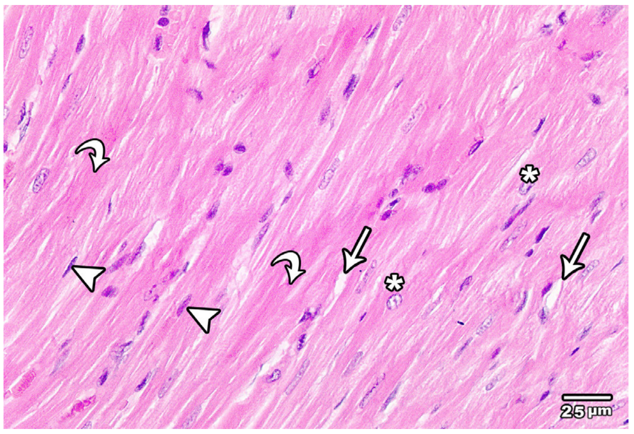


Fig. 1: A photomicrograph of left ventricle of control rat showing regular arrangement of cardiac muscle fibers, acidophilic sarcoplasm (curved arrows) and central oval vesicular nuclei (*) of the cardiac myocytes. Few small blood capillaries (arrows) and elongated nuclei of interstitial cells (arrow heads) are seen in the interfiber spaces (H&E X400).

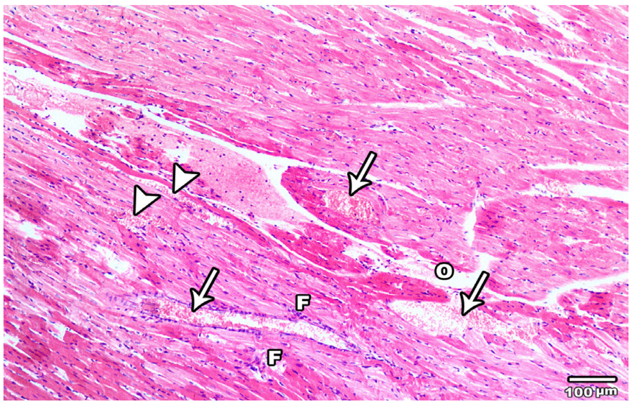


Fig. 2: A photomicrograph of left ventricle of azithromycin treated rat showing distortion of cardiac muscle striation. There is marked increase in the tissue space with interstitial edema (O). The blood vessels are dilated and congested (arrows). Intrafibrillar hemorrhage (arrow heads) and inflammatory cellular infiltration (F) are evident (H&E X100).

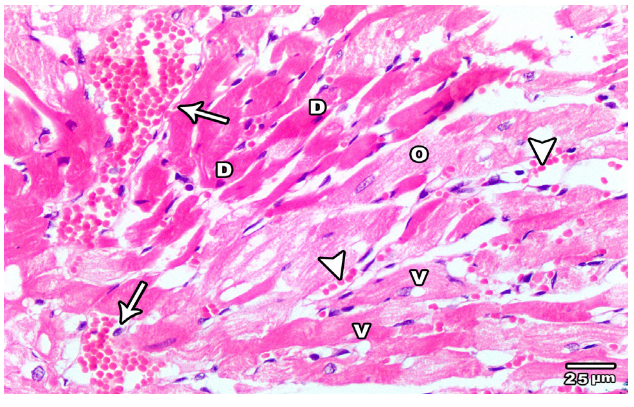


Fig. 3: A photomicrograph of left ventricle of azithromycin treated rat showing marked distortion, fragmentation of cardiac muscle with intrafibrillar hemorrhage (arrow heads). Signs of myocardial necrosis in the form of hypereosinophilia, cytoplasmic vacuolation (V), and peripheral pyknotic nuclei (D) are evident. There are marked increase in the tissue spaces with interstitial edema (O) and dilated and congested blood vessels (arrows) (H&E X100).

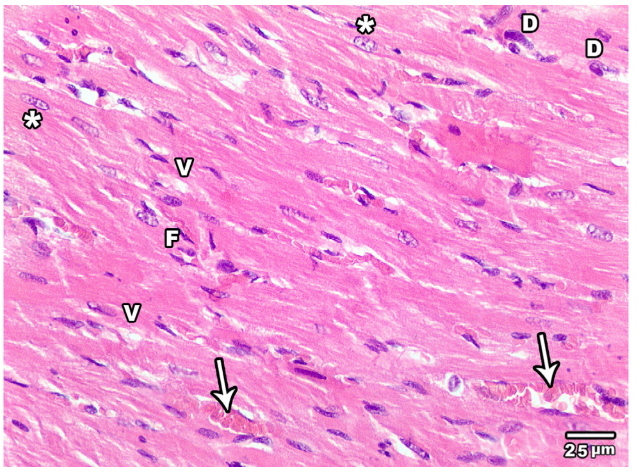


Fig. 4: A photomicrograph of left ventricle of azithromycin and nigella sativa oil co-treated rat showing preserved cardiomyocyte morphology with central oval vesicular nuclei (*). Few myocytes represent degenerated nuclei (D) and mini¬mal vacuolization (V) of the cytoplasm. Nearly normal tissue space with some congested dilated blood vessels (arrows) and little cellular infiltration (F) (H&E X400).

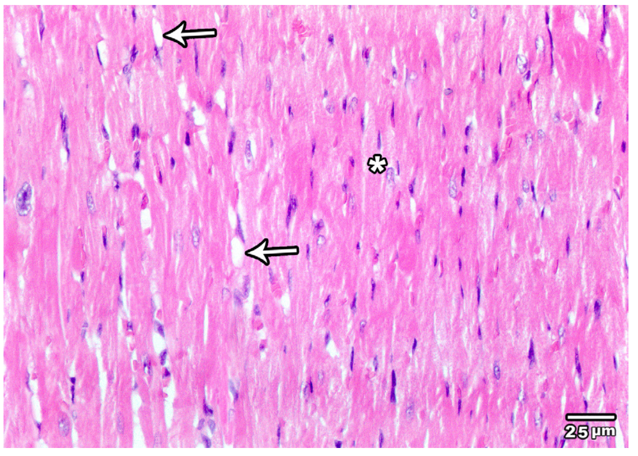


Fig. 5: A photomicrograph of left ventricle of rat treated only by nigella sativa oil showing apparent normal cardiac striation, cardiac myocytes with acidophilic sarcoplasm and central oval vesicular nuclei (*). The intercellular spaces represent few small blood capillaries (arrows) (H&E X400).

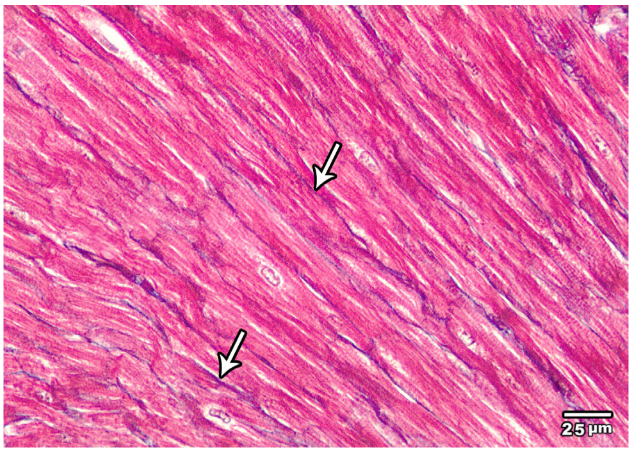


Fig. 6: A photomicrograph of left ventricle of control rat showing scanty blue stained collagen fibers (arrows) between the cardiac muscles (Masson's trichrome X400).

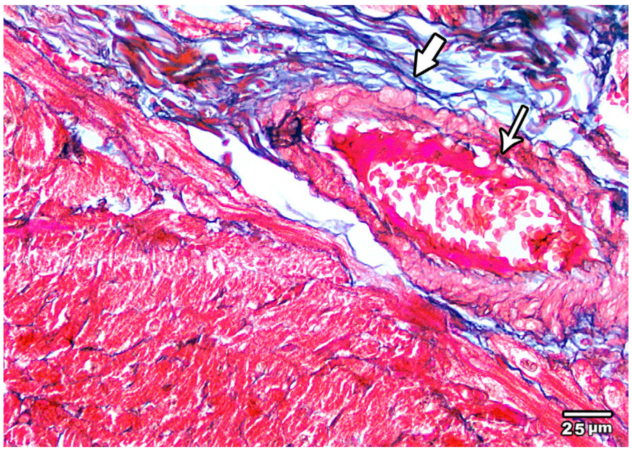


Fig. 7: A photomicrograph of left ventricle of azithromycin treated rat showing large amount of blue stained collagen fibers (thick arrow) around dilated congested blood vessel (thin arrow) (Masson's trichrome X400).

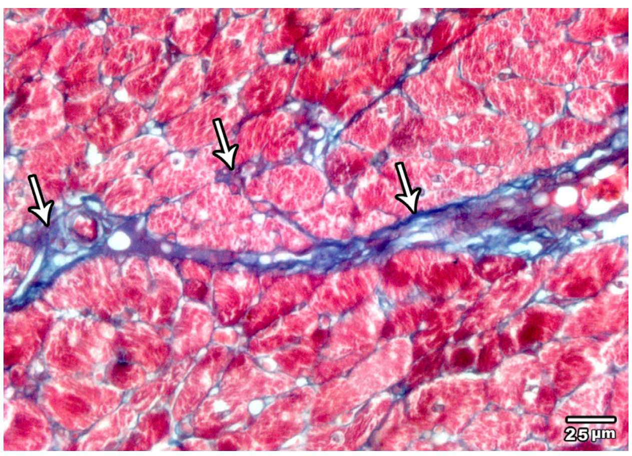


Fig. 8: A photomicrograph of left ventricle of azithromycin treated rat showing thick blue stained collagen fibers (arrows) between the cardiac muscles and around congested blood vessel (Masson's trichrome X400).

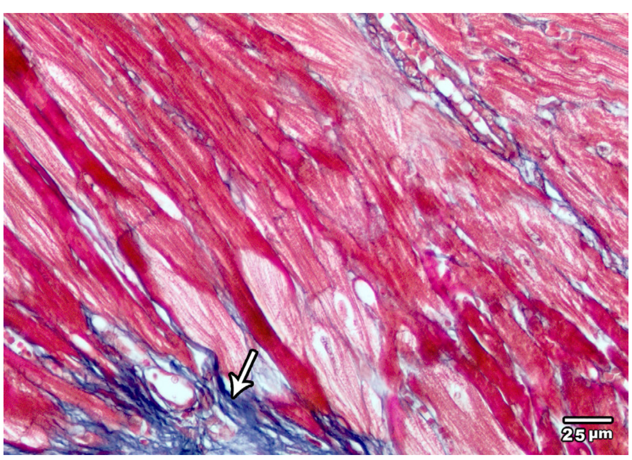


Fig. 9: A photomicrograph of left ventricle of azithromycin treated rat showing blue stained fibrotic area between the cardiac muscles (arrow) (Masson's trichrome X400).

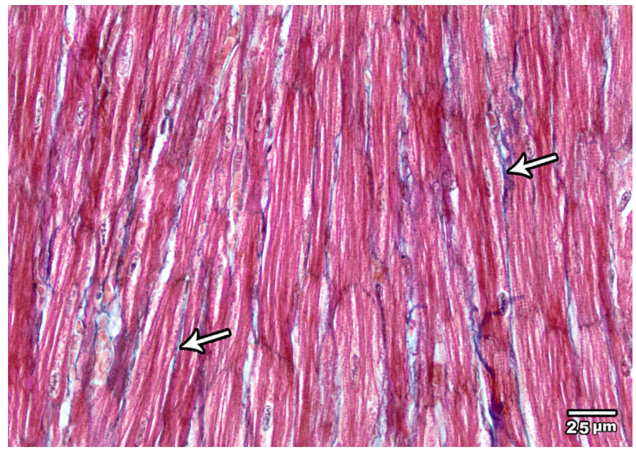


Fig. 10: A photomicrograph of left ventricle of azithromycin and nigella sativa oil co-treated rat showing some blue stained collagen fibers (arrows) between the cardiac muscles (Masson's trichrome X400).

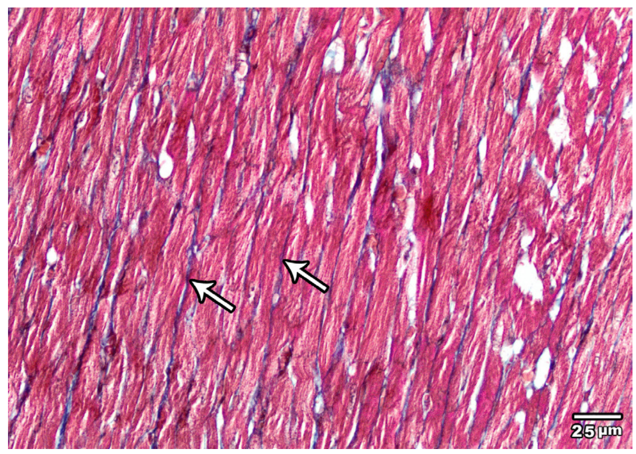


Fig. 11: A photomicrograph of left ventricle of nigella sativa oil treated rat showing few amount of blue stained fibers (arrows) between the cardiac muscles (Masson's trichrome X400).

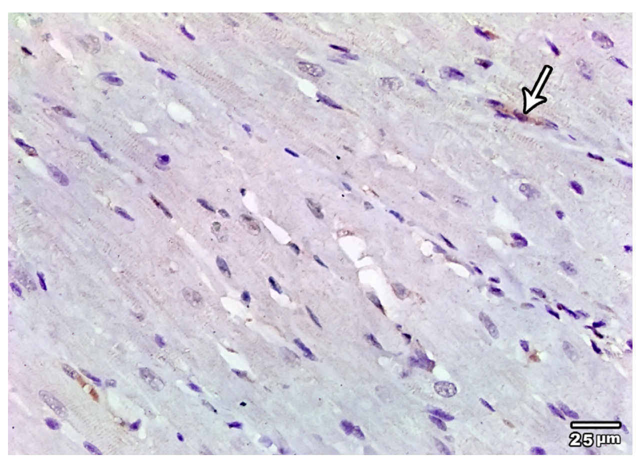


Fig. 12: photomicrograph of left ventricle of control rat showing minimal Caspae-3 expression (arrow) (Caspase-3 immunohistochemistry with H&E counter stain, X400).

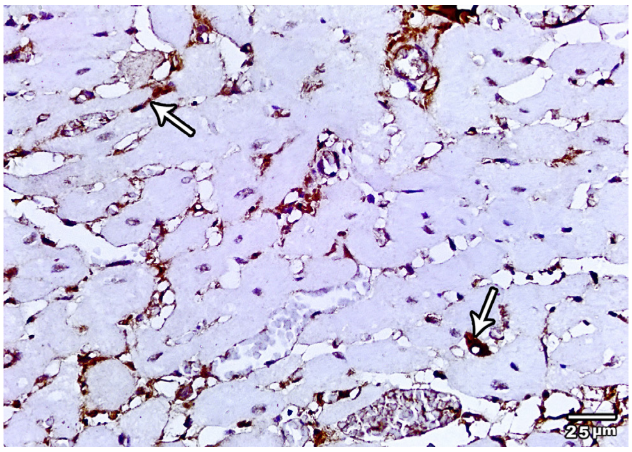


Fig. 13: photomicrograph of left ventricle of azithromycin treated rat showing strong positive Caspase-3 expression (arrows) (Caspase-3 immunohistochemistry with H&E counter stain, X400).

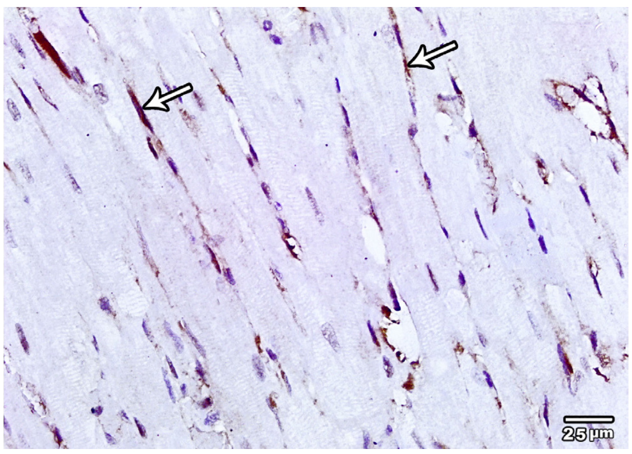


Fig. 14: photomicrograph of left ventricle of azithromycin and nigella sativa oil co-treated rat showing mild positive Caspase-3 expression (arrows) (Caspase-3 immunohistochemistry with H&E counter stain, X400).

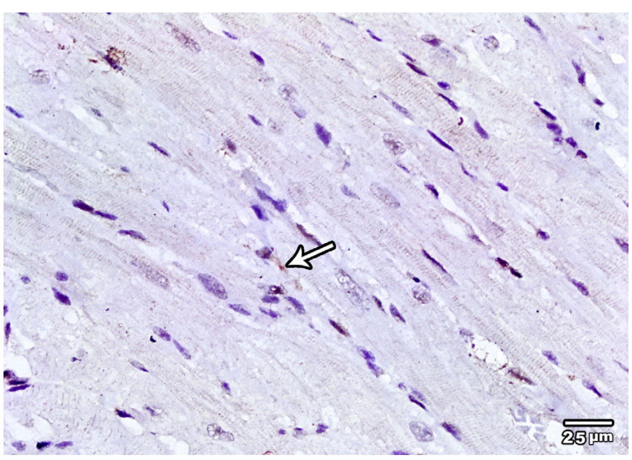


Fig. 15: photomicrograph of left ventricle of nigella sativa oil treated rat showing very weak Caspase-3 expression (arrow) (Caspase-3 immunohistochemistry with H&E counter stain, X400).

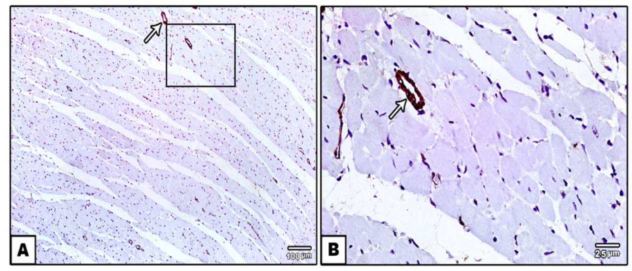


Fig. 16: photomicrograph of left ventricle of control rat showing minimal αSMA expression mainly in the wall of the blood vessels (arrows) (αSMA immunohistochemistry with H&E counter stain, A X100, B X400).

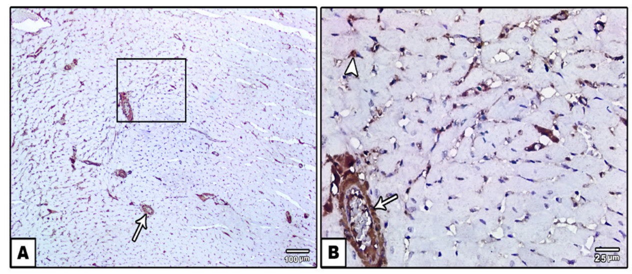


Fig. 17: photomicrograph of left ventricle of azithromycin treated rat showing strong positive αSMA expression in the wall of the blood vessels (arrows) and intensely stained elongated cells among the cardiomyocytes (arrow head) (αSMA immunohistochemistry with H&E counter stain, A X100, B X400).

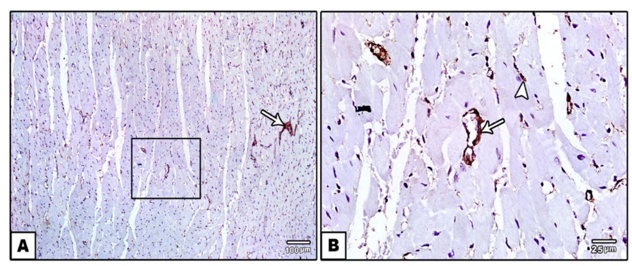


Fig. 18: photomicrograph of left ventricle of azithromycin and nigella sativa oil co-treated rat showing mild positive αSMA expression in the wall of the blood vessels (arrows) and occasionally weak stained elongated cells among the cardiomyocytes (arrow head) (αSMA immunohistochemistry with H&E counter stain, A X100, B X400).

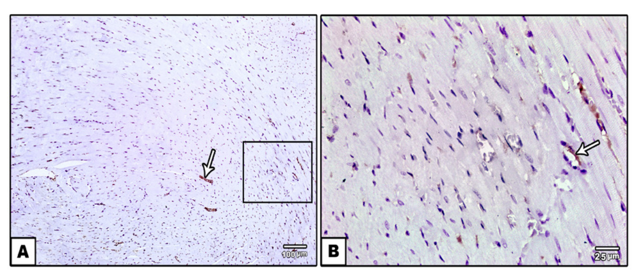


Fig. 19: photomicrograph of left ventricle of nigella sativa oil treated rat showing very weak αSMA expression in the wall of the blood vessels (arrows) (αSMA immunohistochemistry with H&E counter stain, A X100, B X400).

Table 1: Levels of cardiac enzymes (CPK and LDH), TNFα and MDA in different groups

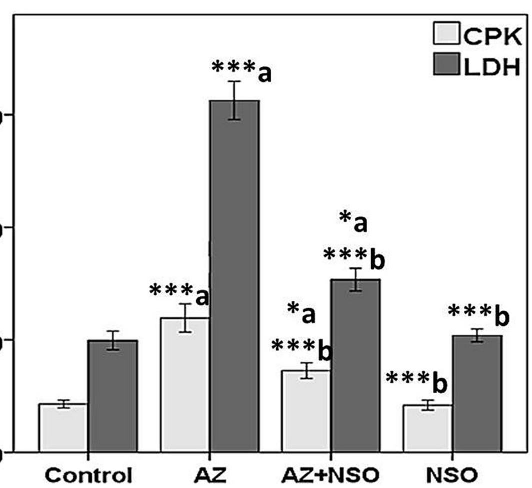
	CN	AZ	AZ+NSO	NSO
Serum CPK (u/l)	86±7	239±25.24	145.33±14.05	84±9
Serum LDH (u/l)	199±16.52	625.33±34.12	307±20.3	208±11.53
Plasma TNFα (pg/ml)	5.8±1.45	25.73±2.76	11.03±1.30	6.03±1.81
Plasma MDA (nml/ml)	6.6±2.16	19.33±3.51	7.17±1.06	5.77±1.44

The above values were expressed as mean ± SD (n = 6), where CN is control group, AZ is azithromycin treated group (30mg/kg/ day for 2 weeks), AZ+NSO is group co-treated with azithromycin (10 mg/kg/ day for 2weeks) and Nigella sativa oil (4ml/kg/day for 2weeks). NSO is Nigella sativa oil treated group (4ml/kg/day for 2weeks).

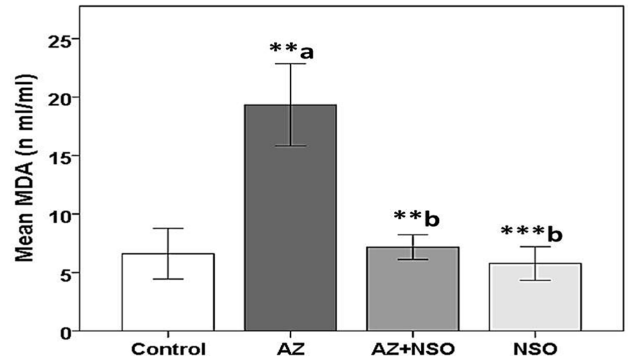
Table 2: Stained area percentage of Masson's trichrome, Caspase-3, and αSMA in different groups

	CN	AZ	AZ+NSO	NSO
Masson's trichrome	2.04±0.58	19.28±.2.36	6.23±1.05	2.15±0.9
Caspase-3	2.96±0.81	11.67±1.03	4.1±1.7	3.12±0.8
αSMA	2.15±0.9	11.1±1.7	3.99±1.92	1.44±0.61

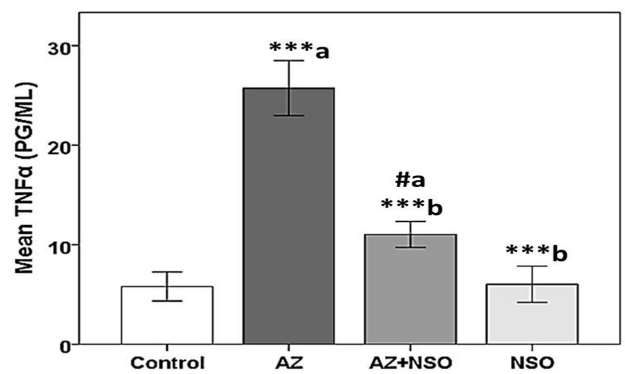
The above values were expressed as mean ± SD (n = 6), where CN is control group, AZ is azithromycin treated group (30mg/kg/ day for 2 weeks), AZ+NSO is group co-treated with azithromycin (10 mg/kg/ day for 2weeks) and Nigella sativa oil (4ml/kg/day for 2weeks). NSO is Nigella sativa oil treated group (4ml/kg/day for 2weeks)



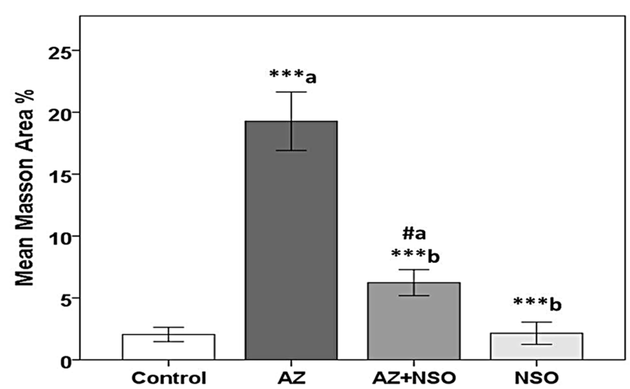
Histogram 1: Histogram showing the mean serum levels of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in different groups. Both CPK and LDH are significantly increased in azithromycin treated group compared to control group. The levels of these enzymes are significantly reduced in azithromycin and nigella sativa oil co-treated group compared to azithromycin group. AZ= azithromycin, NSO=nigella sativa oil. *p<0.01 ,***p<0.0001. a versus control. b versus azithromycin.



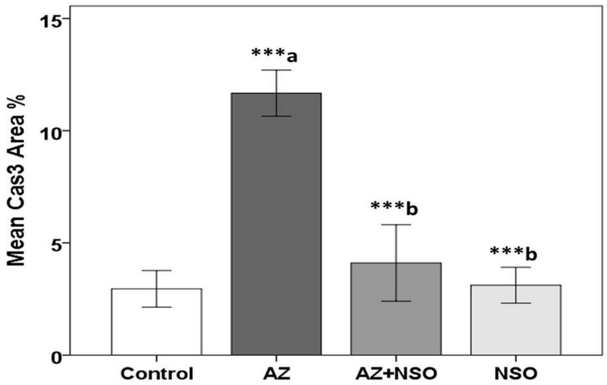
Histogram 2: Histogram showing the mean plasma MDA levels in different groups. MDA level is significantly increased in azithromycin treated group compared to control group. Azithromycin and nigella sativa oil co-treated group shows significant decrease in MDA level to nearly the control group level. AZ= azithromycin, NSO=nigella sativa oil. **p<0.001,***p<0.0001. a versus control. b versus azithromycin.



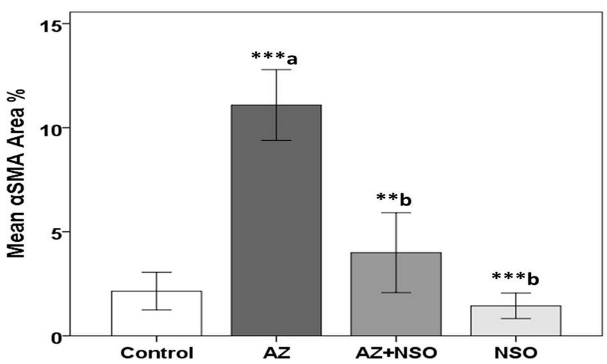
Histogram 3: Histogram showing the mean plasma TNFα levels in different groups. TNFα level is significantly elevated in azithromycin treated group compared to control group. It is significantly decreased in azithromycin and nigella sativa oil co-treated group compared to azithromycin treated group. AZ= azithromycin, NSO=nigella sativa oil. #p<0.05,***p<0.0001. a versus control. b versus azithromycin.



Histogram 4: Histogram showing the mean Masson's trichrome stained area percentage in different groups. The area percentage is significantly increased in azithromycin treated group compared to control group. It is significantly decreased in azithromycin and nigella sativa oil co-treated group compared to azithromycin treated group. AZ= azithromycin, NSO=nigella sativa oil. #p<0.05,***p<0.0001. a versus control. b versus azithromycin.



Histogram 5: Histogram showing the mean Caspase-3 immunostained area percentage in different groups. The area percentage is significantly increased in azithromycin treated group compared to control group. It is significantly decreased in azithromycin and nigella sativa oil co-treated compared to azithromycin treated group. Cas3=Caspase-3, AZ=azithromycin, NSO=nigella sativa oil. ***p<0.0001. a versus control. b versus azithromycin.



Histogram 6: Histogram showing the mean αSMA immunostained area percentage in different groups. The area percentage is significantly increased in azithromycin treated group compared to control group. It is significantly decreased in azithromycin and nigella sativa oil co-treated compared to azithromycin treated group. AZ= azithromycin, NSO=nigella sativa oil. **p<0.001,***p<0.0001. a versus control. b versus azithromycin.

DISCUSSION

Azithromycin (AZ) is an effective macrolide antibiotic that has been used in the treatment of various types of serious bacterial infections. However, the cardiovascular adverse effects associated with AZ have attracted attention recently. Prolonged Q-T interval[22], malignant arrhythmia (torsade de pointes)[23], and even sud¬den deaths due to ventricular arrhythmia[4,24] were reported as a result of AZ use. Therefore, the present work aimed to detect the pathological changes induced by AZ in albino rat myocardium, and the possible protective effect of nigella sativa oil using biochemical, histological, and immunohistochemical methods.

In the current study, AZ administration for two weeks caused significant elevation of serum CPK and LDH which indicated the release of these cardiac biomarkers from the damaged myocardium into the circulation. In addition, AZ administration increased the oxidative stress and inflammatory response as signified by increased plasma MDA and TNFα respectively. The current results were supported with the previous studies that reported elevation of cardiac enzymes, oxidative stress and inflammatory markers following treatment of rats with AZ[5,6].

TNF-α is known to attract leukocytes to the inflammatory sites, enhancing the generation of more reactive species[25]. Moreover, TNF-α could be responsible for production of many inflammatory and apoptotic mediators resulted in structural damage[26].

In this study, the histopathological alternations in the myocardium of the left ventricle were evident by the loss of cardiac muscle striation, cytoplasmic vacuolation, peripheral pyknotic nuclei, interstitial edema, inflammatory cellular infiltration, dilated and congested blood vessels, and evidence of areas of fibrosis. These findings were in agreement with that of El-Shitany and El-Desoky although they used different dose and duration[6]. This could be explained increased free-radical formation induced oxidative damage of cellular lipids, proteins, and DNA[27].

The present work detected Caspase-3 expression in the heart tissue to evaluate the degree of apoptosis after two weeks AZ administration. Caspase-3 is a member of Caspase family that plays a central role in the execution phase of cell apoptosis[28]. In agreement with El-Shitany and El-Desoky[6] there was significant increase in Caspase-3 expression in the left ventricular myocardium. This finding indicates that the AZ-induced cardiotoxicity can lead to apoptotic death of the cardiac cells.

After death of cardiomyocytes, a myocardial remodeling process begins due to uncontrolled proliferation the cardiac fibroblast and excessive deposition of extracellular matrix proteins leading to cardiac dysfunction and fibrosis[29,30]. Cardiac fibroblasts constitute more than 90% of the non-myocytes and perform an essential role in production of extracellular matrix proteins and synthesize angiogenic and cardioprotective factors[31]. Under pathological conditions, these fibroblasts are transformed into myofibroblasts which are responsible for excessive production of fibronectin and collagen resulted in myocardial fibrosis. Since myofibroblasts exhibited characteristics of fibroblasts and smooth muscle cells, their presence in the myocardium can be assessed by immunohistochemical detection of alpha smooth muscle actin (α-SMA)[32].

In agreement with Gava *et al*.[32], the present work showed the only cells expressed α-SMA in the myocardium of control group were vascular smooth muscle cells. However two weeks AZ treatment induced significant increase in α-SMA immunostained elongated cells among the cardiomyocytes indicated intense proliferation of myofibroblasts.

To our knowledge, it is the first time to study the effect of AZ on myofibroblasts proliferation and subsequent fibrosis by demonstrating α-SMA immunohitochemical expression in the heart tissue. It was demonstrated that, myocardial fibrosis is associated with heart rhythm disorders, worsening ventricular systolic function, and increased ventricular wall stiffness[33,34]. This may explain the ventricular arrhythmia associated with AZ treatment in many reported cases.

Nigella sativa oil have been reported to have immunomodulatory, antioxidant[35], anti-inflammatory[36,37], and antiapoptotic[38] effects in different experimental studies. Accordingly, it was hypothesized that co-treatment with this agent might decrease or prevent AZ-induced cardiotoxicity.

The present study showed that concomitant administration of NSO (4ml/kg daily) could effectively lowered the levels of cardiac injury markers (CPK and LDH), oxidative stress (MDA) and inflammatory mediator (TNF-α), and caused a significant improvement in the histopathological structure of the cardiac tissue. This antioxidant and antiinflammatory effects of NSO were confirmed with the previous study of Ahmed *et al*. who reported cardioprotective effect of NSO (4 ml/kg daily) against lead-induced cardiotoxicity in rats by decreasing oxidative stress, proinflammatory cytokine levels and cardiac tissue damage[12]. Also, Ebru *et al*. found that pretreatment with NSO (2 ml/kg daily) decreased lipid peroxidation, normalized cardiac histopathology, improved antioxidant enzyme status and cellular protein oxidation in cyclosporine cardiac injury in rats[39].

The current work demonstrated significant reduction in Caspase-3 expression in AZ-NSO co-treated group which indicated the antiapoptotic effect of NSO against AZ-induced cardiotoxicity. This finding was in line with the result of Adali *et al*. who insured the antiapoptotic effect of NSO active ingredient (thymoquinone) against cisplatin-induced cardiotoxicity[40].

In agreement with the result of Ayuob *et al*. who found that thymoquinone effectively reduced myocardial αSMA expression in hypothyroidism-induced cardiomyopathic rats[41], the present study demonstrated significant decrease in αSMA expression and reduction in myocardial fibrosis in AZ-NSO co-treated group. This antifibrotic effect of NSO which could prevent the myocardial fibrosis and ventricular arrhythmia reported in many AZ treated cases.

CONCLUSION

This study concluded that AZ induced cardiotoxicity represented by elevated plasma cardiac biomarkers and histopathological abnormalities. This cardiac adverse side effect may be related to oxidative stress, inflammatory reaction, and apoptosis with subsequent myofibroblasts proliferation, collagen deposition and myocardial fibrosis. Based on the current study, NSO exerted an effective role in prevention of AZ-induced cardiotoxicity, and its mechanism may be related to antioxidant, antiinflammatory, antiapoptotic and antifibrotic properties.

RECOMMENDATIONS

It is recommended to determine the presence of cardiovascular risk factors in patients under AZ treat¬ment. When prescribed with high or repeated doses, cardiac enzymes follow up and daily ECG recordings are recommended. Also, further studies are required to determine the efficacy of NSO as a protective agent in human AZ intoxication.

CONFLICTS OF INTEREST

There are no conflict of interest.

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الملخص العربى

تقييم السمية القلبية الناتجة عن استخدام الأزيثرومايسين في ذكر الجرذ الابيض والتأثير الوقائي المحتمل لزيت حبة البركة

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الخلفية: الأزيثروميسين هو مضاد حيوي واسع المجال، يستخدم بتكرار في علاج العدوى البكتيرية. اثبتت الدراسات السريرية السابقة العديد من الاثار الجانبية الضارة للأزيثرومايسين على القلب، ومع ذلك، لا يوجد الا القليل جدا من الابحاث التجريبية المنشورة التي توضح تاثيره على قلب الجرذان.

هدف الدراسة: هدف هذا العمل إلى دراسة التغيرات الهستوباثولوجية في قلب ذكور الجرذان البيضاء الناتج عن العلاج بالأزيثروميسين. أيضا لتوضيح الاليات المتعلقة بالإجهاد التأكسدي، والإلتهاب، وموت الخلايا المبرمج، وتليف عضلة القلب. أيضا لتقييم الآثار الوقائية المحتملة لزيت حبة البركة.

المواد والطرق: قسمت أربعة وعشرون من ذكور الجرذان البيضاء إلى أربعة مجموعات متساوية: المجموعة الأولى (الضابطه)، المجموعة الثانية التى تلقت ٣٠ مجم / كجم من الأزيثروميسين بالفم يوميا لمدة أسبوعين. المجموعة الثالثة التى تلقت ٣٠ مجم / كجم من من الأزيثروميسين بالإضافة الى ٤مل / كجم من زيت حبة البركة بالفم يوميا لمدة أسبوعين. المجموعة الرابعة والتى تلقت ٤مل / كجم زيت حبة البركة بالفم يوميا لمدة أسبوعين. تم أخذ عينات من الدم والقلب للدراسة الكيميائية، و النسيجية، والهستوكيميائية المناعية كما تم إجراء تحليل مورفومتري وإحصائي.

النتائج: أظهرت المجموعة التي عولجت بالأزيثروميسين ارتفاعا ملحوظا في إنزيمات عضلة القلب، ودلالات الأكسدة والإلتهاب. كما أنه تسبب في زيادة ملحوظة في نخر، وتليف، والموت المبرمج لخلايا عضلة القلب. وتعد هذه هي المرة الأولى لدراسة تأثير الأزيثروميسين على تكاثر الخلايا الليفية العضلية من خلال تقييم التعبير الكيميائي المناعي لأكتين العضلات الملساء ألفا والذي أظهر زيادة ملحوظة بعد العلاج بالأزيثروميسين. ادى العلاج المشترك بزيت حبة البركة مع الأزيثروميسين الى انخفاض في مستوى انزيمات القلب, ودلالات الأكسدة والإلتهاب، بالاضافة الى تحسن ملحوظ في شكل نسيج القلب، وتقليل التعبير الكيميائي المناعي لكاسباس ٣، ولأكتين العضلات الملساء ألفا مقارنة بالمجموعة التي تم علاجها فقط بالأزيثروميسين.

الخلاصة: يمكن الوقاية من الاثار الضارة على عضلة القلب الناتجة من العلاج بالأزيثروميسين باستخدام زيت حبة البركة، وقد يرجع ذلك الى كونه مضاد للأكسدة، و للإلتهاب، والتليف، وموت الخلايا المبرمج.