Original Article

Evaluation of The Defensive Role of Nigella Sativa Oil and Omega-3 Against Cardiac Damage Mediated by the Nandrolone in Adult Male Albino Rats: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Nandrolone (ND) is one of the Anabolic-androgenic steroid widely used among athletes in doses that result in severe adverse effects on many organs specially heart. Nigella sativa oil and Omega-3 have antioxidant effect.

Aim of the Work: To evaluate the potential defensive effect of Nigella sativa oil and Omega-3 on Nandrolone-induced cardiac muscle histological changes in rats.

Materials and Methods: Forty adult male rats, were divided into four groups, Group I (control group). Group II; treated with ND only (10 mg/kg b.w.) 3 times per week injection for 6 weeks. Group III; treated with ND as group II and N. sativa oil orally (400 mg/kg b.w.) daily for 6 weeks. Group IV; treated with ND as group II and omega- 3 orally (400 mg/kg b.w.) daily for 6 weeks. Then cardiac muscle specimens prepared for histological and immunohistochemical studies by light and electron microscopes.

Results: Group II showed marked distortion, fragmentation, loss of cardiac muscle striation, inflammatory cellular infiltrations, significantly increased deposition of collagen fibers, interruption in intercalated disc. Significantly increased immuno- expressions of Caspase-3 and alpha –Smooth muscle actin (α-SMA) when compared to control group. Group III & IV that treated with either Nigella sativa oil or Omega-3 respectively showed improvement of the histological architecture of cardiac muscle and significantly decreased immune- expressions of Caspase-3 and alpha –Smooth muscle actin (α-SMA) when compared to group II.

Conclusion: Nigella sativa oil and Omega-3 could protect the heart from the injurious effect of ND. The results of Nigella sativa oil were better than Omega-3 at histological and immunohistochemical levels.

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Key Words: Cardiac muscle, nandrolone, nigella sativa, omega-3.

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INTRODUCTION

Anabolic-androgenic steroid (AAS) is a synthetic products of testosterone, were generated as an effort to manufacture a testosterone-like steroid that is supposed to have powerful anabolic effects in bone and muscle[1]. Around the world, abuse of AAS become very common and one of the major health problem[2].

One of the most widely used AAS between athletes is the nandrolone decanoate, which is derived from 19-nortestosterone[3]. It may use in the treatment of senile and postmenopausal osteoporosis[4]. Now, it is popular among young men swimmer, weightlifters and bodybuilders, as it increases the muscle mass of the body. This leads to severe damage on many organ systems[3].

The most repeated adverse effect of the administration of AAS is the cardiac disease, due to its sensitivity to oxidative stress as compared to the other organs[5]. The bad effects of AAS on cardiovascular system including cardiac dysfunction, abnormalities of impulse conduction and contractility[6]. It acts on androgenic receptors in the cytoplasmic compartment of the cardiomyocytes leading to ventricular hypertrophy[7].

Recently natural agents extracted from plants became highly accepted as strategy instead of traditional therapeutic drugs. One of these herbal medicines is the Black seed or Nigella sativa seed[8]. It is used widely in the management of diverse diseases for ex. gastrointestinal problems, hypertension, obesity, cardiac diseases and numerous types of cancer. Moreover, Nigella sativa can decrease fatigue and depression and accelerate the body strength[9].

Thymoquinone (TQ) and polyphenols are the essential bioactive major oil components of Nigella sativa, which explain its therapeutic properties[10]. The antioxidant effects of thymoquinone are due to the prevention of lipid peroxidation as well as scavenging of hydroxyl radicals and superoxide radical ions[11]. Other properties of Nigella sativa including anti-inflammatory and immunomodulatory[12].

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One of the polyunsaturated fatty acids (PUFA) family is the omega-3, which cannot be produced in the body, but found in seafood such as salmon, mackerel, anchovies and sardines mostly[13]. Omega-3 contains three types of acids: eicosapentaenoic acid (EPA), alpha-linolenic acid (ALA), and docosahexaenoic acid (DHA)[14]. It maintains the cholesterol levels within the normal range and is crucial for healthy heart, brain, and immune system[15].

Omega-3 PUFAs has a major role in reduce the oxidative stress and defense the cell against ROS by increasing the action of antioxidant enzymes levels of superoxide dismutase (SOD) and glutathione (GSH)[16]. Also it has anti-apoptotic, and immunomodulatory role and decreases metabolic disturbances[17].

So, the present work intended to clarify the histopathological changes in the cardiac muscle of male albino rats treated with Nandrolone and the supposed defensive effects of N. Sativa and Omega-3 were assessed.

MATERIALS AND METHODS

Animals

The present study was conducted on forty apparently healthy male adult albino rats, its weight ranged between 180g to 220g. They were obtained from laboratory animal's unit in Veterinary Medicine Faculty, Zagazig- University, Egypt. Rats were lodged in individual plastic cages that were cleaned daily and were exposed to a 12 h light/12 h dark cycle at (23± 1 °C) and qualified humidity. The animals were provided with their need of water and feed.

Ethical Consent: The procedures in this experiment were carried out according to the guidelines and principles of the Animal Ethics Committee of the Faculty of medicine, Benha University, confirmed to the Guide for the care and use of Laboratory Animals, Publication No. Rc.35.9.2022.

Drugs

Nandrolone decanoate (ND): Nandurabolin 50 mg/ml, oily solution, provided from the Nile Company pharmaceuticals –Cairo (Egypt). 5 ml of corn oil used to dilute the ampoule.

Nigella sativa (N. Sativa): BARAKA capsule, 450 mg of Nigella sativa oil, soft gelatin capsules, gained from PHARCO Company pharmaceuticals –Cairo (Egypt). The capsule was dissolved in 5 ml corn oil.

Omega-3: Omega-3, fish oil 1200 mg, soft gelatin capsule was supplied from MISR PHARMA, Company, Egypt. The capsule was diluted in 15 ml of corn oil.

Experimental protocol

After 1 week of adaptation. The rats were distributed into 4 groups;10 animals each as follows:

Group I (control (C); subdivided equal into two subgroups:

- Ia: Rats with no medication.
- Ib: Rats were received corn oil, by intra-muscular injection with the same volume and times of ND group.

Group II (ND- treated group); each rat in this group was treated with ND only at a dose 10 mg/kg body weight by intra-muscular- injection 3 times per week for 6 weeks[18].

Group III (ND and N. sativa treated group); each rat in this group treated with both ND as group II, and N. sativa oil at a dose 400 mg/kg body weight daily via gastric gavage for 6 weeks[19].

Group IV (ND and omega 3 treated group); each rat in this group treated with both ND as group II, and omega-3 at a dose 400 mg/kg body weight daily through gastric gavage for 6 weeks[20].

All rats were sacrificed after 24 hours from the end of the trial, under ether anesthesia. Heart of each animal was excised after a midline incision of the anterior aspect of the chest at the stern costal junctions. The specimens of the heart were obtained from the anterior-lateral left ventricular wall for light and electron microscopic study.

For light microscopic study

Half of the cardiac muscle specimens for each group were fixed in 10% neutral buffered formalin and handled for paraffin sections preparation of paraffin sections (5µm thick) for:

1- Histological study: Hematoxylin and Eosin (H&E) stain for ordinary histological investigation and Masson's trichrome stain for recognition of collagen fibers[21].

2- Immunohistochemical study: Alpha-smooth muscle actin immunostain for detection of myofibrosis, and Caspase-3 immunostain for detection of apoptosis. We use the streptavidin-biotin complex immunoperoxidase system for the immunohistochemical reaction. Successive sections of paraffin-implanted specimens were de-paraffinized on charged slides. Step of blocking the endogenous peroxidase was done by incubate the sections in hydrogen peroxide 0.1% for 30 min, then incubated with the primary antibody. For anti-alpha-smooth muscle actin, incubation with (abcam, ab5695, 1:100) primary antibody at room temperature for half an hour. Later, secondary antibody was added for 8 minutes. Finally, one to two drops of DAB (diaminobenzidine) was added for 8 minutes. At last, sections were counterstained with Mayer's hematoxylin, then dehydrated and cleared[22]. for caspase-3, used rabbit polyclonal antibody (CAT-No: RB-3425-R2 as primary antibody. Then incubate the slide with the secondary anti-rabbit antibody versal kits (Zymed laboratories), diluted 1: 200 for 30 minutes, staining was completed by incubation with chromogen, called diamiobenzidine (DAB). Use Mayer's hematoxylin as a counterstain[23].

Photomicrographs were taken to assess the histopathological changes by light microscopic examination using a light microscope camera (Olympus CX 41, Japan) at Anatomy & Embryology department, Faculty of Medicine, Benha University.

Electron microscopic study

The other half specimens that used for ultrastructural examination, were fixed in 4% phosphate- buffered-glutaraldhyde (pH 7.2–7.4) immediately and then post-fixed in 1% osmium tetroxide (osmic acid) in phosphate buffer at 4°C, for 2 hours dehydrated, and in epoxy resin were embedded. 1 µm thick semi-thin sections were stained with 1% toluidine blue for examination by light microscope to determine the part. Ultra-thin sections were stained with 4% uranyl acetate and lead citrate[24]. The grids were examined and photographed by JEOL-JEM-100 transmission electron microscope (Tokyo, Japan) at the unit of electron Microscope, Faculty of Medicine, Tanta University, Egypt.

Digital morphometric study

Computer assisted digital image analysis. Slides (Ten non overlapping fields from each groups) that photographed using Olympus ® digital camera installed on Olympus ® microscope with 0.5 X photo adaptor, using 40 X objective and saved as TIFF. The result images were analyzed on Intel ® Core I7 ® based computer using VideoTest Morphology ® software (Russia) with a specific built-in routine for:

- Area measurement for Masson's Trichrome.
- Integrated density for Alpha smooth muscle actin
- % positive cells for Caspase-3.

Results were exported to Excel Sheet for Statistical analysis.

Statistical analysis

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical Package for Social Science) version 26.0 to obtain: Descriptive statistics were calculated in the form of Mean ±Standard deviation (SD). The statistical comparison between the different groups, the significance of difference was tested using One-way ANOVA (Analysis of variance). Compare between more than two different groups of numerical (parametric) data followed by post-hoc tukey. *P value* <0.05 was considered statistically significant.

RESULTS

Examination of all control subgroups revealed the same finding.

Results of light Microscope

Haematoxylin and Eosin (H & E) stain

Slices of group I, revealed regularly arranged cardiac muscle fibers which branch and anastomose together giving the appearance of a sheet, with acidophilic cytoplasm and central oval nuclei. Small blood capillary was evident in the intercellular spaces, and normal flat peripheral nuclei of the interstitial connective tissue (Figure 1a).

In Nandrolone treated group, there was marked loss of normal architecture of the cardiac muscle fibers, the slices showed fragmentation of the cardiomyocytes and its vacuolation, wide endomysium, pyknotic nucleus, perinuclear vacuolation, interfibrillar haemorrhage and inflammatory cell infiltration. Some cardiac fibers are wavy. (Figures 1 b,c).

In Nandrolone and N. sativa treated group, there was marked restoration of normal shaped cardiac fibers, minimal vacuolization of the cytoplasm. Some cardiac nuclei appeared normal, others were pyknotic (Figure 2 a).

In Nandrolone and Omega-3 treated rats, some cardio myofibrils appeared normal, others were wavy. The cardiac cell nuclei still pyknotic (Figure 2 b).

Masson's trichrome stain

Slices rats of the control subgroups revealed minimal amount and normal distribution of blue colored collagen filaments in the interstitium between the cardio myofibrils (Figure 3 a). While that of ND- treated group, there was a high deposition of collagen filaments between the cardiomyofibres (Figure 3 b).

In nandrolone and N. sativa supplemented rats, few fine collagen filaments were observed in the endomesium between the cardiac muscle fibers (Figure 4 a). While slices from rats of omega 3 with nandrolone supplemented group presented, moderate amount of collagen filaments between the cardio myofibrils (Figure 4 b).

Immunohistochemical stain results

Alpha smooth muscle actin stain

Examination of the αSMA immunostained sections of control groups showed, negative αSMA expression in the myocardium and minimal stained smooth muscle fibers of the blood vessels (Figure 5 a). Nandrolone treated group showed high intensely stained smooth muscle fibers of the blood vessels and within the cardiomyocytes intensely stained elongated cells (Figure 5 b). Nandrolone with N. sativa treated group showed, markedly minimal stained smooth muscle fibers in the wall of the blood capillary and between the cardiomyocytes (Figure 5 c). Also section of Nandrolone and omega 3 treated group, revealed, decreased stained smooth muscle fibers in the wall of the blood vessels and between the cardiac muscle cells (Figure 5 d).

Caspase-3 stain

Sections from control group showed minimal caspase-3 reaction (Figure 6 a). Sections from Nandrolone treated group which presented marked positive (+ve) caspase-3 reaction in the cytoplasm of the cardiomyocytes (Figure 6 b). Group of Nandrolone with N. sativa showed mild +ve caspase- 3 reaction as compared with group II (Figure 6 c). Sections of Nandrolone and omega 3 treated group, there was moderate +ve caspase-3 reaction (Figure 6 d).

Electron microscopic results

Examination of the ultrathin sections of the left ventricular myocardium of group I revealed, regularly arranged and parallel bundles of myofibrils, which had successive dark A and light I bands with H line and Z line bisecting them respectively. Cardiomyocytes have euchromatic nuclei with scattered chromatin, joined by straight intercalated discs. Regular sheets of mitochondria between the myofibrils with closely packed cristae and glycogen granules could be detected also in the sarcoplasmic reticulum between the muscle fibers. (Figure7 a,b).

Sections treated with nandrolone for 6 weeks showed, marked disintegration and decomposition of the myofibrils with loss of its normal striation, interruption in intercalated disc and Z line. The mitochondria were swollen between the myofibrils, dilated smooth endoplasmic reticulum. Nuclear changes in the form of irregular outline and condensed marginally arranged chromatin were also observed. (Figure 8 a,b).

Sections from rats of nandrolone and N. sativa treated group showed, marked restoration of normally arranged myofibrils, with regular appeared Z and H lines. The sarcoplasm is dilated and edematous. (Figure 9 a).

Sections from nandrolone and omega 3 treated group showed, some cardio myofibrils return to its normal pattern, but some of them still degenerated. Sarcoplasm is dilated markedly, and the mitochondria not regularly arranged between the muscle fibers. (Figure 9 b).

Histomorphometric and statistical results

MTC % area

About the Masson's trichrome stained sections, the mean ±SD of area percent of collagen fibres deposition is represented in (Table 1, Histogram 1) for all groups. There was a statistically marked increase in the mean area percent of collagen fibres in the 2nd group as compared to the 1st one. There was a marked decrease in the mean area percent of collagen fibres in Nandrolone +N. sativa -treated rats when compared to Nandrolone - treated group. There was a significant decrease in the mean area % of collagen fibres in Nandrolone + omega 3-supplemented rats when related to Nandrolone -treated group. In both Nandrolone +N. sativa -supplemented group and Nandrolone + omega 3-treated group, there was a marked increase in the area percent of collagen fibres when related to control one. As well as there was statistically marked decrease in the mean area percent of collagen fibres of Nandrolone +N. sativa -treated group when related to Nandrolone and omega 3 treated group.

αSMA (Integrated Density x 10^5)

About the αSMA immunostained sections, the mean± SD of αSMA immunostained Integrated Density is represented in (Table 1, Histogram 2) for all groups. A statistically marked increase in integrated density of αSMA immunoexpression in Nandrolone -supplementd group was observed as related to control one. There was a marked decrease in the mean integrated density of αSMA immunoexpression in Nandrolone +N. sativa -treated rats when compared to the 2nd group. There was a significant decrease in the mean integrated density of αSMA immunoexpression in Nandrolone + omega 3-supplementd rats when compared to Nandrolone -treated group. In both Nandrolone +N. sativa -treated group and Nandrolone + omega 3-treated group, there was a marked increase in the mean integrated density of αSMA immunoexpression when compared to control group. As well as there was statistically significant decrease in the mean integrated density of αSMA immunoexpression in Nandrolone +N. sativa -supplemented group when compared to Nandrolone and omega 3 supplemented group.

Caspase 3 (% +ve Cells)

About the immuno-expression for Caspase -3, the mean ±SD of Caspase- 3 immunostained % positiive Cells is represented in (Table 1, Histogram 3) for all studied groups. There was a statistically marked increase in mean Caspase-3 (% +ve Cells) in ND - treated group when related to control one. There was a significant decrease in the mean Caspase-3 (% +ve Cells) in ND +N. sativa -treated rats when compared to ND - supplemented group. There was a marked decrease in the mean Caspase- 3 (% +ve Cells) in Nandrolone + omega 3 supplemented rats when related to ND -supplemented group. In both Nandrolone +N. sativa -treated group and Nandrolone + omega 3-treated groups, there was a marked increase in the mean Caspase- 3 (% +ve Cells) when related to the control one. As well as there was statistically marked decrease in the mean Caspase-3 (% positive Cells) in Nandrolone +N. sativa -supplemented group when compared to ND and omega 3 treated group.

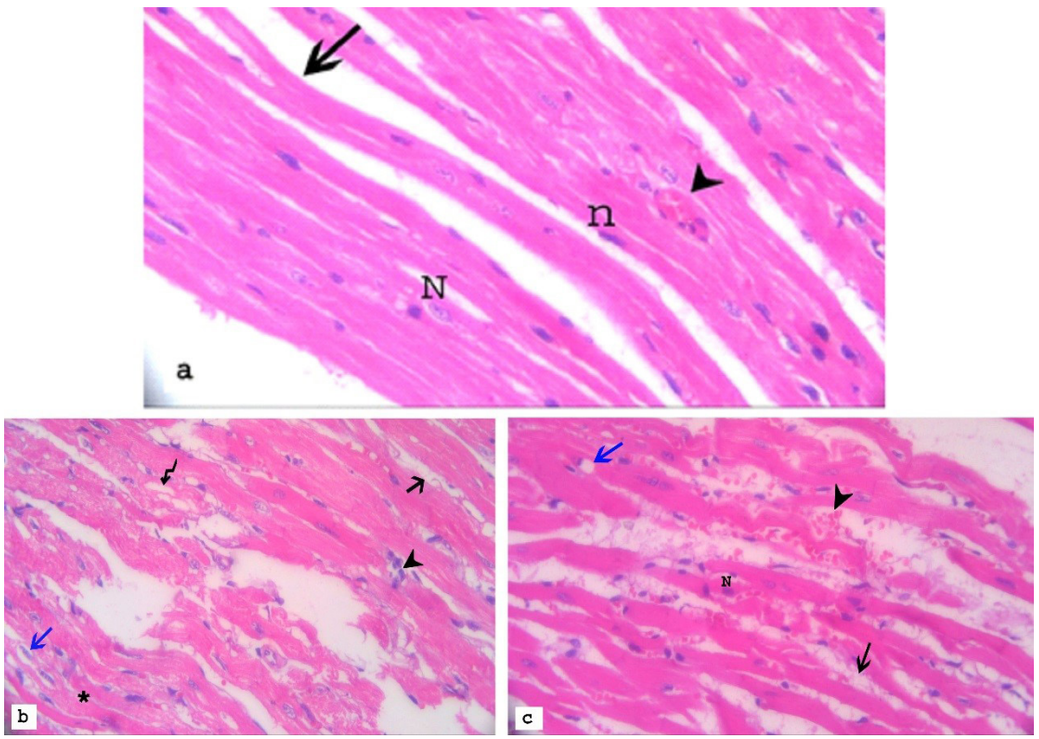


Fig. 1: A photomicrograph of H& E stained slices of ventricular myofibril (1a): Section of control group presenting: branched, anastomosed and parallel cardiac muscle fibers (thick arrow), with acidophilic cytoplasm and centrally-located nuclei (N), blood capillary between the myofibril (arrow head), nuclei of interstitial connective tissue (n). (1b): Section of the 2nd group revealed loss of normal pattern of the cardiao myofibril, wide endomysium (black arrow), vacuolation of the cardiomyocyte (zigzag line), wavy cardiac fibers (star), inflammatory cell infiltration (arrow head), and perinuclear vacuolation (blue arrow). (1c): another section of nandrolone treated group showing, pyknotic nucleus (N), prinuclear vacuolation (blue arrow), interfibrillar hemorrhage (arrow head), and wide endomysium (black arrow). (H& E X 400)

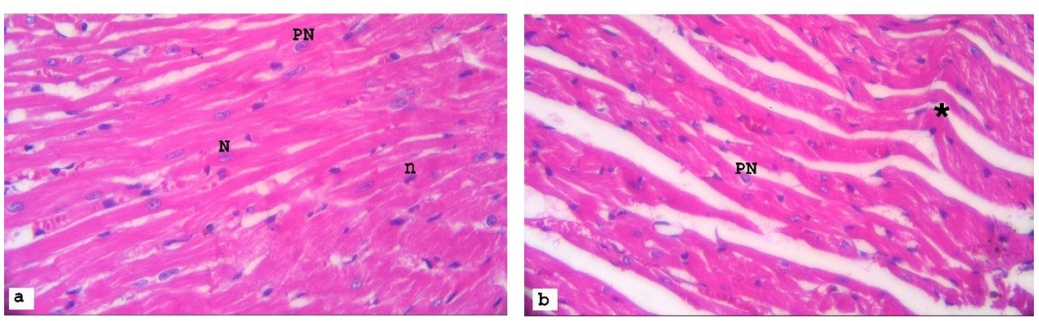


Fig. 2: A photomicrograph of H& E stained slices of ventricular myofibril, (2a): Section of Nandrolone and N. sativa treated group showing: nearly normal appearance of the cardio myofibrils with acidophilic cytoplasm and normal nuclei (N), pyknotic nuclei (PN), and nuclei of connective tissue (n). (2b): Section of Nandrolone and omega 3 treated group showing, normal branched fibers of the myocardium, some fibers are wavy (star), and pyknotic nucleus (PN). (H&E X400).

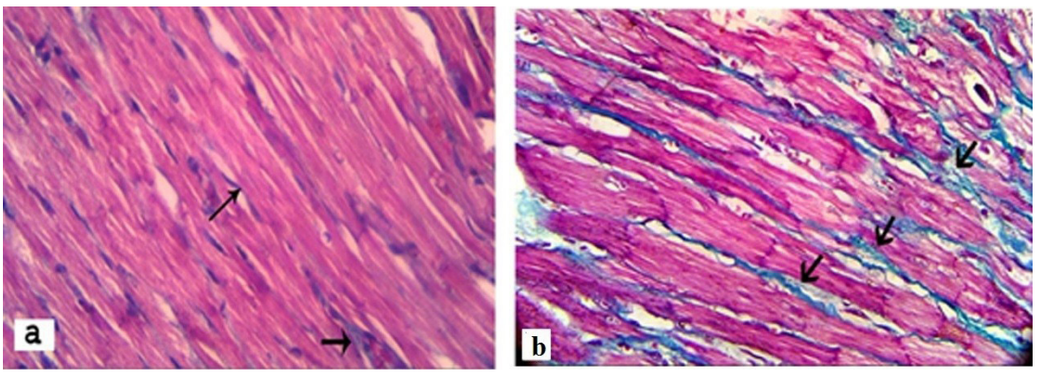


Fig. 3: A photomicrograph of Masson's trichrome stained slices: (3a): Section of Group I presenting, collagen filaments between the fibers (thick arrows). (3b): Section of Nandrolone treated group showing, greater amount of collagen (arrows) than the group I. (Masson's trichrome X400)

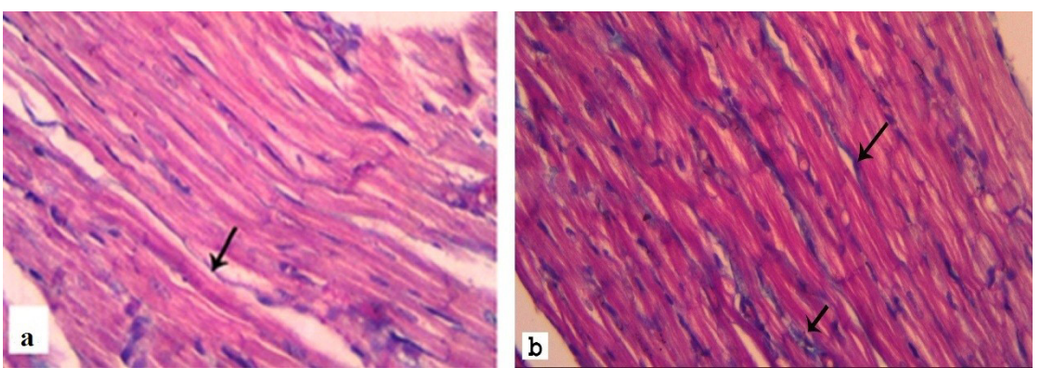


Fig. 4: A photomicrograph of Masson's Trichrome stained slices (4 a): Nandrolone and N sativa-supplemented rats showing, few amount of collagen (arrow) between the myofibrils. (4 b): Nandrolone and omega 3treated group showing, moderate amount of collagen between the cardiac myofibrils (arrows). (Masson's trichrome X400)

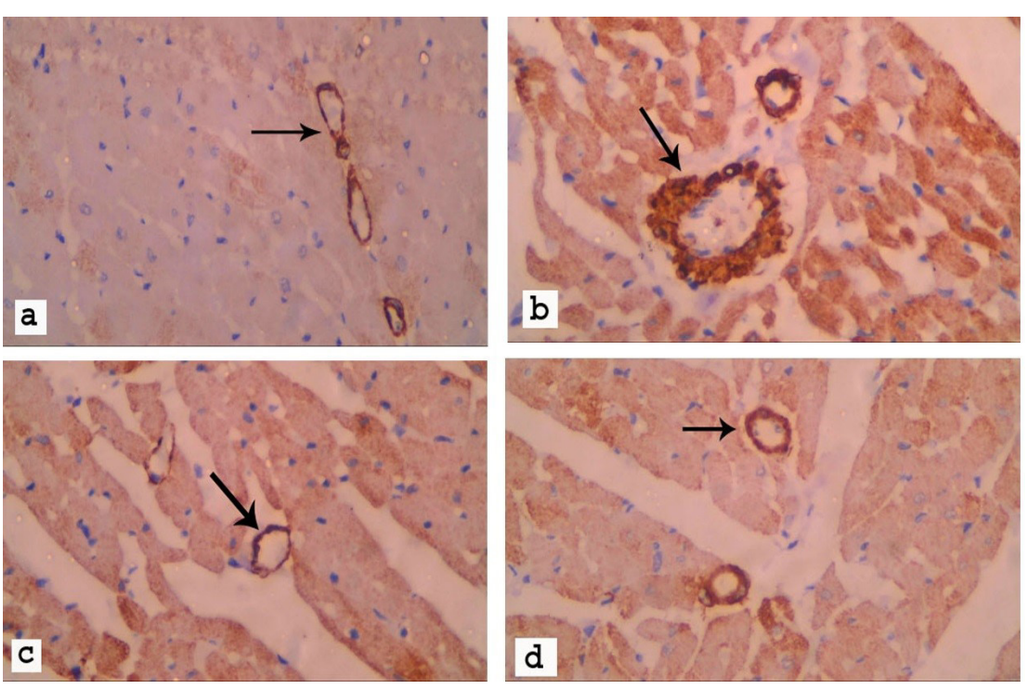


Fig. 5: A photomicrograph of alpha smooth muscle actin (a-SMA) immunostaining sections of (a): Group I presenting minimal αSMA expression mainly in the wall of the blood vessels (arrow). (b): ND- treated group presenting: High positive αSMA expression in the wall of the blood vessels (arrow) and intensely stained cardiomyocytes (c): Nandrolone and N. sativa treated group and (d) Nandrolone and omega 3 treated group showing: weak αSMA expression in the wall of blood vessels (arrow) and occasionally weak stained cardiomyocytes (α SMA immunohistochemistry with H&E counter stain X400).

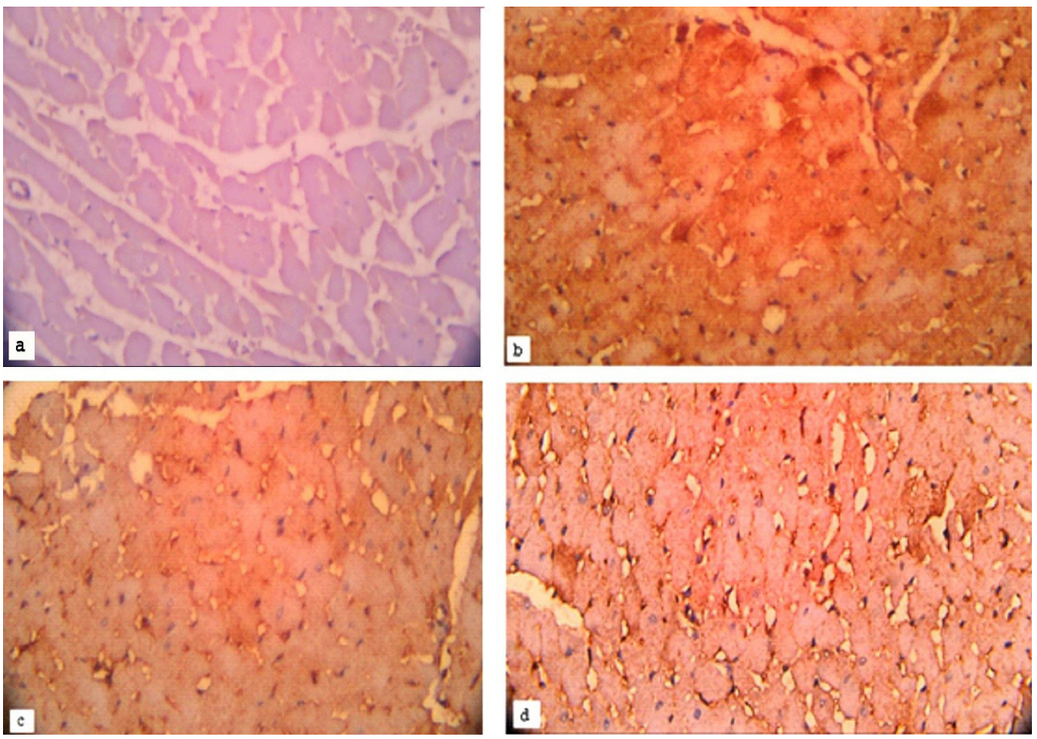


Fig. 6: A photomicrograph of caspase-3 reaction stained slices (6a): Group I presenting, negative caspase- 3 immunoreaction. (6b): ND-treated group presenting, strong caspase-3 +ve reaction. (6c): nandrolone with N. sativa treated rat showing mild caspase-3 reaction. (6d): nandrolone and omega- 3 treated rats showing moderate caspase-3 +ve reaction (Caspase-3 immunostaining; X 400).

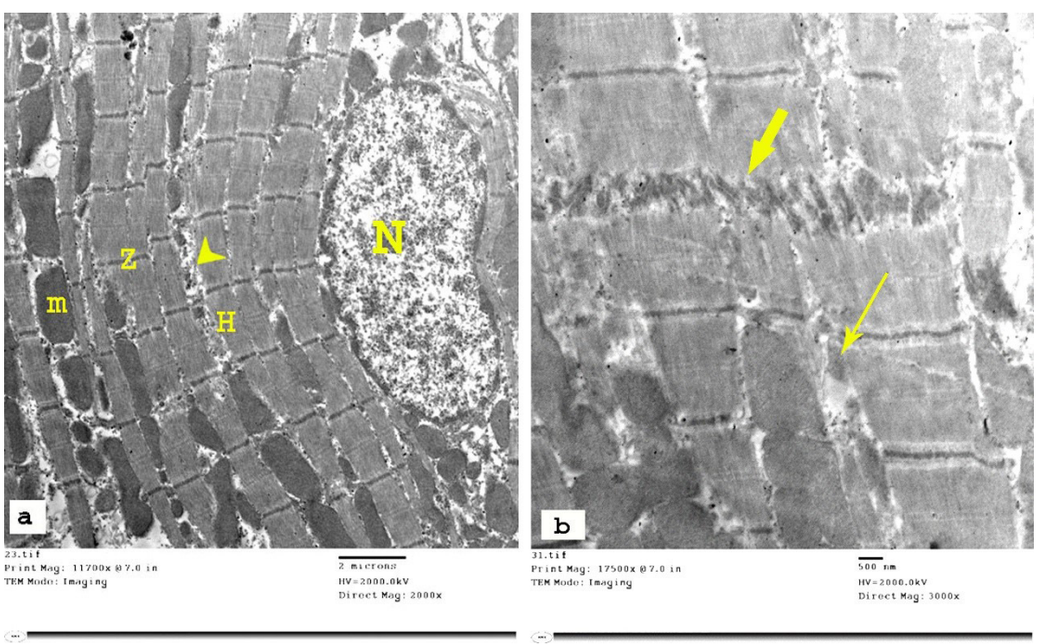


Fig. 7: Scanning electron microscopy images of cardiac muscle slices from rats of group I presenting (7a): Cardiomyocyte with euchromatic nucleus (N), Bundles of myofibrils with Z lines (Z) and H lines (H). Mitochondria (m) and sarcoplasmic reticulum with collagen granules (arrow head). (7b): Two cardio myocytes connected at an intercalated disc (thick arrow), Glycogen particles (thin arrow) are appear in-between the myofibrils.

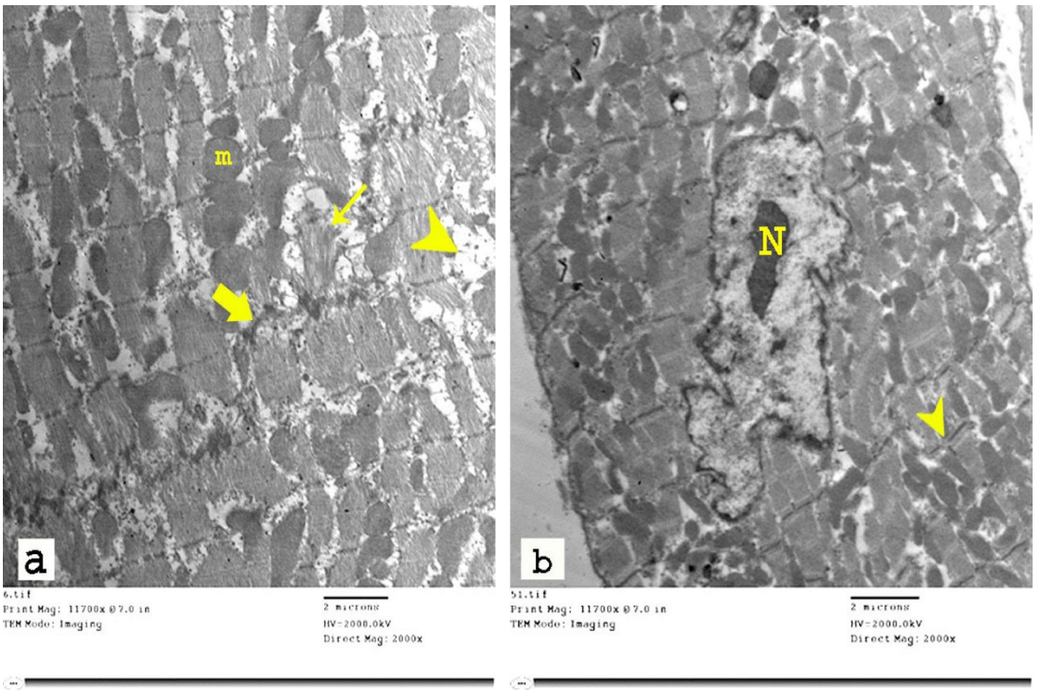


Fig. 8: Scanning electron microscopy images of the cardiac muscle slices from ND- treated group presenting: (8a): degenerated cardio myofibrils (thin arrow), interrupted intercalated disc (thick arrow), dilated sarcoplasmic reticulum (Arrow head), and swollen mitochondria (m). (8b): Nucleus with irregular outline and heterochromatin (N), disrupted Z line (Arrow head).

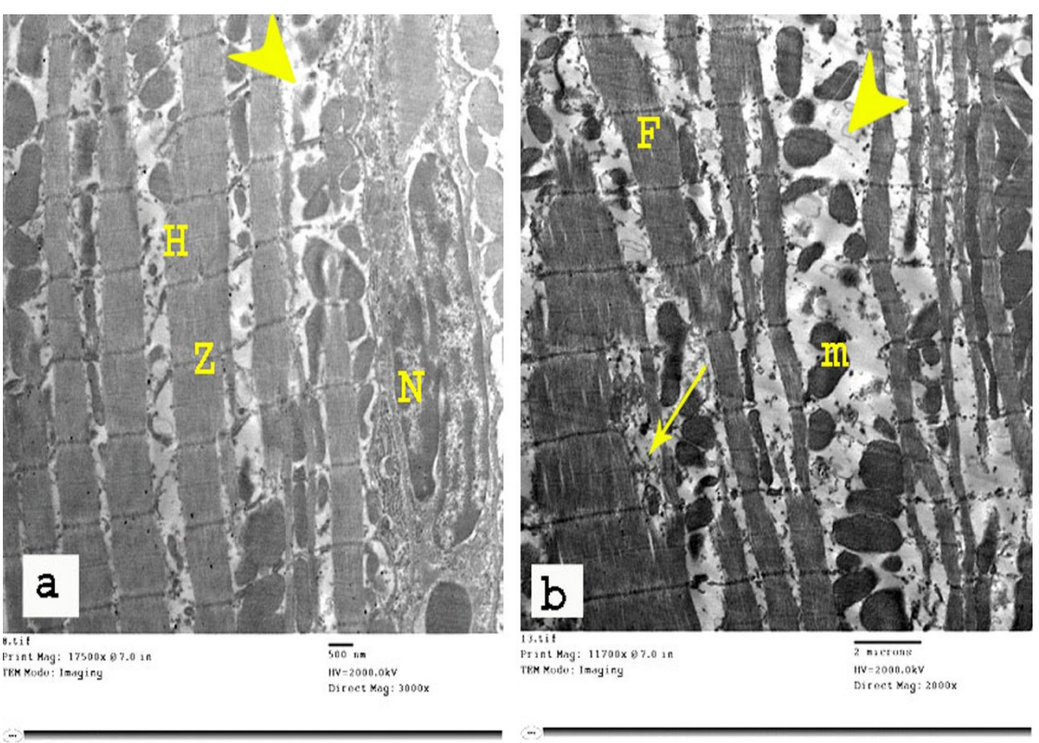
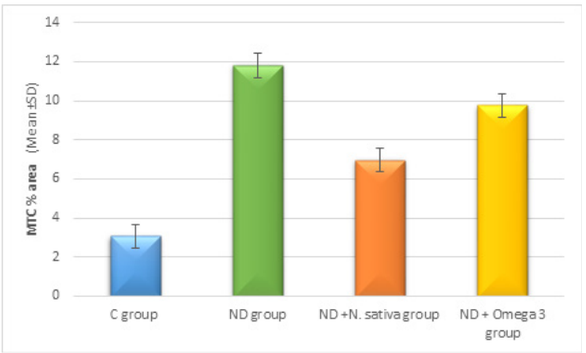


Fig. 9: Scanning electron microscopy images of ventricular myocardium (9a): Nandrolone and N. sativa treated group showing: Regular myofibrils with Z line (Z), H line (H), dilated sarcoplasm (Arrow head). Notice: nucleus of interstitial connective tissue (N). (9b): Nandrolone and omega 3 treated group showing: Some normally arranged myofibril (F), others are degenerated (Arrow). Dilated sarcoplasm (Arrow head), dispersed mitochondria(m).

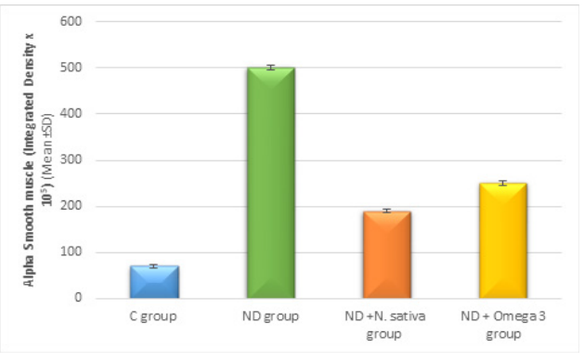
Table 1: Comparison of MTC % area, αSMA (Integrated Density x 105) & Caspase 3 (% +ve Cells) between different studied groups

	Control (C) group	Nandrolone (ND) Treated group	Nandrolone +N. sativa treated group	Nandrolone + omega 3 treated group
MTC % area	3.06±0.61	11.81±1.71[a]	6.97±1.02[ab]	9.76±1.31[abc]
IDx10[5]	70.41±4.44	500.80±4.20[a]	189.90±1.65[ab]	250.30±2.69[abc]
Caspase 3 (% +ve Cells)	2.09±0.36	16.83±2.16[a]	9.15±1.42[ab]	12.52±1.64[abc]

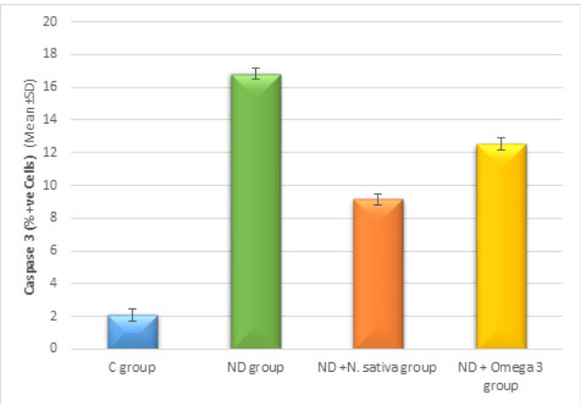
Data expressed as Mean± SD
SD: standard deviation
P: Probability *: significance <0.05
Test used: One-way ANOVA followed by post-hoc tukey
a: significance vs Control (C) group, b: significance vs Nandrolone (ND) Treated group: significance vs Nandrolone +N. sativa treated group



Histogram 1: Showing the mean Masson's trichrome stained area percentage in different groups



Histogram 2: Showing the mean αSMA (Integrated Density x 105) in different groups.



Histogram 3: Showing the mean of Caspase 3 (% +ve Cells) in different groups

DISCUSSION

The current work aimed to throw further light on the histological, histochemical and ultrastructural changes that made in the myocardium of albino rats treated with Nandrolone decanoate. and evaluated the possible protective role of N. sativa and omega 3 against the injury induced by nandrolone injection. Also it aimed to allow the possibility to determined which one of them give better result at histological, histochemical and ultrastructural levels.

In this study, administration of nandrolone was 3 times /week for 6 weeks in a dose of 10 mg/kg b.w.. This dosage was designated and matched to inappropriate dose taken by athletic person, 10 to 100 times greater than therapeutic dose[18].

In this study, we reported that, nandrolone induces pathological changes in cardiac muscle detected by histopathological examination by light microscopic examination, such as the loss of striation of cardiac muscle striation, cytoplasmic vacuolation, pyknotic nuclei, inflammatory cellular infiltration and evidence of areas of fibrosis. These findings were in agreement with previous studies[18,25], although some of them used different duration and dose. Franquni *et al*[26] revealed that Nandrolone increased the cardiac hypertrophy through enhancing the collagen deposition and suggested that due to increasing pro-inflammatory cytokines/ anti-inflammatory cytokines ratio. Emer *et al*[27] suggested that inflammatory pathways can explain the pathological findings of leukocyte infiltrates and disorganized myocardial fibers.

In current study, we reported another pathological finding in Nandrolone treated group, significant increased collagen content, deposited between cardiac myocytes when compared to control group. These results in agreement with the findings of previous studies[25,26,28]. Joukar *et al*[29] registered that Nandrolone causes progressive increase in the collagen fibers and this may lead to heart failure due to cardiac hypertrophy and decreased compliance of the ventricular wall. Collagen deposition that led to fibrosis of cardiac muscle tissue also investigated after intramuscular administration of nandrolone on rabbits in study by Vasilaki, *et al*[5] who suggested that was mediated by oxidative stress.

The present work revealed significant increase in immunoexpression of α-SMA immunostained sections in Nandrolone treated group when compared to control group. this was in line with other studies as El-kader[30] who showed that the only cells expressed α-SMA in the myocardium of control group were vascular smooth muscle cells, but after treatment with AZ, there was significant increase in α-SMA immunostained elongated cells among the cardiomyocytes indicated strong myofibroblasts proliferation. This may have explained by Gava *et al*[31] who had indicated that the increase in myofibroblasts number occurs as an attempt to avoid rupture of the damaged area and maintain myocardial compliance during the contraction.

The present work revealed significant increase in caspase-3 expression in cardiac muscle tissue in Nandrolone treated group when compared to control group. Caspase-3 expression evaluate the degree of apoptosis according other studies[32]. Our results were in agreement with results of other researchers who reported that AAS can lead to apoptotic death of the cardiac cells[33,34,35]. Shirpoor *et al*[36] had registered that Nandrolone abuse has been associated with myocardial infarction. Lopes *et al*[37] reported that apoptosis might be explained by ROS generation and oxidative stress that increasing apoptotic mediators and activate program of cell death, leading to cardiotoxic effect in treatment with testosterone and its synthetic derivatives.

In this presenting work, examination by electron microscope of the nandrolone- treated group exposed that, the cardiac muscle displayed destructive changes, fragmentation and lytic changes of some myofibrils, marked changes of the nucleus, destroyed mitochondria, and also the intercalated discs showed disturbance in-between the adjacent cardio myocytes. These results were obtained by other researchers[25,38]. Pinheiro *et al*[39] explained that both small and great doses of nandrolone decanoate inducing oxidative stress which causes myocardial tissue damage by increase lipid peroxidation and making imbalance between ROS and antioxidants. Also our findings were in line with study by Pozzi *et al*[40] who suggested that there was relation between oxidative stress and DNA damage when they evaluated the damage produced by nandrolone in heart, liver, and kidney in rats.

The present study showed that concomitant administration of Nigella sativa daily for six weeks decreased nandrolone-induced cardiotoxicity as examination of light and electron stained sections of this group revealed great improvement and reduction of the pathological findings. Masson's trichrome stained sections of ND+ N. sativa group revealed significant decrease of the collagen fibers between the cardiomyocytes when compared to ND treated group. These observations were in agree with study by Abeer *et al*[33] who had registered that, the use of N. sativa protect heart against the harmful effects of nandrolone injection. Also study by El-kader[30] reported that N. sativa deceased the levels of inflammatory mediator, markers of cardiac injury and oxidative stress and so caused a significant improvement in the histopathological structure of the cardiac tissue against the toxic effect of Azithromycin. Study by Bocsan *et al*[41] reported that N. sativa oil have an anti-inflammatory and cardio-protective effect in ischemia inducted by ISO.

The present study demonstrated significant decrease in αSMA expression in ND+ N. sativa group when compared to ND treated group, and this was in agreement with the result of other study [30]. Also study by Ayuoba *et al*[42] who found that in hypothyroidism, the N. Sativa can diminished myocardial αSMA immunoexpression effectively in rats.

The existing work demonstrated a significant drop in Caspase-3 expression in ND+ N. sativa group which indicated the anti-apoptotic effect of N. sativa oil against nandrolone induced cardiotoxicity. These finding was in same line with the result of Adali *et al*[43] who insured the anti-apoptotic effect of N. sativa active ingredient (thymoquinone) in cardiotoxicity induced by cisplatin. Also Altun *et al*[19] suggested that NSO can suppress the apoptosis and protect the myocardium in streptozotocin-induced diabetic rats.

The existing study displayed that, omega- 3 alleviates the effect of nandrolone on cardiac muscle at histological and immmunhistochemical levels. Significant decrease in collagen fiber, αSMA immunoexpression and Caspase-3 expression when compared to ND treated group. Another studies confirmed the protective role of omega-3 when it used as cardio-protective against several cardiotoxic agents as Gopal *et al*[44] who proved the ability of omega-3 to restore the structure and function of the myocardium as it reduced the oxidative stress injury induced by isoproterenol on myocardium. Also study by Franekova *et al*[45] and Campos-Staffico *et al*[46] who suggested that, the anti-inflammatory effect of Omega- 3 protects the myocardial cells from damage. The study by Saleh *et al*[47] showed improvement in biomarkers of cardiac and renal function and histopathological findings by Omega-3 in Doxorubicin treated rat by showing an improvement in oxidative and apoptotic biomarkers in both heart and kidney tissues.

CONCUSIONS

Cardiac damage mediated by Nandrolone treatment

for 6 weeks is noted and proven by histological and immunohistochemical observations. Either Nigella sativa or Omega-3 had beneficial role and could protect the myocardium, reduced their injury and fibrosis. The improving effect of Nigella sativa was better than that of omega-3 as confirmed by histological and immunohistochemically methods.

CONFLICT OF INTERETS

There are no conflicts of interest.

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الملخص العربى

تقييم الدور الدفاعي لزيت حبة البركة وأوميغا ٣ ضد الأضرار القلبية بوساطة الناندرولون في ذكور الجرذان البيضاء البالغة: دراسة نسيجية وكيميائية مناعية

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المقدمة: يعتبر عقار الناندرولون هو أحد الستيرويد الابتنائي الأندروجيني المستخدم على نطاق واسع بين الرياضيين بالجرعات التي تؤدي الى اثار ضارة شديدة على العديد من الاعضاء وخاصة القلب.

الهدف من الدراسة: تقييم التأثير الوقائي لزيت حبة البركة وأوميجا ٣ على التغيرات النسيجية لعضلة القلب التي يسببها الناندرولون في الفئران.

المواد والطرق: تم تقسيم اربعين من ذكور الجرذان البالغة الى مجموعات، المجموعة الاولى هي المجموعة الضابطة. المجموعة الثانية خضعت للعلاج بعقار الناندرولون حقنا بجرعة ١٠ مجم / كجم من وزن الجسم، ثلاث مرات في الاسبوع لمدة ست اسابيع. المجموعة الثالثة تم علاجها بعقار الناندرولون على نفس النحو كالمجموعة الثانية بالاضافة الى العلاج بزيت حبة البركة عن طريق الفم بجرعة ٤٠٠ مجم / كجم من وزن الجسم، مرة واحدة يوميا لمدة ست اسابيع. المجموعة الرابعة تم علاجها بعقار الناندرولون على نفس النحو كالمجموعة الثانية بالاضافة الى العلاج بالأوميجا ٣ عن طريق الفم بجرعة ٤٠٠ مجم / كجم من وزن الجسم، مرة واحدة يوميا لمدة ست اسابيع. اعدت عينات عضلة القلب وخضعت للدراسات النسيجية و هستوكيميائية مناعية باستخدام كل من المجهر الضوئي والاليكتروني.

النتائج: اظهرت المجموعة الثانية تشوها واضحا وتفتتا وفقدان للتخطيط المنتظم لنسيج عضلة القلب،ارتشاح خلوي التهابي وزيادة واضحة في ترسيب الياف الكولاجين وزيادة واضحة في التعبيرالمناعي عن (α-SMA) و Caspase-٣ واظهرت نتائج المجموعة الثانية و الثالثة تقليل التاثيرات السامة لعقار الناندرولون على عضلة القلب وتحسين البنية النسيجية وتقليل التعبير المناعي بشكل واضح.

الخلاصة: يمكن ان يحمي زيت حبة البركة و الأوميجا ٣ عضلة القلب من التاثير الضار لعقار الناندرولون. لكن نتائج زيت حبة البركة كانت الافضل على مستوى الدراسةال نسيجية و هستوكيميائية مناعية