Effect of Nandrolone on Rat Cardiac Muscle and the Possible Protective Role of Vitamin E: A Light & Electron Microscopic Study

Maha El-Sayed Soliman, Ghada Hasan El-saify, Nadia S. Badawy khair, Mona A. Mohamed Soliman, Soad S. Abo-Habsa

Department of Histology, Faculty of Medicine, Menoufia University, Menoufia, Egypt
gh_hasan_2010@yahoo.com

Abstract: Objectives: Evaluating the effects of nandrolone on cardiac muscle of adult male albino rats and the possible protective role of vitamin E. **Background:** Nandrolone is an anabolic androgenic steroid used to increase the strength and improve the physical appearance. **Material and Methods:** Adult male albino rats (n=50) were used & classified into four groups: Group I (n= 10) served as control, group II(n= 10) treated with vitamin E (400 mg/kg/day) orally for 4weeks,group III (n= 20) treated with nandrolone (10 mg/kg/week) IM injection for 4weeks then, half the animals were killed (subgroup IIIA), and the other half were left without treatment for another 2 weeks (subgroup IIIB) & group IV(n= 10) treated with both nandrolone & vitamin E. At the end of the experiment, the heart was excised & processed for light, electron microscopic and statistical analysis. **Results**: Cardiac muscles of nandrolone treated rats showed considerable histological, ultrastructural & histochemical changes in the form of fragmentation, pyknotic nuclei, loss of striations, dehiscent intercalated disc, vacuolation, hemorrhage & dilated endomysium. There were increased collagen fibers in the endomysium & glycogen deposition. Intracellular cessation of nandrolone administration resulted in mild improvement of the changes. Co-administration of vitamin E with nandrolone revealed minimal changes. **Conclusion:** Nandrolone had toxic effect on rat cardiac muscle. This effect is partially reversible after cessation of treatment. Vitamin E had marked protective effect when co-administrated with nandrolone.

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Key words: Cardiac muscle, intercalated disc, nandrolone, fragmentation, vitamin E.

1. Introduction

In recent years, abuse of anabolic androgenic steroids (AAS) has become very common and represents a major health problem around the world **[1, 2].** AAS are synthetic derivatives of testosterone with similar effects on the body **[3]**. Nandrolone decanoate is one of the most popular AAS among athletes with the purpose of enhancing their physical performance. It is a derivate of 19-nortestosterone.It can be taken as oral pills, injectable steroid, creams and gel **[4].** Nandrolone was used as supportive therapy in some pathological conditions as cachexia that associated with some chronic diseases to increase body weight. Also it was used for treatment of anemia **[5].** But now, it is widely used in community for non medical causes, mostly by young men to improve building of their body muscles that lead to increase in their self confidence, energy and motivation **[6] .**Abuse of anabolic androgenic steroids has become a major health problem around the world. They have many adverse effects especially when used by otherwise healthy people. Serious adverse effects can be resulted from long –term use of excessive doses of anabolic steroids. These effects include hypercholestrolemia, hepatic dysfunction, hypertension and serious changes in structure of the heart **[7].** The most important is the increased cardiovascular diseases, as they have led to death of several young male body builders **[8].** The most commonly reported adverse effects of nandrolone decanoate on the heart were increase in atherosclerosis, cardiac dysfunction, tachycardia, arrhythmia, premature acute ischaemic heart diseases, myocardial infarction and even sudden death **[9]** Other effects as depression, nervousness, testicular atrophy and liver damage are also found by other authors **[10].**

Vitamin E includes a group of compounds which are tocopherols and tocotrienols. Clinically, vitamin E is used to prevent and repair cell and tissue damage during the radiation therapy. Vitamin E also used with adjuvant evening primrose oil to reduce breast pain. Nowadays, vitamin E had important role in the treatment of some types of cancer **[11].** Alpha tocopherol is an important lipid –soluble antioxidant. As it is fat –soluble, it is incorporated into the cell membrane, which protects it from oxidative damage. It also acts as a peroxyl radical scavenger, reducing the production of free radicals which damage the tissues by binding with them to form a tocopherol

radical **[12].**So vitamin E prevents the oxidation reaction through removal of the free radical intermediates **[13].**Since few and limited studies on animal models were existed to prove and establish the negative effects of AAS especially nandrolone on myocardium structure. Therefore, the aim of this current study was to throw more light on the histological, histochemical and ultrastructural alterations that produced in the myocardium of adult male albino rats treated with high –dose nandrolone decanoate and the possible protective role of vitamin E against the injury induced by nandrolone injection.

2. Material and Methods

A-Materials:

Animals:

This study was carried on fifty (50) adult male albino rats**.** Strict care and hygiene were maintained to keep them in normal and healthy conditions. They had their needs of diet and water. All animal procedures were done in accordance with the animal ethical committee of faculty of medicine, Menoufia University.

Drugs and chemicals:

Nandrolone decanoate (Deca –Durabolin): The drug was in the form of ampoules (25 mg/ ampoule). It was provided by the Nile Company pharmaceuticals –Cairo, under license of N.V. Organon-oss-Holland.

Vitamin E: Each ampoule contains 25 mg /ml. Vitamin E: the drug was in the form of soft gelatin capsules from Pharo Pharmaceuticals company.

Experimental protocol:

The animals were divided into four groups as follows:

Group I (control group): included 10 animals, half of them received no treatment **(IA)** while the other half received corn oil daily orally **(IB)**.

Group II (**vitamin E treated group):** Included 10 animals which receive vitamin E. One capsule of vitamin E (400mg) was dissolved in corn oil to produce 5cm of desired concentration (80mg /cm). It was given at a dose of 400mg/kg/day orally by modified plastic syringe for four weeks **[14].**

Group III (Nandrolone – Treated group):

Included 20 rats to which nandrolone was given by intra-muscular injection at a dose of 10 mg/kg/week for four weeks **[15].**

Then rats of this group were further subdivided into equal two subgroups (10 rats in each sub group).

Subgroup IIIA: animals of this subgroup sacrificed 24 hour after the last injection.

Subgroup IIIB: animals of this subgroup sacrificed two weeks after arrest of nandrolone treatment.

Group IV (Nandrolone and Vitamin E-treated group):

Composed of 10 rats, each was given nandrolone and vitamin E simultaneously at a dose similar to the previous groups. Then they were sacrificed 24 hour after the last injection.

B-Methods:

At the end of the determined period of each group, Animals from all groups were anaesthetized using ether inhalation then were sacrificed. Heart of each animal was excised.

Light microscopic study

Half of the cardiac muscles were fixed in 10% formol saline for light microscopy using Haematoxyline & eosin (H&E) stain for routine histological examination **(16)**, Masson trichrome stain for detection of collagen fibers **(17)** and Periodic acid schiff's reaction (PAS) for detection of glycogen**(18)**.

Electron microscopic study

The other half of the cardiac muscles were fixed in 1% glutaraldehyde in phosphate buffer for electron Microscopic Study **(19).**

Statistical analysis

The data (body weight and heart weight) were expressed as mean± SD. The p-value was used to test the significant change in each parameter in the experimental animals (treated groups in comparison with the control group). The data collected were tabulated and analyzed using statistical package for the Social Science Software (SPSS) software (version 17.0 on an IBM compatible computer; SPSS Inc., Chicago, Illinois, USA). *P* value was set at 0.05, *P*>0.05 non-significant, *P* value<0.05 significant and *P* value <0.01 highly significant **(20).**

3. Results

General appearance of animals:

All animals of the control group and vitamin E treated groups were in a good general condition and showed a normal behaviour, activity, eating and growth. Animals treated with nandrolone for four weeks showed decreased activity and progressive diminution in their appetites. Some animals became morbid and weak during the experiment. While the animals left two weeks after arrest of nandrolone treatment**,** gradually regained their activity at the end of the experiment. Animals treated with nandrolone plus vitamin E were in a somewhat good general condition and had a moderate appetite compared with the nandrolone treated group.

Body weight:

There was a highly significant decrease in the body weight observed in nandrolone treated group (subgroup IIIA) compared with animals of control group (*p* value < 0.01) while there was non-significant decrease in body weight in both vitamin E (group II) and protected (group IV) groups (*p* value > 0.05). There was a significant decrease (*p* value <

0.05) in the body weight of recovery group (subgroup IIIB) when compared with control animals of the same age (Table 1).

There was a significant decrease (p value < 0.05) in the body weight of recovery group (subgroup IIIB) when compared with protected group (group IV) (Table 2).

Table (1): Statistical means of body weight (gm) of various experimental groups.

Group	Mean ± SD	T-test	*P* value
Group I (Control)	**203.9 ± 14.9**		
Group II (Vitamin E)	**204.1± 11.9**	**0.033**	**0.974***
Subgroup IIIA (Nandrolone)	**188.1± 9.6**	**.883**	**0.011*****
Subgroup IIIB (Recovery)	**189.2± 15.3**	**2.210**	**0.043****
Group IV (Protected)	**203.1 ± 11**	**0.137**	**0.893***

Non significant * (P> 0.05) Significant** (P>0.05) Highly significant*** (P>0.01) t: Student test.

Table (2): Statistical means of body weight (gm) of recovery and protected groups.

Group	Mean ± SD	T-test	*p*-value
Subgroup IIIB (recovery)	**189.2 ± 15.3**	**2.376**	**0.031****
Group IV(Protected)	**203.1±11**		

Significant**(P>0.05) t : Student test.

Heart weight:

There was a highly significant increase in the heart weight observed in nandrolone treated group (subgroup IIIA) compared with animals of control group (p value < 0.01) while there was no significant increase in heart weight in both vitamin E (group II) and protected (group IV) groups (p value > 0.05). There was a significant increase (p value < 0.05) in the heart weight of recovery group (subgroup IIIB) when compared with control animals of the same age (Table 3).

There was a significant increase (p value < 0.05) in the heart weight of recovery group (subgroup IIIB) when compared with protected (group IV) group (Table 4).

Table (3): heart weight (gm) of various experimental groups.

Group	Mean ± SD	T-test	*P*-value
Group I (Control)	**0.9 ±0.1**		
Group II (vitamin E)	**0.9 ±0.1**	**1.755**	**0.1***
Subgroup IIIA (Nandrolone)	**1.1 ±0.3**	**2.994**	**0.009*****
Subgroup IIIB (Recovery)	**1 ±0.2**	**2.240**	**0.041****
Group IV (Protected)	**0.94± 0.1**	**0.120**	**0.906***

Non significant *(P> 0.05) Significant**(P>0.05) Highly significant*** (P>0.01) t: Student test.

Table (4): heart weight (gm) of recovery and protected groups.

Group	Mean ± SD	T-test	*p*-value
Subgroup IIIB (Recovery)	**1 ± 0.2**	**2.308**	**0.036****
Group IV (Protected)	**0.94± 0.1**		

Significant** (P>0.05) t : Student test.

Light Microscopic Results:

1-Histological Results:

Hematoxylin and Eosin stain:

Sections of the myocardium of control rats of both subgroups A & B showed the normal histological structure of cardiac muscle with transverse striations of muscle fibers**,** intercalated discs and centrally located oval vesicular nuclei **(Fig.1a).** Sections of vitamin E treated myocardium showed normal appearance of muscle fibers and nuclei **(1b).** Sections of nandrolone treated myocardium showed fragmented muscle fibers, vacuolation**,** loss of transverse striations, disorganized intercalated disc and widened endomysium **(1c&1d).** Sections of myocardium of rat sacrificed two weeks after arrest of nandrolone treatment showed fragmented muscle fibers, hemorrhage and pyknotic nuclei**(1e).** Sections of myocardium of rats treated with nandrolone plus vitamin E showed wavy appearance of muscle fibers, widened endomysium and dilated blood vessel **(1f)**.

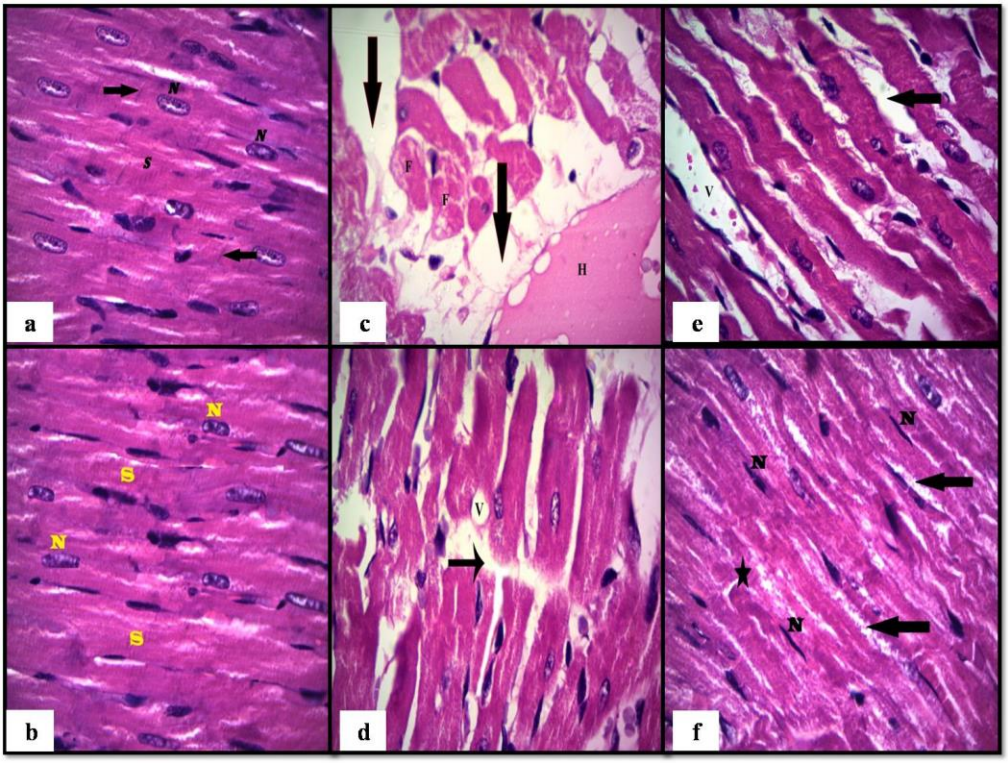

Fig.1: A photomicrograph of Hx & E stained sections of the myocardium of

(1a): control rat showing the transverse striations of the muscle fibers (S), the intercalated discs (arrows) and the centrally located oval vesicular nuclei with prominent nucleolus (N). **(1b):** vitamin E treated rat showing transverse striation of the muscle fibers (S) and normal appearance of nuclei (N). **(1c):** nandrolone treated rat showing fragmented muscle fibers with vacuolation (V), loss of the transverse striation and disorganized intercalated disc (arrow). **(1d):** nandrolone treated rat showing widened endomysium (arrow), fragmented dissoluted muscle fibers (F) and acidophilic homogenous exudate (H). **(1e):** nandrolone treated rat & sacificed two weeks after arrest of treatment showing fragmented muscle fibers (star) which acquire a wavy appearance with intramuscular hemorrahgic areas (arrow). Most of nuclei are still pyknoic (N). **(1f):** nandrolone plus vitamin E treated rat showing wavy appearance of the cardiac muscle fibers, widened endomysium (arrow) and dilated blood vessel (V). **(Hx. & E. x 1000).**

Masson Trichrome Stain:

Sections of the myocardium of a control rat showed normal distribution of collagen fibers (green colour) in the endomysium. **(2a).** Sections of vitamin E treated myocardium showed small amount of collagen fibers in the endomysium**(2b).** Sections of nandrolone treated myocardium showed marked amount of the collagen fibers around the blood vessels and in the endomysium **(2c).** Sections of myocardium of rat sacrificed two weeks after arrest of nandrolone treatment showed moderate amount of the collagen fibers in the endomysium**(2d)**. Sections of myocardium treated with nandrolone plus vitamin E showed mild amount of collagen fibers in the endomysium **(2e).**

2-Histochemical stain:

Periodic Acid Schiff (PAS) reaction:

Sections of the myocardium of control rat and vitamin E treated rats showed mild PAS +ve reaction **(3a& 3b).** Sections of the myocardium of nandrolone treated rats showed strong PAS +ve reaction and weak reaction in some degenerated fibers **(2c).** Sections of the myocardium of rats sacrificed two weeks after arrest of nandrolone treatment showed moderate to strong PAS +ve reaction **(3d).** Sections of myocardium treated with nandrolone plus vitamin E showed moderate PAS +ve reaction **(3e).**

Electron microscopic results:

Sections of control myocardium showed normal banding pattern of the myofibrils. Mitochondria with apparent cristae appeared between them **(4a).** Sections of vitamin E treated myocardium showed normally appeared myofibrils with interposed mitochondria **(4b).** Sections of nandrolone treated myocardium showed dilated sarcoplasmic reticulum, destructed myofibrils and mitochondrial membrane (moth eaten appearance) **(4c & 4d).** Sections of myocardium of rat sacrificed two weeks after arrest of nandrolone

treatment showed that some of the myofibrils restored their normal appearance**,** but most of them were still degenerated**.** There was abnormal distribution of degenerated mitochondria **(4e).** Sections of rat treated with nandrolone plus vitamin E showed nearly normal myofibrils with slightly degenerated mitochondria **(4f).**

Sections of myocardium of control rat and vitamin E treated rats showed normal organization of the intercalated disc (step like pattern) **(5a&5b).** Sections of two cardiomyocytes of myocardium treated with nandrolone showed marked disorganization of intercalated disc with nearly separation of the two cardiomyocytes**(5c).** Sections of myocardium of rats sacrificed two weeks after arrest of nandrolone treatment showed disorganized intercalated disc and disturbance of the normal banding pattern of the myofibrils **(5d).** Sections of two cardiomyocytes of rats treated with nandrolone plus vitamin E showed slight disorganization of intercalated disc with slight separation of the cardiomyocytes**(5e).**

Sections of the myocardium of control rat showed a euchromatic nucleus with dispersed chromatin **(6a).** Sections of vitamin E treated myocardium showed normal appearance of the nucleus and the myofibrils **(6b).** Sections of myocardium of rat treated with nandrolone showed abnormal appearance of the nucleus with peripherally condensed marginated chromatin and corrugated membrane. There was increased amount of collagen fibers in the widened endomysium. Large vacuole appeared near the nucleus **(6c).** Sections of myocardium of rat sacrificed two weeks after arrest of nandrolone treatment showed abnormal nucleus **(N)** with peripherally condensed marginated chromatin **(6d).** Sections of myocardium treated with nandrolone plus vitamin E showed nearly normal appearance of the nucleus **(6e).**

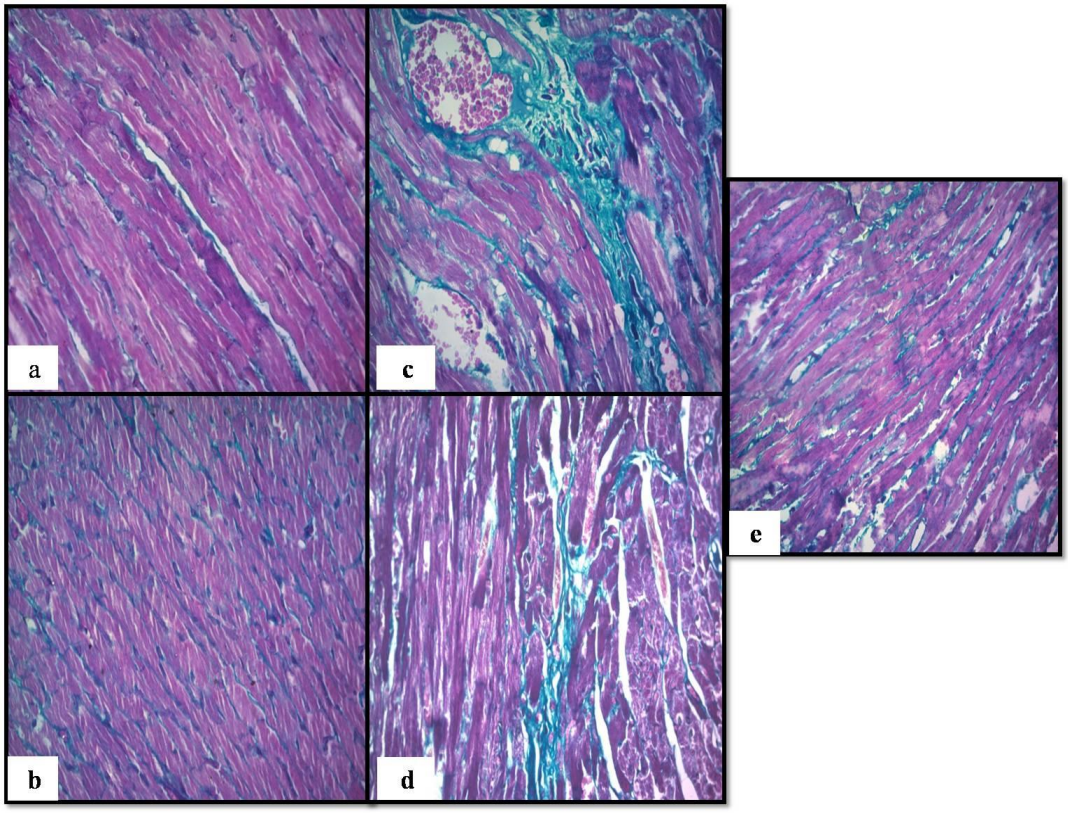

Fig.2: A photomicrograph of Masson Trichrome (M.T.) stained sections of the myocardium of:
(2a): control rat showing normal distribution of collagen fibers (small amount) in the endomysium.
(2b): vitamin E treated rat showing small amount of collagen fibers in the endomysium.
(2c): nandrolone treated rat showing marked amount of the collagen fibers around the blood vessel and in the endomysium.
(2d): rat sacrificed two weeks after arrest of nandroloneteatment showing moderate amount of the collagen fibers in the endomysium.
(2e): rat treated with nandrolone plus vitamin E showing mild amount of collagen fibers in the endomysium. **(M.T X 400)**.

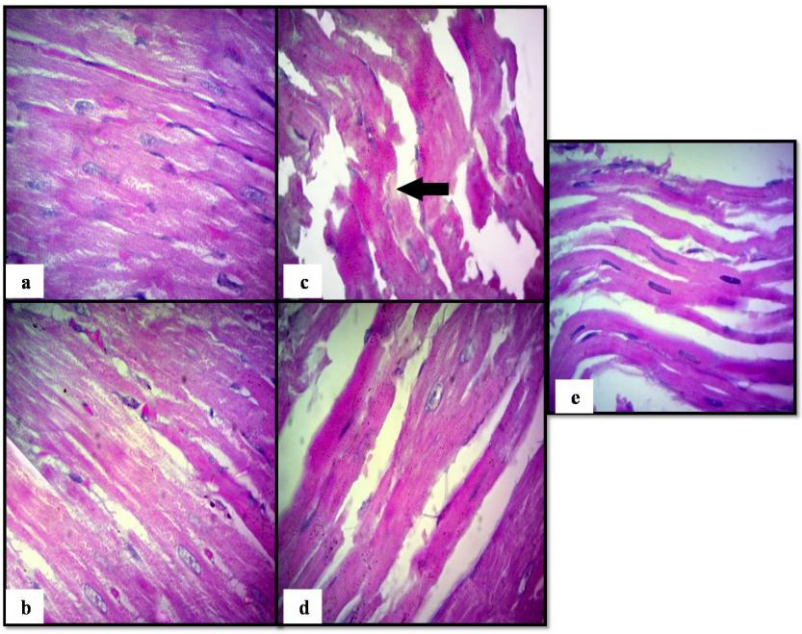

Fig.3: A photomicrograph of Periodic Acid Schiff' reaction (PAS) stained sections of the myocardium of: **(3a):** a control rat showing mild PAS +ve reaction. **(3b):** vitamin E treated rat showing weak to mild PAS +ve reaction. **(3c):** nandrolone treated rat showing strong PAS +ve reaction and weak reaction in some degenerated fibers (arrow). **(3d):** rat sacrificed two weeks after arrest of nandolone treatment showing moderate to strong PAS +ve reaction. **(3e):** rat treated with nandrolone plus vitamin E showing moderate PAS +ve reaction.**(PAS x 1000)**

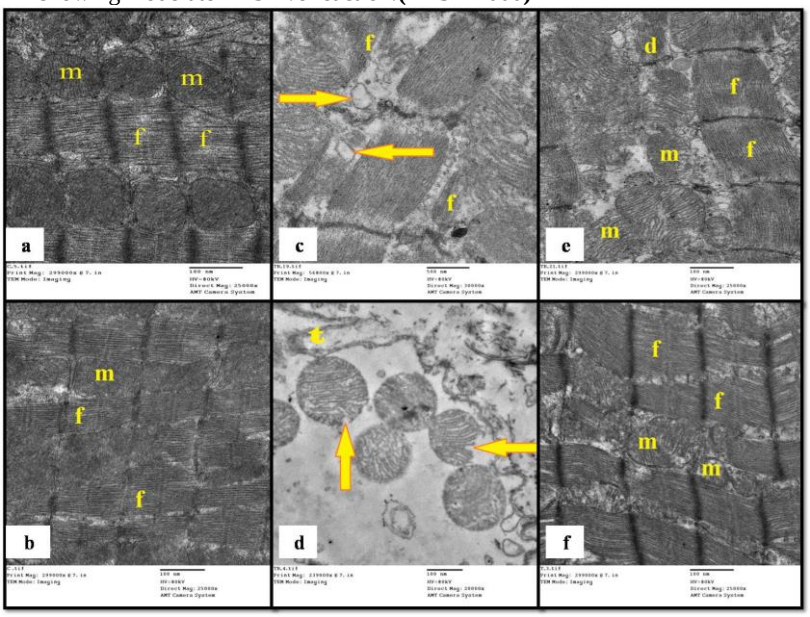

Fig.4: Anelectron micrograph of the myocardium of:

(4a): a control rat showing nomal banding patten of the myofibrils **(f).** Rounded to oval mitochondria **(m)** with apparent cristae arranged in rows between them. **(TEMx25000)**. **(4b):** vitamin E treated rat showing normally appeared myofibrils **(f)** with transverse striations and rows of interposed mitochondria **(m). (TEM x 25000)**. **(4c):** nandrolone treated rat showing dilatation of sarcoplasmic reticulum **(arrow)** and destruction of some myofibrils **(f). (TEM x 30000)**.

(4d): nandrolonetreated rat showing destruction of mitochondrial membrane **(moth eaten appearance) (arrows)** and dilatation of t tubule **(t).** The myofibrils are nearly absent. **(TEM x 20000)**. **(4e):** rat sacrificed two weeks after arrest of nandoloneteatment showing that some of the myofibrils restored their normal appearance **(f),** but most of them are still degenerated **(d).** There is abnormal distribution of degenerated mitochondria **(m). (TEM x 25000)**. **(4f):** rat treated with nandrolone plus vitamin E showing nearly normal architecture of the myofibrils **(f)** with slightly degenerated mitochondria **(m). (TEM x 25000)**.

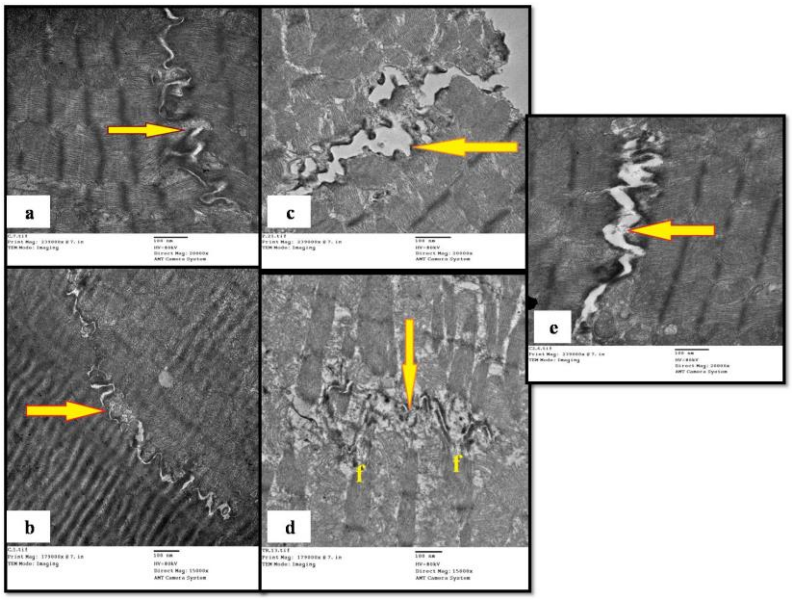

Fig.5: An electron micrograph of two cardiomyocytes of:

(5a): a control rat showed normal appearance **(step like patten)** of the intercalated disc **(arrow). (TEM x 20000)**.
(5b): vitamin E treated rat showing normal organization of the intercalated disc **(arrow). (TEM x 15000)**.
(5c): nandrolone treated rat showing marked disorganization of intercalated disc **(arrow)** with nearly separation of the. **(TEM x 20000)**.
(5d): rat sacrificed two weeks after arrest of nandoloneteatment showing disorganized intercalated disc **(arrow).** There is disturbance of the normal banding pattern of the myofibrils **(f). (TEM x 15000)**.
(5e): rat treated with nandrolone plus vitamin E showed slightly disorganized intercalated disc **(arrow)** with slight separation of the cardiomyocytes. **(TEM x 20000)**.

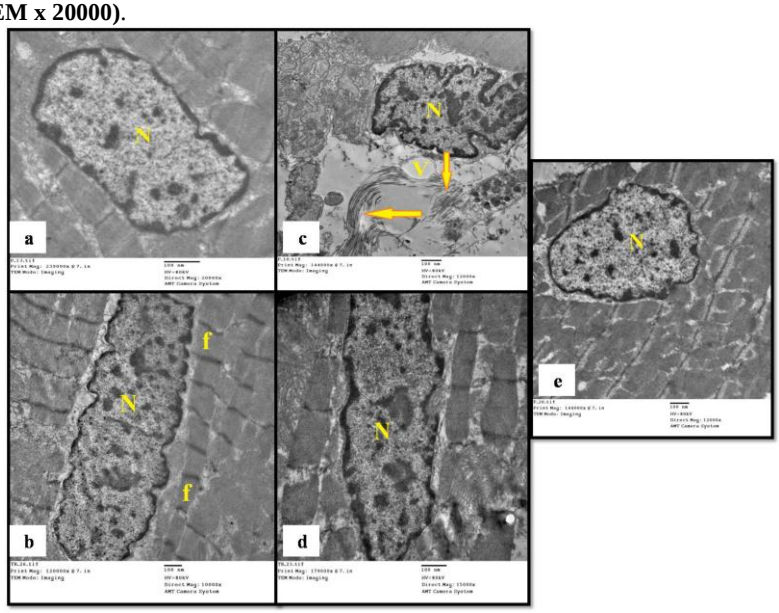

Fig.6: Anelectron micrograph of the myocardium of:

(6a): a control rat showing aneuchromatic nucleus (N) with dispersed chromatin. **(TEM x 20000)**.
(6b): vitamin E treated rat showing normal appearance of the nucleus (N) and the myofibrils (f). **(TEM x 10000)**.
(6c): nandrolone treated rat showing abnormal appearance of nucleus of the cardiaomyocyte (N) with peripherally condensed marginated chromatin and corrugated membrane. There is increased amount of collagen fibers (arrow) in the widened endomysium with the presence of a large vacuole near the nucleus (V). **(TEM x 12000)**.
(6d): rat sacrificed two weeks after arrest of nandoloneteatment showing abnormal nucleus (N) with peripherally condensed marginated chromatin. **(TEM x 15000)**.
(6e): rat treated with nandrolone plus vitamin E showing nearly normal appearance of nucleus (N). **(TEM x 12000)**.

4. Discussion

Anabolic androgenic steroids (AAS) are drugs synthesized from testosterone, produced to maximize anabolic effects and minimize the androgenic. Their anabolic action is mainly due to increase synthesis and reduction in degradation of the muscle proteins **[21, 22].** AAS are used to improve physical performance and appearance of individuals perform physical training. However, the AAS non-therapeutic use in humans is related to many health problems **[23].**

AAS abuse elicited several adverse effects. The increased risk of cardiovascular diseases is the most important one. The heart is the most frequently affected organ due to abuse of these steroids **(24).**

Franquni *et al.* [25] concluded that high doses of nandrolone induced cardiotoxic effects. The most commonly reported side effects of AAS were increase in atherosclerosis, tachycardia, cardiac hypertrophy, impaired cardiac function and sudden death **[9, 10].**

In the present study, nandrolone was used at a dose of 10mg/kg b.wt./week for 4 weeks by intramuscular injection according to previous study done by **Hemmat [15]** who studied the effect of nandrolone on cardiac muscle in adult male albino rats.

Antioxidants are substances that present in minerals, vitamins and other compounds. They had a role in preventing diseases by fighting free radicals which had harmful effect on the body when left unchecked. Free radicals are formed by environmental pollutants like smoking and also by normal bodily processes such as breathing. These free radicals circulate throughout the body, damaging it in absence of adequate amounts of antioxidants**[26]**.

Vitamin E which is a fat soluble vitamin was used in our study. It contains good medical properties, associated with health benefits against multiple diseases including inflammation, cancer, atherosclerosis and cardiovascular disorders. It also acts as an antioxidant **[27].** But its protective effect on nandrolone induced cardiac damage has not been well studied.

In our study, vitamin E was used in a dose of 400mg/Kg body weight daily orally according to previous study done by **Songthaveesin *et al.* [14]** who studied the radioprotective effect of vitamin E on spermatogenesis in mice exposed to gamma irradiation.

In our study, rats treated with nandrolone for four weeks showed marked statistical changes in body weight and heart weight & histological, ultrastructural and histochemical changes in cardiac muscle compared with control rats. These changes showed little improvement after arrest of nandrolone treatment for two weeks. Minimum changes were detected when vitamin E was used in combination with nandrolone.

In the current study, rats treated with nandrolone showed less weight gain as compared to control group. The body weight was highly significantly decreased in rats treated with nandrolone and sacrificed 24 hour after the last injection. While those sacrificed two weeks after arrest of nandrolone treatment showed significant decrease in their body weight as compared with control group. Both vitamin E and nandrolone plus vitamin E treated groups showed non significant decrease in their body weights as compared to control group.

These findings are in agreement with different authors **[28, 29, 30]** who reported that AAS reduce body weight.

Such reduction in body weight could be due to decreased the appetite and an increase in fat metabolism in adipose tissue as had been previously explained by**(31)** in their study on the effect of anabolic steroids on cardiac renin-angiotensin system in rats.

In contrary, previous studies demonstrated that the anabolic steroid, nandrolone decanoate, may increase muscle mass and body weight **[32].**

In the present study, administration of AAS caused a highly significant increase in the heart weight (HW) in comparison with the control group. Animals sacrificed two weeks after arrest of nandrolone treatment showed significant increase in heart weight compared to that of control group. While vitamin E only and nandrolone plus vitamin E treated groups showed non- significant increase in heart weight in comparison to control group.

This coincided with recent studies which reported that high dose AAS administration in mice produce significant increase in HW **[24, 33].**

AAS resemble the testosterone hormone and work like it in some ways but not in others. One of the ways they don't work like testosterone is in terms of toxicity **[34].**

Although testosterone has beneficial effects on the cardiovascular system, some studies linked the exogenous supra-physiological doses of AAS with the development of cardiovascular diseases as hypertension, increased interventricular septum thickness, dilated cardiomyopathy, arrhythmia, altered lipoprotein profiles, thrombosis, erythrocytosis, heart failure and sudden death **[8, 35]. Melchert and Welder [36]** categorized the effects of AAS on the cardiovascular system into four groups of activities: atherogenic, thrombotic, vasospastic, and direct myocardial injuries. Studies on isolated hearts from rats treated chronically with nandrolone decanoate (ND) have shown also increase the myocardial susceptibility to ischemia/reperfusion injuries **[37, 38]. Frati *et al.* [39]** and **Tanno *et al.* [40]** reported that steroid abuse induces also changes in heart

structure, ventricular thickness and size, as well as heart connective tissue content. Also left ventricular hypertrophy, associated with fibrosis and myocytolisis was reported by **Montisci *et al.*[41]** and **Paolo *et al.*[42].**

Riezzo *et al.* [24] observed that exogenous AAS administration induce cardiac hypertrophy, and when combined with exercise, these steroids change exercise-induced physiological cardiac hypertrophy to pathophysiological cardiac hypertrophy. These results are in harmony with previous studies **[43].** In addition to that, **Nahrendorf *et al.* [28]** reported an increase in the left ventricle mass in rats treated with testosterone during 10 weeks.

Androgens may act by receptor-dependent and receptor-independent mechanisms. Androgen receptors are present in the cardiovascular system, including human vascular endothelium, macrophages, smooth muscle cells and cardiac myocytes **[44].**

The presence of androgen receptors in cardiac myocytes can directly mediate a significant hypertrophic response to androgens in heart as had been previously reported by **Hannan *et al.*[45]** and **Sethi *et al.*[46]**.

Our results are in agreement with **Ren [47]** who showed that steroids alone cause cardiomyocyte hypertrophy.

Karhunen *et al.* [48] found that abuse of anabolic steroids led to increased peripheral vascular resistance and cardiac hypertrophy with depressed contraction ability of the heart. **Trifunovic *et al.*[49]** reported similar finding but without altered contractility of the heart.

Our finding was in contrast to **Hemmat [15]** who found that administration of nandrolone caused a significant decrease in the heart weight during the experimental period in comparison with the control group. However, **Pereira *et al.*[50]** reported that no significant change in the heart weight of rats treated with nandrolone.

Maísa *et al.* [30] reported that these variations in AAS effects may depend on the dose, treatment duration and method of drug administration. Also their effects depend on, the user age and performance level.

In the present study, light and electron microscopic results of cardiac muscle in nandrolone treated rats showed fragmented, distorted and highly degenerated muscle fibers. Also hypertrophied muscle fibers with intramuscular haemorrhagic areas, vacuolation were seen. Numerous pyknotic and karyolytic nuclei were obviously detected. Widened endomysium with increased the collagen fibers were also observed. Pronounced disturbance of banding pattern of myofibrils with variation in the mitochondrial size with ruptured cristae and ruptured outer and inner membranes were detected.

These findings support and confirm those of **Golestani *et al.*[51]** who reported that AAS indirectly led to sarcomere disruption, mitochondrial damage and apoptosis.

Cavasin *et al.*[52] also reported that the use of an anabolic steroid caused serious injuries in the rodent heart

Heart protection include antioxidant enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and others as well as non-enzymatic antioxidants, such as alpha-tocopherol and reduced glutathione (GSH) that directly scavenge unpaired electrons of free radicals avoiding damage propagation **[53].**

The ability of nandrolone to disturb normal antioxidant defenses and induce oxidative stress was previously reported by **Celec *et al.*[54]** and **Ahmed [55].**

The cardiotoxic effects associated to AAS abuse could be mediated by reduced heart antioxidant capacity. Nandrolone administration caused significant reductions in rat heart GPx (glutathione peroxidase), GR (glutathione reductase) and SOD (super oxide dismutase) activities as had been previously explained by several authors **[56]**.

Apoptosis has been also observed in heart diseases, and constitutes a key factor in the pathogenesis of cardiac failure. Previous findings demonstrate that cardiomyocyte apoptosis is a critical event in the transition between compensatory cardiac hypertrophy and heart failure **[57**]. AAS increase primarily anabolic and growth-promoting effects in cardiac tissue, however, they also cause ultrastructural alterations of cardiomyocytes similar to the changes observed in the early stages of congestive heart failure **[58].**

Another adverse effect of nandrolone abuse is the increased collagen content of the heart. Our study showed that AAS caused marked increase in the amount of collagen between the cardiac myocytes. Our results are in accordance with those of **Franquni *et al.* [25]** who found that administration of nandrolone alone cause a 10 fold increase in heart collagen.

Increased collagen fibers in the present study could be due to increase collagen synthesis, especially in soft connective tissues by high doses of AAS. This effect tended to be dose-dependent. These short-term changes in collagen metabolism may be due to increased anabolic effects in muscle or may be secondary effects of increased working capacity as had been previously explained by **Parssinen *et al.* [59]**.

All of the cardiovascular effects of nandrolone have been demonstrated to be fully reversible within

several months after cessation of the steroid use **[60, 61].**

The PAS value reported in this study was generally higher than the control in all the treated groups. The lowest rate of PAS positive material was observed in control and vitamin E groups, while the highest rate was observed in the nandrolone treated group. In the group treated with vitamin E + nandrolone the reaction was intermediate between the two groups.

The increased glycogen content of the cardiac muscle fibers in presence of AAS could be due to the ability of these steroids to change the tissue response to other hormones, such as insulin-like growth factor as had been previously reported by **Silva *et al.*[62]**.

Goodwin and **Yaegtmeye [63]** reported that the pattern of energy use by the heart is combined competition between fatty acids, lactate and glucose and differs according to hormonal status and workload.

These findings were in harmony with the results of **Falkenberg *et al.* [64]** and **Foss and Keteyian [65]** who also reported that steroids can increase glycogen synthase activity with subsequent increase in glycogen synthesis in male animal.

On the contrary; **Asmaa and Manal [66]** reported that AAS administration alone did not increase cardiac glycogen content in steroid group as compared to control group.

Light and electron microscopic examination of rat cardiac muscle fibers treated with vitamin E and nandrolone revealed marked protection of myofibers against the deleterious changes induced by nandrolone, however, mild widened endomysium, some pyknotic and karyolytic nuclei and few degenerated mitochondria were still present.

Verhagen *et al.* [67] demonstrated that vitamin E and other antioxidants protect cells from the damaging effects of free radicals. Free radicals damage cells and might contribute to the development of cardiovascular diseases and cancer.

In summary the present results suggested that the high dose of nandrolone not only elicit measurable increase in performance, but also is able to induce adverse effects on cardiac muscle fibers at the histological, histochemicals and ultrastructural levels.

On the other hand vitamin E supplementation elicited better maintenance of muscle strength and doesn't cause any damaging effects to the cardiac muscle fibers. The application of vitamin E to the nandrolone-treated animals could provide a possible protective role from these adverse effects of nandrolone injection. So, nandrolone must be used under clinical supervision especially in the young misinformed athletes.

Conclusion

From the foregoing, it is concluded that chronic administration of supra physiological doses of nandrone had toxic effect on rat cardiac muscle. This effect is partially reversible after cessation of treatment. Vitamin E had marked protective effect when co-administrated with nandrolone. So, it's use with nandrolone is recommended.

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