Original Article

Evaluation of Nandrolone Decanoate Effects on Sinoatrial Node in Experimental Rat Model (Histological and Immunohistochemical study)

Marwa M. El Sawy, Reham F. Tash and Ahmed M. Desouky

Department of Anatomy, Faculty of Medicine, Ain Shams University, Egypt.

ABSTRACT

Background: Nandrolone decanoate (NA) is one of the anabolic steroids used medically and abused by athletes but has several harmful effects.

Aim: Evaluate the effect of nandrolone decanoate on sinoatrial node structure of albino rat and the effect of its withdrawal.

Materials and Methods: Thirty adult albino rats were divided into three groups each containing ten rats. Group I (control): ten rats were divided into the negative control was left untreated and the positive control was injected by castor oil, twice weekly/8 weeks. Group II (NA group): ten rats were injected by NA 5 mg/kg twice/week/8 weeks. Group III (recovery group): ten rats were injected by NA 5 mg/kg twice/week /8 weeks, then were left 12 weeks to evaluate recovery. At the end, the sinoatrial node were dissected out, and subjected to histological and immunohistochemical examination.

Results: Examination of right atrial sections of NA injected group showed wide separation of sinoatrial node structure and disordered cell arrangement. P cell degeneration with increased cytoplasmic vacuoles was noted. Some cardiomyocytes exhibited degenerative changes including wavy sarcoplasm and fragmented pyknotic nuclei. After discontinuation of NA, the sinoatraial node did not regain its normal architecture. Mean area percentage % of collagen fiber deposition, SYN and iNOS immunoexpressing in NA injected group was significantly increased relative to control group which did not recovered after drug withdrawal.

Conclusion: Use of NA caused serious changes in sinoatrial node tissue which will threaten life of their users especially the athletes. Thus, NA must be administered only under medical supervision.

Key Words: Nandrolone decanoate, rat, recovery, sinoatrial node.

Revised: 25 October 2022, **Accepted:** 26 November 2022.

Corresponding Author: Marwa M. El Sawy, PhD, Department of Anatomy, Faculty of Medicine, Ain Shams University, Egypt, **Tel.:** +202 01066709220, **E-mail:** drmarwaelsawy@gmail.com

ISSN: 2536-9172, December 2021, Vol. 5, No. 2

INTRODUCTION

Nandrolone decanoate (NA) are synthetic testosterone derivatives with functional and structural hormonal elements which increase muscle mass and strength (anabolic activity)[1]. It is rapidly absorbed from the small intestines and metabolized in the liver by the enzyme 5α-reductase, into, 19-norandrosterone and 5α-dihydronandrolone, which can be detected in urine[2]. NA are utilized in significant clinical setting to treat different circumstances including osteoporosis, growth deficiency and hypogonadism[3], aplastic anemia and cachexia-associated conditions as cancer and burns[4], renal and hepatic failure[5].

The abuse of these agents among young adult athletes and body builders with the aim to enhance athletic performance and muscular development is a great health problem[6]. The world-wide prevalence of NA use is difficult to estimate but several countries have in the last years reported increased problems with these substances[7,8&9].

Several studies on animals confirmed cardiovascular effects of NA including blood vessel disorders, increased erythropoiesis, platelet aggregation, hyperviscosity[10] and hypertension[11], but NA may have direct effects on cardiac muscle and its function and altering repolarization[12], heart failure, arrhythmia, and sudden cardiac death[13].

Evidence indicates direct relationship between NA and heart disorders by performing animal experiments and identifying autonomic dysfunction[14], fibrosis, hypertrophy, and myopathy[15&16] impaired ionic balance[17].

High doses of NA can lead to sudden cardiac death[13]. Therefore the effect of nandrolone decanoate on sinoatrial node of the rat was studied. Furthermore, effects of its withdrawal were investigated as a controversy exists regarding improvement of the side effects produced by NA upon withdrawal.

 DOI: 10.21608/jmh.2022.170705.1105

MATERIALS AND METHODS

Drug:

Nandrolone decanoate, commertially known as Deca-Durabolin, produced, by the Nile Co. for Pharmaceuticals-Cairo-A.R.E. In 1ml ampoules packaging, each contained 25mg of the drug in the form of oily substance. Choosing the dose and the route of administration of the drug was based on athletes' usage of the NA at fitness centers. NA was injected intramuscularly to the treated group (5 mg/kg twice/week dissolved in 0.5 ml of castor oil) and the dose of the drug was calculated according to the average weight of the rats[18].

Animals:

Thirty adult male albino rats (six-months old), weighing 180-200 gm, housed at the animal house of the Research Institute (MASRI), Faculty of Medicine, Ain-Shams University were used in the present study. Rats were housed with regular dark/light cycles, every 12 hours and were fed the standard rat chow diet and supplied water ad libitum. All rats were kept under the same circumstances throughout the experiment. The experimental protocol was approved by the ethical Committee for Scientific Research, Faculty of Medicine, Ain Shams University.

Experimental design:

All animals were divided into three groups, ten rats each:

Group I: (control group) ten rat were further divided into two subgroups (5 rats each):

Group I-a (negative control): five rats were left without any intervention.

Group I-b (positive control): five rats were injected intramuscularly with 0.5ml of castor oil twice/week for eight weeks.

Group II: (NA group) rats were given intramuscular injection of nandrolone decanoate in a dose of 5 mg/kg body weight twice/week for eight weeks.

Group III: (recovery group) rats were given intramuscular injection of nandrolone decanoate in a dose of 5 mg/kg body weight twice/week for eight weeks then were left for twelve weeks to evaluate recovery.

Specimen collection:

Rats were anesthetized by ether inhalation then sacrificed by cervical dislocation. The sinus node samples were dissected from the junction between the superior vena cava and the right auricular appendage and subjected to the following techniques:

1- For histological study:

Small pieces (1cm^3) of all the extracted tissues were fixed in 10% formalin for one week, processed and embedded in paraffin. Paraffin sections (4-5-µm thick) were cut and were stained with Haematoxylin & Eosin and Masson's trichrome stain[19]. The sections were examined using an Olympus light microscope and were photographed in Anatomy department, Faculty of Medicine, Ain Shams University.

2- For immunohistochemistry technique:

Five µm thick sections were obtained, deparaffinized, and washed with phosphate buffered saline. The sections were incubated overnight in a humidified chamber with the primary antibody; mouse monoclonal synaptophysin antibody (1:200; Sigma-Aldrich Chemicals) for detection of synaptophysin glycoprotein which present in synaptic vesicles[20] and anti inducible nitric oxide synthase antibodies (iNOS) (1:100 dilution, ;Sigma-Aldrich, USA) for evaluation of the oxidative stress[21]. Then, the atrial sections were rinsed with buffered saline and treated with the biotinylated antibody for one hour. The sections were then incubated with streptavidin combined to horseradish peroxidase (Sigma, USA) and finally the reaction was established using DAB (3,3-diaminobenzidine tetrahydrochloride, Fluca). Stained atrial sections were examined using Olympus binocular microscope and photographed using a Canon camera connected to an IBM computer system.

3- Image Analysis and Statistics:

Quantification of mean surface area percentage (%) of the immunoreactivity of synaptophysin per microscopic field (400 X magnification), and the number of iNOS positive cells between different groups (400X magnification) were performed. In Masson's trichrome stained sections at 400 X magnification, the sections were examined to measure the collagen fiber surface area percentage (%). Measurement in the three groups was recorded and analyzed using an image analyzer and the collected data were statistically analyzed using SPSS statistical package version 13. One-way ANOVA test followed by Tukey's multiple comparison tests was used to compare the means of the various groups. *P values <0.05* were considered significant and those *< 0.001* were considered highly significant[22].

RESULTS

Histological findings of the sinoatrial node:

Group I (control group):

Light microscopic examination of longitudinal sections of the right atrial walls from positive and negative control groups revealed a similar histological picture. The sinoatrial node (SAN) tissue is oval shaped structure of special cardiac muscle. The SAN was located beneath the

epicardium on crista terminalis and in close proximity to the superior vena cava (Fig.1a). The SAN substance was arranged around a central nodal artery that sometimes bifurcates in the SAN substance (Fig.1a-d). The SAN cells were composed of oval or spherical shaped P cells (typical nodal pacemaker cells) and rod-shaped or long cylindrical T cells (transitional cells). The cytoplasm of P cells were paler than those of myocardiocytes, were located in the center and around the nodal artery; with a single rounded nuclei. The elongated T cells were pale, and located at the periphery of the node, with a single dark round nucleus. Cardiomyocytes were darker than P cells of the node, which unite together to form fascicles of sarcoplasm parallel to one another (Fig. 1c). Its nuclei are central, dark and rounded. Masson trichrome sections showed little amount of fine collagen fibers in between the nodal cells (Fig. 1d).

Group II (NA group):

Sections of sinus nodes of this group showed splitting of SAN structure (Fig. 2a) with disordered cell arrangement (Figs. 2b & 2c). Appearance of congested blood vessels was observed (Figs. 2a & 2b). P cell degeneration was characterized by fragmented cytoplasm (Fig. 2b) and increased cytoplasmic vacuoles (Fig.2c). Some cardiomyocytes exhibited degenerative changes including wavy sacroplasm and deeply stained cytoplasm with fragmented nuclei (Fig. 2c). Increased collagen deposition was noted between nodal cells especially in areas surrounding cardiomyocytes (Fig. 2d).

Group III (rats treated with NA for 8 weeks followed by 12 weeks withdrawal):

Sections of sinus nodes of this group showed splitting of SAN structure and the spacing between the nodal cells appeared similar to that of the NA injected group (Fig. 3a). Occasionally, the cellular injuries tended to recover and the blood vessels lying between the nodal cells were not congested. Areas of mononuclear cellular infiltrations were rarely noted (Fig. 3b). Regular arrangement in most of the nodal cells was observed with rounded shaped central nuclei of P cells together with elongated T cells and parallel sarcoplasm of cardiomyocytes (Fig. 3c). Collagen fibers between the nodal cells appeared especially in areas surrounding central nodal artery (Fig. 3d).

Immunohistochemical findings:

Synaptophysin immunoreactivity (SYN): minimal SYN immunoreactivity was noticed in between nodal cells, as well as around central nodal artery of control group. SYN was detected as tiny spots with immunopositivity reaction (Fig. 4a). Numerous SYN immunopositivity reaction was observed in NA injected group (Fig.4b), whereas the SYN immunoreactivity were reduced significantly in recovery group compared to NA group (Fig.4c).

Induced nitric oxide synthase immunoreactivity (iNOS): weak light brown positive iNOS expression detected in the cytoplasm of some nodal cells of control group (Fig.5a). iNOS expression levels of nodal cells in general were significantly increased in NA injected group compared to the control group (Fig. 5b). Recovery group showed moderate positive iNOS immunoreaction detected among the nodal cells (Fig.5c).

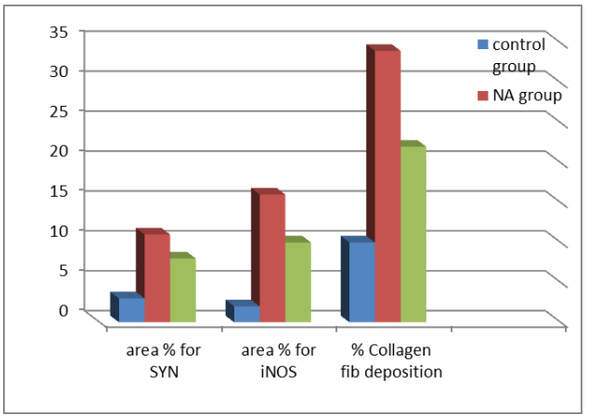
Statistical Results

Upon computer image analysis, the mean area percentage % of collagen fiber deposition (fibrosis), SYN and iNOS immunoexpressing in NA injected group was significantly increased relative to the control group. In contrast, recovery group showed significant decrease if compared to NA group but showed significant increase when compared to control group (Table 1, Histogram 1).

Table. 1: Comparison between the three experimental groups as regards % of collagen fibers deposition, and mean area % of SYN & iNOS immunoreactivity per microscopic field

Groups	area % for SYN	area % for iNOS	% Collagen fib deposition
Control group	3.2±0.4	2.2±0.31	9. 33 ± 1.8
NA injected group	11.5±0.9*	16.7±2.3*	34.43± 9.2*
Recovery group	7.6±1.3*,#	9.3±0.4*,#	22.4± 1.5*,#

Values are mean±SD; One-way ANOVA followed by Tukey's multiple comparison test.
* $P < 0.05$ compared to control group.
# $P < 0.05$ compared to NA group.



Histogram 1: Comparison between the three experimental groups as regards % of collagen fibers deposition, and mean area % of SYN & iNOS immunoreactivity

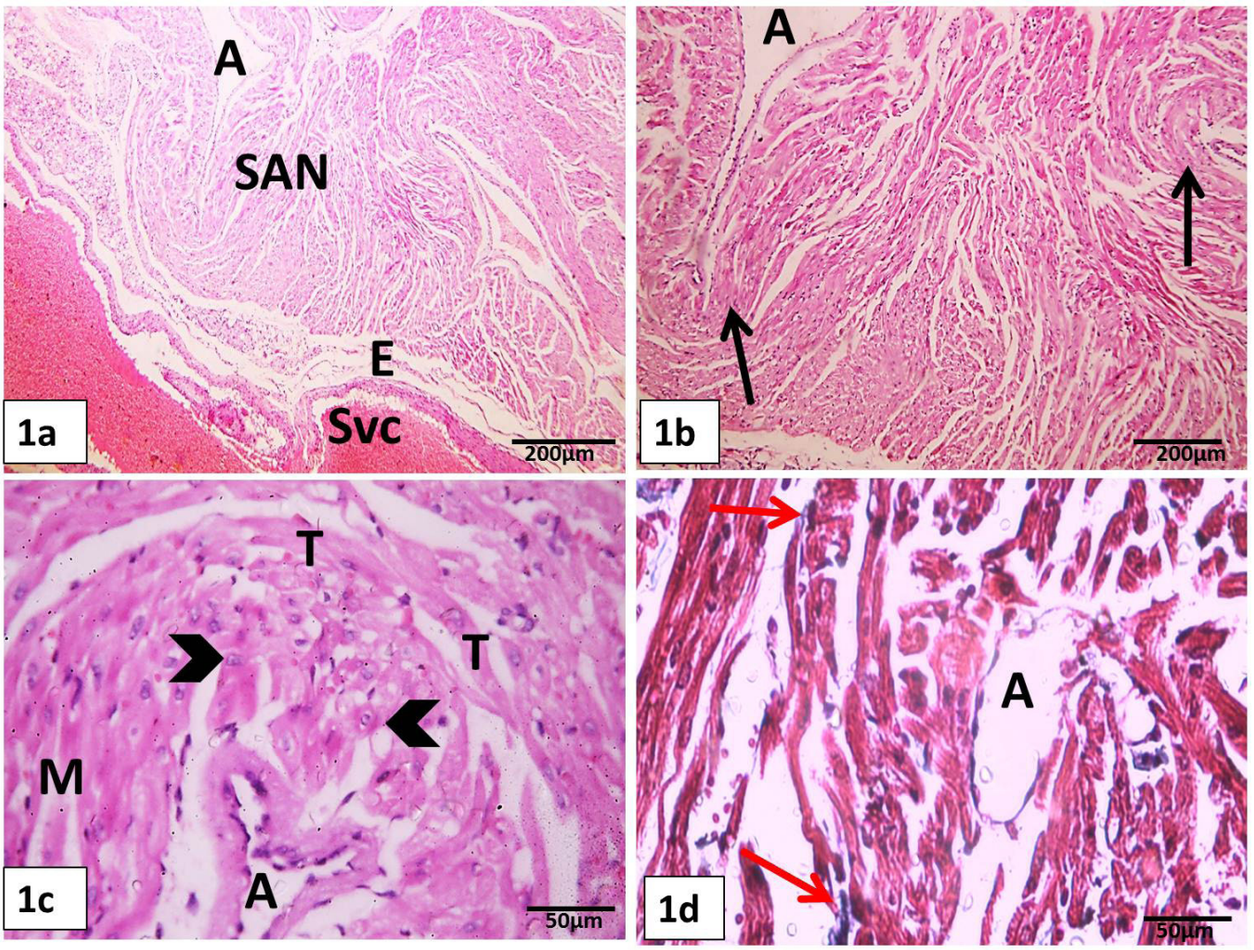


Fig. 1: Photomicrographs of atrial wall sections of control group showing (1a) The sinoatrial node (SAN) is located beneath the epicardium (E) and in close proximity to the superior vena cava (Svc). (1b) The SAN substance (black arrows) is arranged around a central nodal artery (A) (1c) The SAN cells are composed of oval or spherical shaped P cells (arrowhead) and rod-shaped or long cylindrical T cells (T) surrounding a central nodal artery (A). Cardiomyocytes (M) unite together to form fascicles of sarcoplasm parallel to one another. (1d) little amount of fine collagen fibers (red arrows) in between the nodal cells [Scale bar: (1a) x 40 H & E stain, (1b) x 100 H & E stain, (1c) x 400 H & E stain (1d) x400 Masson's trichrome stain]

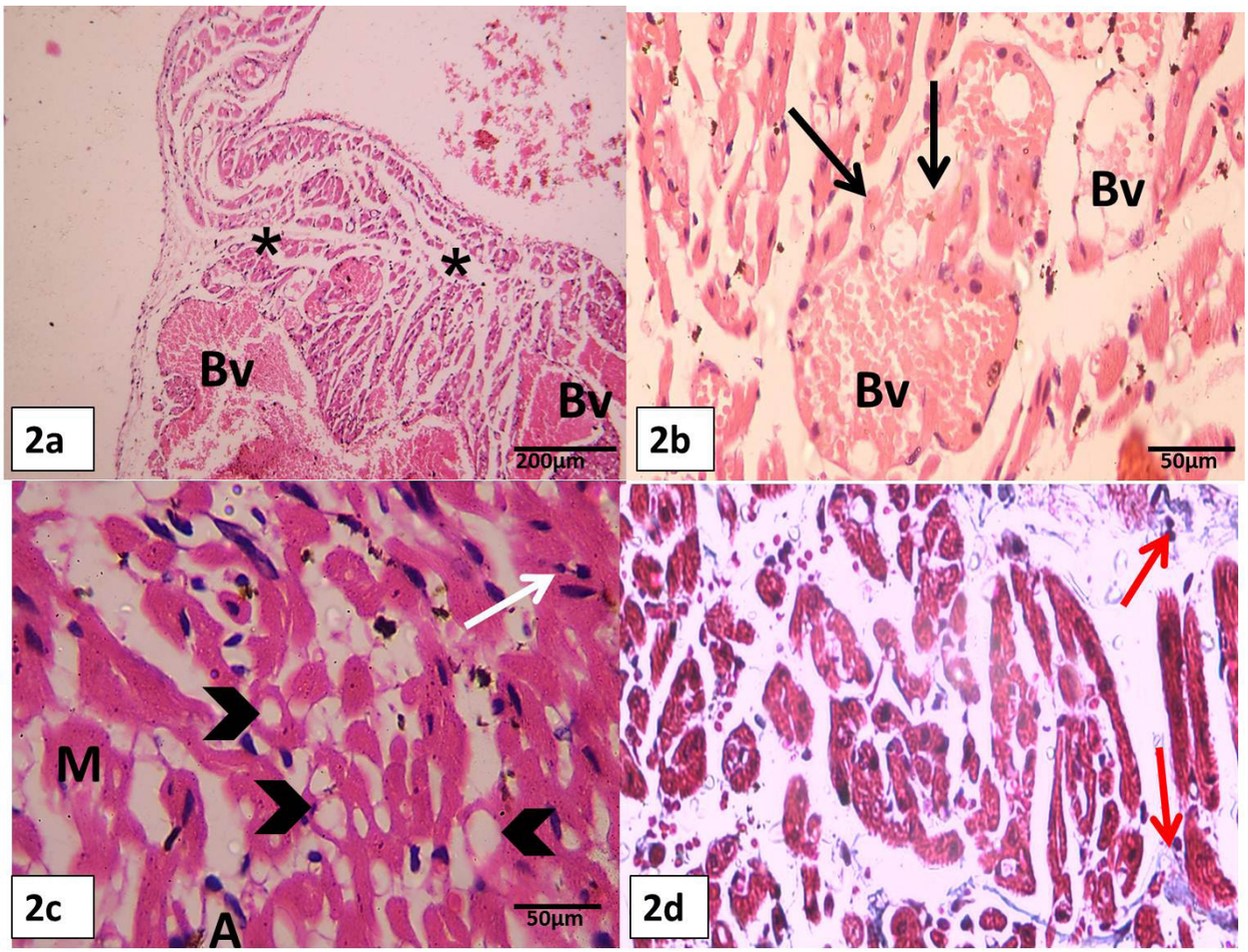


Fig. 2: photomicrographs of atrial wall sections of Nandrolone decanoate injected group showing (2a) splitting of SAN structure (asterisks) and appearance of congested blood vessels (Bv) (2b) P cells with fragmented cytoplasm (black arrows) (2c) P cells with cytoplasmic vacuoles (arrowhead). Notice cardiomyocytes with wavy sacroplasm (M) and deeply stained cytoplasm with fragmented nuclei (white arrow). (2d) increased amount of collagen fibers deposition (red arrow). [Scale bar: (2a) x 40 H & E, (2b& 2c) x 400 H & E, (2d) x400 Masson's trichrome stain]

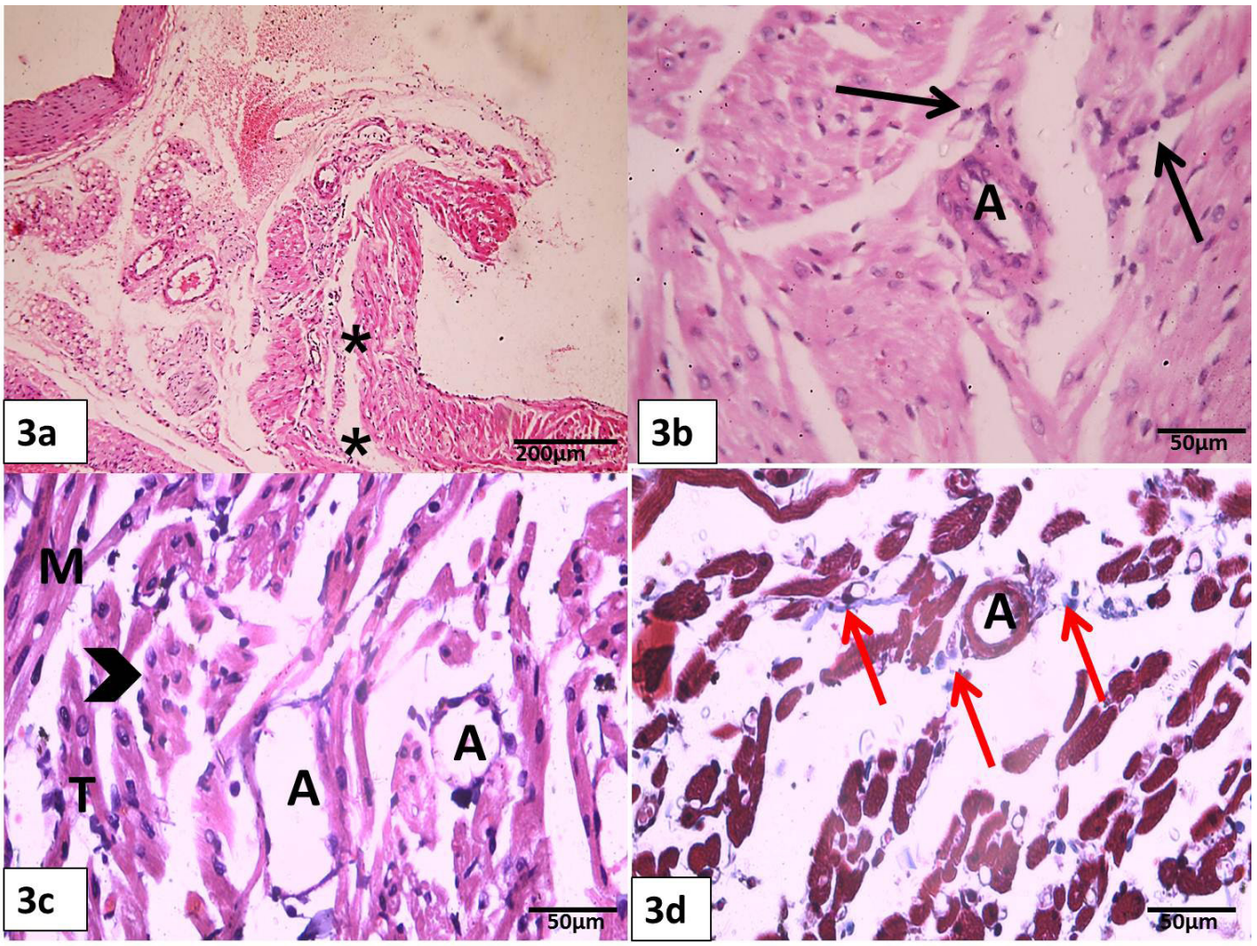


Fig. 3: photomicrographs of atrial wall sections of recovery group showing (3a) spacing between the nodal cells (asterisks) (3b) Areas of mononuclear cellular infiltrations (black arrows) (3c) regular arrangement in most of the nodal cells with rounded shaped central nuclei of P cells (arrowhead) together with elongated T cells (T) and parallel sarcoplasm of cardiomyocytes (M). (3d) Collagen fibers between the nodal cells (red arrow)especially in areas surrounding central nodal artery (A). [Scale bar: (3a) x 40 H & E, (3b& 3c) x 400 H & E, (3d) x400 Masson's trichrome stain].

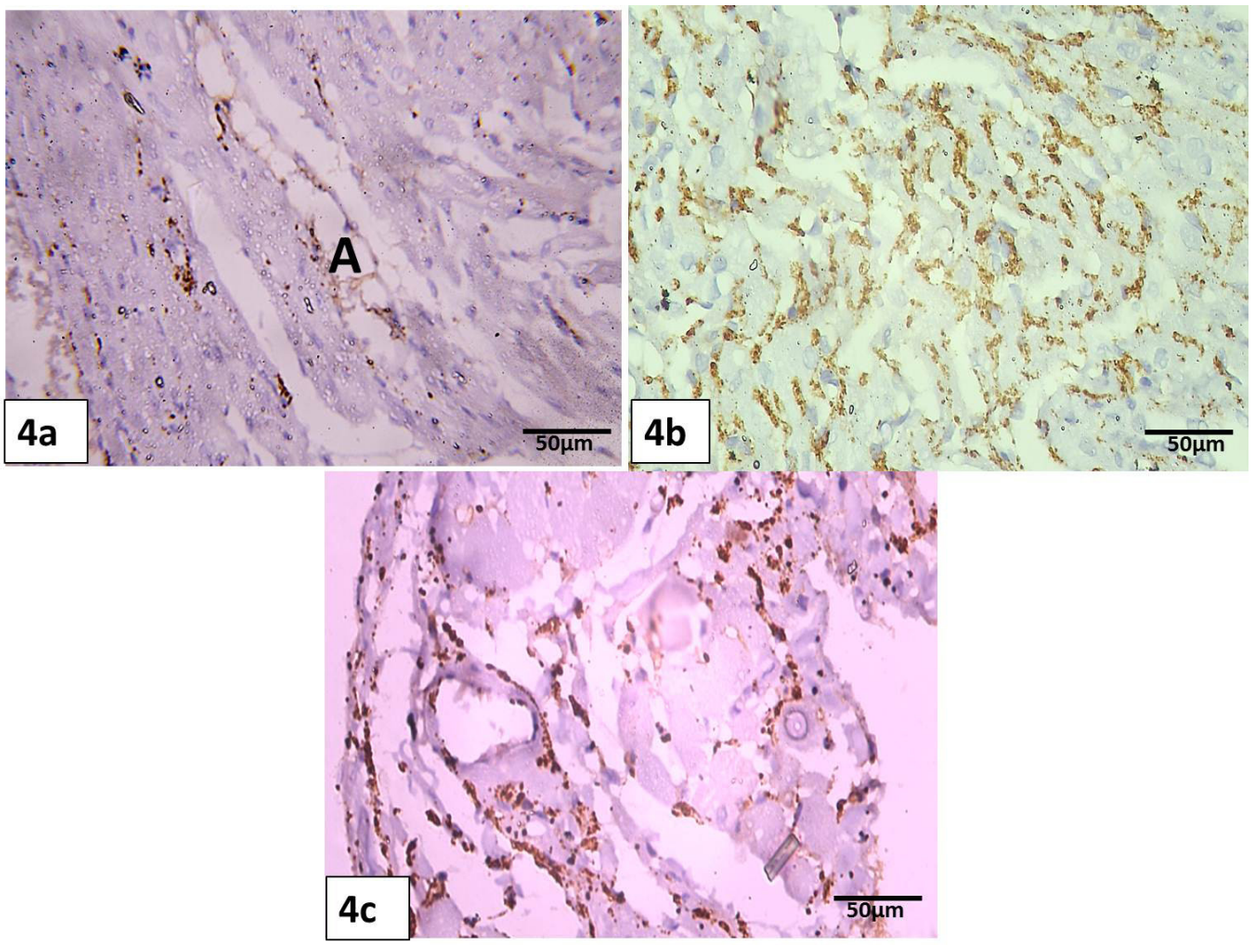


Fig. 4: Photomicrographs of SYN immunostaining atrial wall sections of control and experimental groups showing (4a) control group with minimal tiny spots of SYN immunopositivity reaction. around central nodal artery (A). (4b) NA group with numerous SYN immunopositivity reactions. (4c) recovery group with mild SYN immunoreactivity. (SYN IHC × 400).

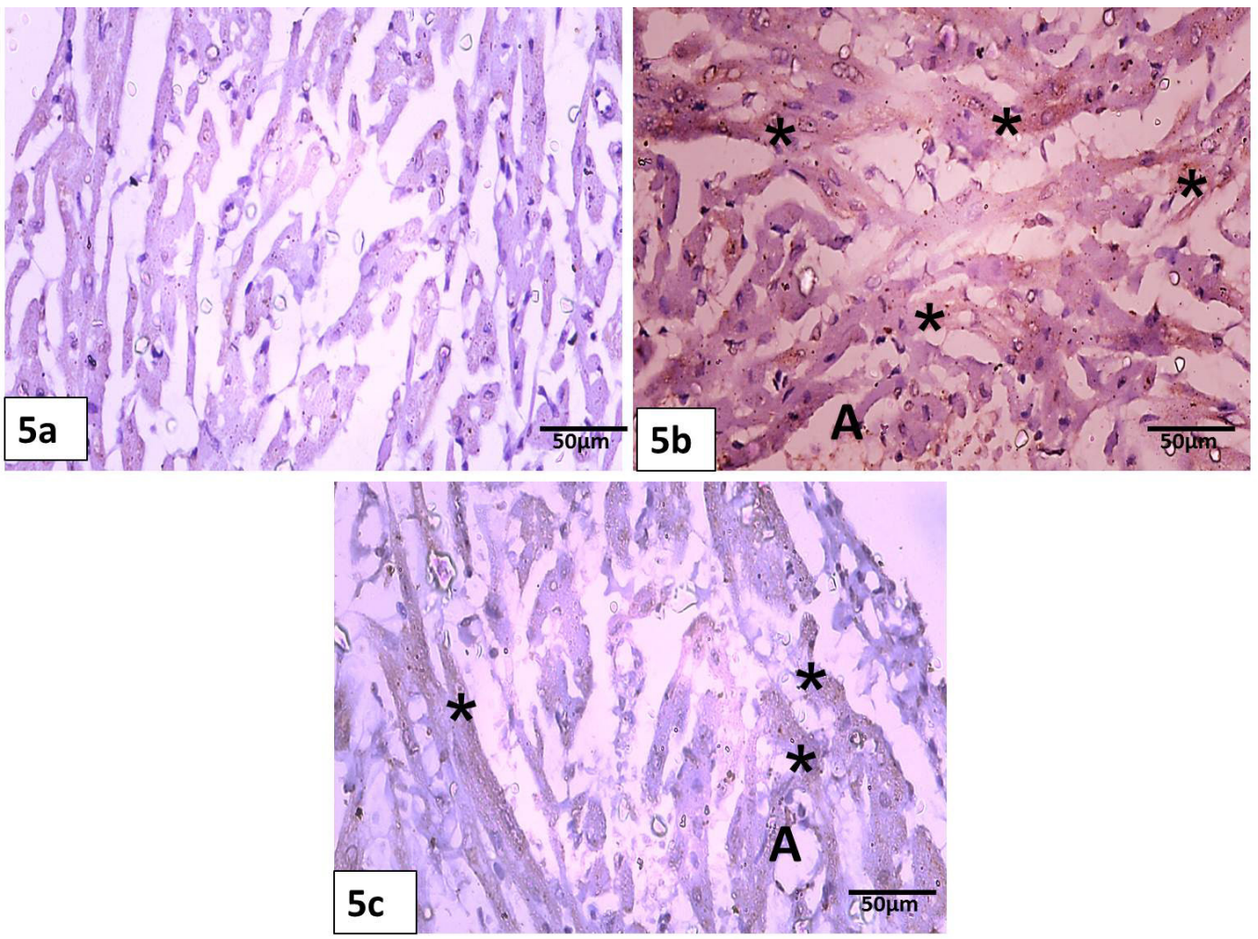


Fig. 5: Photomicrographs of iNOS immunostaining atrial wall sections of control and experimental groups showing (5a) control group with minimal light brown positive iNOS expression in the cytoplasm of some cardiomyocytes. (5b) NA group with strong iNOS expression (asterisks). around central nodal artery (A). (5c) Recovery group with moderate iNOS expression (asterisks). around central nodal artery (A). (iNOS IHC × 400).

DISCUSSION

The present study showed histological and immunohistochemical alterations in the sinoatrial node structure in NA injected group if compared with the control group. Abdollahi *et al*.[23] found electrocardiogram (ECG) abnormalities with Nandrolone plus severe exercise including decrease the heart rate and increase the RR and JT intervals of ECG that increases the risk of ventricular fibrillation (VF). Furthermore, Santos *et al*.[24] reported that Nandrolone had a higher binding affinity to the androgen receptor (AR) and might induce its effects by alternative mechanisms other than the classical AR pathway.

After discontinuation of NA for twelve weeks, the sinoatraial node did not regain its normal architecture. This was in agreement with Kahal *et al*.[25] who reported that androgenic anabolic abuse might lead to irreversibly destroy the heart tissue.

In the current work, NA injection induced wide separation of sinoatrial node structure and disordered cell arrangement. P cell degeneration with increased cytoplasmic vacuoles was noted. Some cardiomyocytes exhibited degenerative changes including wavy sacroplasm and deeply stained cytoplasm with fragmented pyknotic nuclei. Such abnormalities could result from NA induced oxidative stress with reactive oxygen species [ROS] generation. Similarly, Frankenfeld *et al*.[26] stated that androgenic anabolic abuse increased production of reactive oxidative species and such radicals could induce cellular DNA damage with subsequent cell apoptosis or necrosis. Also, Magalhães *et al*.[27] proved that high doses of NA caused oxidative stress and increase in the hydrogen peroxide production, together with lower activities of the antioxidant enzymes.

Marked vascular congestion was noted with extravasated blood in between the nodal cells following NA treatment. Vascular congestion could be due to thrombus

formation and impairment of circulation. Similarly, Omar *et al*.[10] recorded the androgenic causes of increased blood viscosity through either increased production of thromboxane A2 or decreased cyclooxygenase activity and prostacyclin, with polycythemia, and increased platelet count thus increase platelet aggregation. However, these congested vessels returned to their normal morphology after withdrawal of NA. This was similarly noted by Easori *et al*.[28] who reported normality of appearance of muscular blood vessels after discontinuation of NA.

The current study showed areas of inflammatory cell infiltration after withdrawal of NA and this was in agreement with Magalhães *et al*.[27]. Magalhães *et al*.[27] detected higher inflammatory signaling in retroperitoneal fat pad of albino rats and greater levels of IL-6, IL-1β and TNF-α induced by high doses of NA.

In the current investigation, an increase in collagen fibers was observed between nodal cells. Also, excess collagen fiber deposition was obvious in the sinoatrial node as well as perivascular after discontinuation of NA. Moreover, high significant elevation in area percentage of collagen fiber deposition was observed in NA-treated group in contrast to the control group. Similar findings were reported that there was a highly significant increase in heart collagen content in the nandrolone group compared to the control group[29, 30]. It was stated that the increase of intrafasicular connective tissue usually represent a response to a loss of cardiomyocytes where fibroblasts replace the damaged area, with subsequent formation of collagen fibers[31]. Furthermore, Lu *et al*.[32] reported a positive linear correlation between myocardial fibrosis activity and immune cell infiltration.

Pacemaker activity of the SAN is constantly modulated by input from autonomic nervous system[33], so we evaluate SAN sections to quantify expression of synaptophysin. Increased synaptophysin (SYN) immunoreactivity within the SAN in the NA group of the present study were indicative signs of autonomic imbalance[34]. Previous experimental findings indicate that anabolic steroid excess promotes autonomic imbalance characterized by sympathetic hyperactivity. Seara *et al*.[17] observed cardiac rhythmic and mechanical abnormalities associated with autonomic imbalance, increased contractile sensitivity to Calcium (Ca2+) and abnormal Ca2+ mobilization induced by NA overdose.

The current work clarifies significant INOS immmunoreactivity in NA group. This was in accordance with Lucas-Herald *et al*.,[35] who detected androgen-induced cardiac damage, either nuclear or cytoplasmic factors along with increased oxidative stress and pro-apoptotic effects. NA affects ionic balance in several ways, including downregulated K+ channel-interacting proteins[36] and altered Ca2+ mobilization[17] that cause longer QT repolarization time[36].

In the present study, INOS immunohistochemical examination of recovery group showed moderate reaction which was significantly higher than the control group. This result was approved previously by Andrade *et al*.,[37] who stated that NA promoted histological alterations in female genital organs in a dose-independent manner, despite recovery from treatment.

CONCLUSION

It was concluded that the use of nandrolone decanoate, one of the (AAS), caused serious changes in sinoatrial node tissue which will threaten life of their users especially the athletes who use them in very high non-medical doses. Thus, these compounds must be administered only when indicated under medical supervision.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Angell P, Chester N, Green D, Somauroo J, Whyte G, George K. (2012) Anabolic steroids and cardiovascular risk. Review. Sports Med. 42(2):119-34.

2. Monda V., Salerno M., Fiorenzo M., Villano I., Viggiano A., Sessa F., Triggiani A.I., Cibelli G., Valenzano A., Marsala G., *et al*. (2017) Role of sex hormones in the control of vegetative and metabolic functions of middle-aged women. Europe PMC.;8:773. doi: 10.3389/fphys.2017.00773.

3. Anawalt BD (2019) Diagnosis and Management of Anabolic Androgenic Steroid Use. J Clin Endocrinol Metab. Jul 1;104(7):2490-2500. doi: 10.1210/jc.2018-01882

4. Magdalena Celichowska, Miłosz Miedziaszczyk & Katarzyna Lacka (2022) Pharmacotherapy in Cachexia: A Review of Endocrine Abnormalities and Steroid Pharmacotherapy, Journal of Pain & Palliative Care Pharmacotherapy, 36:2, 117-131, DOI: 10.1080/15360288.2022.2063469

5. Johansen KL, Painter PL, Sakkas GK, *et al*. (2006) Effects of resistance exercise training and nandrolone decanoate on body composition and muscle function among patients who receive hemodialysis: a randomized, controlled trial. J Am Soc Nephrol.;17:2307–2314.

6. Kanayama G., Kaufman M.J., Pope H.G. (2018). Public health impact of androgens, Curr. Opin. Endocrinol. Diabetes Obes. 25:218–223.

7. Hearne E., Wazaify M., Van Hout M.C., Atkinson A., McVeigh J. (2021). Anabolic-androgenic steroid use in the Eastern Mediterranean Region: a scoping review of extant empirical literature, Int. J. Ment. Health Addict. 19: 1162–1189.

8. Mullen C., Whalley B.J., Schifano F., Baker J.S. (2020). Anabolic androgenic steroid abuse in the United Kingdom: an update, Br. J. Pharm. 177: 2180–2198.

9. Sagoe D., Pallesen S. (2018). Androgen abuse epidemiology, Curr. Opin. Endocrinol. Diabetes Obes. 25: 185–194.

10. Omar M., Abdul R., Panday A., Teelucksingh S. (2017). Anabolic steroid abuse: What shall it profit a man to gain muscle and suffer the loss of his brain? QJM: Int. J. Med. 110:747–748. doi: 10.1093/qjmed/hcx129.

11. Shirpoor A., Heshmatian B., Tofighi A., Eliasabad S.N., Kheradmand F., Zerehpoosh M. (2019). Nandrolone administration with or without strenuous exercise increases cardiac fatal genes overexpression, calcium/calmodulin-dependent protein kinaseiiδ, and monoamine oxidase activities and enhances blood pressure in adult wistar rats. Gene.; 697:131–137. doi: 10.1016/j.gene.2019.02.053.

12. Liu P.Y., Death A.K., Handelsman D.J. (2003). Androgens and Cardiovascular Disease. Endocr. Rev. 24:313–340. doi: 10.1210/er.2003-0005.

13. Torrisi M, Pennisi G, Russo I, Amico F, Esposito M, *et al*. (2020) Sudden Cardiac Death in Anabolic-Androgenic Steroid Users: A Literature Review. Medicina 56:11, pages 587.

14. Marocolo M., Silva-Neto J.A., Neto O.B. (2018). Acute interruption of treatment with nandrolone decanoate is not sufficient to reverse cardiac autonomic dysfunction and ventricular repolarization disturbances in rats. Steroids. 132:12–17. doi: 10.1016/j.steroids.2018.01.005.

15. Akbari Z., Esmailidehaj M., Avarand E., Shariati M., Pourkhalili K. (2018). Ischemic Preconditioning Efficacy Following Anabolic Steroid Usage: A Clear Difference Between Sedentary and Exercise-Trained Rat Hearts. Cardiovasc. Toxicol. 19:287–296. doi: 10.1007/s12012-018-9497-4.

16. Vasilaki F., Tsitsimpikou C., Tsarouhas K., Germanakis I., Tzardi M., Kavvalakis M., Ozcagli E., Kouretas D., Tsatsakis A.M. (2016). Cardiotoxicity in rabbits after long-term nandrolone decanoate administration. Toxicol. Lett. 241:143–151. doi: 10.1016/j.toxlet.2015.10.026.

17. Seara F.D.A.C., Arantes P.C., Domingos A.E., Barbosa R.A., Olivares E.L., Sudo R.T., De Carvalho A.C.C., Nascimento J.H. (2019). Cardiac electrical and contractile disorders promoted by anabolic steroid overdose are associated with late autonomic imbalance and impaired Ca2+ handling. Steroids. 148:1–10. doi: 10.1016/j.steroids.2019.04.001.

18. Joukar S, Yoosefnia M, Naderi-Boldaji V, Nasri H, Rafie F. (2018). Heart Reaction to Nandrolone Decanoate plus Two Different Intensities of Endurance Exercise: Electrocardiography and Stereological Approach. Addict Health. 10(3):180-189. doi: 10.22122/ahj.v10i3.587. PMID: 31105916; PMCID: PMC6511393.

19. Bancrofit J. D. and Layton C. (2012). The Hematoxylins and Eosin, Ch: 10 and Connective and mesenchymal tissues with their stains, Ch: 11. In: Theory and Practice of histological techniques, 7 th edn, (eds S.K. Suvarna, C. Layton, J.D. Bancrofit), London: Churchill Livingstone. PP: 173- 214.

20. Yao I, Iida J, Nishimura W, Hata Y. (2002). Synaptic and nuclear localization of brain-enriched guanylate kinase-associated protein. J Neurosci. 22(13):5354-64.

21. Ahmed A. M. (2017) Inhibition of inducible nitric oxide synthase (iNOS) by simvastatin attenuates cardiac hypertrophy in rats. Folia Morphol 2017;76(1):15-27. DOI: 10.5603/FM.a2016.0043

22. Sawilowsky, S. (2005) Misconceptions leading to choosing the t test over the Wilcoxon Mann-Whitney U test for shift in location parameter. Journal of Modern Applied Statistical Methods.; 4 (2): 598–600. DOI: 10.22237/jmasm/1130804700

23. Abdollahi F., Joukar S., Najafipour H., Karimi A., Masumi Y., Binayi F. (2016).The risk of life-threatening ventricular arrhythmias in presence of high-intensity endurance exercise along with chronic administration of nandrolone decanoate. Steroids, 105 , pp. 106-112, 10.1016/j.steroids.2015.12.002

24. Santos, H.O.; Haluch, C.E.F. (2022). Downregulation of Androgen Receptors upon Anabolic-Androgenic Steroids: A Cause or a

Flawed Hypothesis of the Muscle-Building Plateau? Muscles, 1, 92–101. https://doi.org/10.3390/ muscles1020010

25. Kahal A., Allem R., Zahzeh T., Koriche S., Kouri A., Douani A., Kassoul H., Ababou A. (2020). Evolutions in cardiac and gonadal ultra-structure during a "cycle" of androgenic anabolic abuse in adult male mice. Steroids. 155:108571. doi: 10.1016/j.steroids.2019.108571.

26. Frankenfeld SP, Oliveira LP, Ortenzi VH, *et al*. (2014). The anabolic androgenic steroid nandrolone decanoate disrupts redox homeostasis in liver, heart and kidney of male Wistar rats. PLoS One. 9:e102699. doi: 10.1371/journal.pone.0102699.

27. Magalhães SC, de Oliveira KA, Freiras PA, Gomes MD, Pereira LM, Boa LF, *et al*. (2020). High-dose Nandrolone Decanoate induces oxidative stress and inflammation in retroperitoneal adipose tissue of male rats. J Steroid Biochem. Mol Biol 203 (2):105728

28. Easori J., Dodd S. and Powcis S. (2003) Use of anabolic steroids to attenuate the effects of glucocorticoids on the rat muscle. Physical Therapy ,83: 29-36.

29. Sretenovic J., Zivkovic, V., Srejovic, I. and Milosavljevic, Z. (2016). "The Effects of High Doses of Nandrolone Decanoate on Cardiac Muscle Tissue" Serbian Journal of Experimental and Clinical Research, vol.17, no.4, 2016, pp.303-308. https://doi.org/10.1515/sjecr-2016-0021

30. Franquni JV, do Nascimento AM, de Lima EM, Brasil GA, Heringer OA, Cassaro KO, da Cunha TV, Musso C, Silva Santos MC, Kalil IC, *et al*. (2013) Nandrolone decanoate determines cardiac remodeling and injury by an imbalance in cardiac inflammatory cytokines and ACE activity, blunting of the Bezold-Jarisch reflex, resulting in the development of hypertension. Steroids.78(3):379-85.

31. Talman V., Ruskoaho H. (2016). Cardiac fibrosis in myocardial infarction-From repair and remodeling to regeneration. Cell Tissue Res. 365:563–581. doi: 10.1007/s00441-016-2431-9.

32. Lu W., Li Y., Dai Y., Chen K. (2022). Dominant Myocardial Fibrosis and Complex Immune Microenvironment Jointly Shape the Pathogenesis of Arrhythmogenic Right Ventricular Cardiomyopathy. Front. Cardiovasc. Med. 9:900810. doi: 10.3389/fcvm.2022.900810.

33. Soltysinska, E., Speerschneider, T., Winther, S.V. *et al*. (2014). Sinoatrial node dysfunction induces cardiac arrhythmias in diabetic mice. Cardiovasc Diabetol 13, 122. https://doi.org/10.1186/s12933-014-0122-y

34. Seara F. A.C. Pereira-Junior., P. P, Silva-Almeida C., Dos-Santos R. C., Souza R. N., Costa C. R.M., Domingos A. E., Barbosa R. A.Q. *et al* (2020). Anabolic steroid excess promotes hydroelectrolytic and autonomic imbalance in adult male rats: Is it enough to alter blood pressure?, Steroids,Volume 163, 2020, 108711, ISSN 0039-128X, https://doi.org/10.1016/j.steroids.2020.108711.

35. Lucas-Herald A.K., Alves-Lopes R., Montezano A.C., Ahmed S.F., Touyz R.M. (2017). Genomic and non-genomic effects of androgens in the cardiovascular system: Clinical implications. Clin. Sci. 131:1405–1418. doi: 10.1042/CS20170090.

36. Medei E., Marocolo M., Rodrigues D.D.C., Arantes P.C., Takiya C.M., Silva J., Rondinelli E., Goldenberg R.C.D.S., De Carvalho A.C.C., Nascimento J.H.M. (2010). Chronic treatment with anabolic steroids induces ventricular repolarization disturbances: Cellular, ionic and molecular mechanism. J. Mol. Cell. Cardiol. 49:165–175. doi: 10.1016/j.yjmcc.2010.04.014.

37. Andrade G., Simão V., Souza B., Chuffa L.G.A., Camargo I.C.C. (2018). Sex steroid receptors profiling is influenced by nandrolone decanoate in the ampulla of the fallopian tube: Post-treatment and post-recovery analyses. Tissue Cell. 50:79–88. doi: 10.1016/j.tice.2018.01.001

الملخص العربى

تقييم تأثير الناندرولون ديكانوات على العقدة الجيبية الأذينية في نموذج الجرذ التجريبي (دراسة هستولوجية وهستوكيميائية مناعية).

مروة محمد الصاوي, ريهام فتحي طاش, احمد محمد دسوقي
قسم التشريح كلية الطب بجامعة عين شمس

خلفية البحث: الناندرولون ديكانوات (NA) هو أحد المنشطات البنائية المستخدمة طبيا والتي يسيء استخدامها الرياضيون ولكن لها آثار ضارة عديدة.

الهدف من البحث: دراسة تأثير ديكانوات الناندرولون على البنية الدقيقة للعقدة الجيبية الأذينية للذكور الجرذان البيضاء وتأثير انسحابها.

المواد والطرق: تم تقسيم ثلاثين من ذكور الجرذان البالغة إلى ثلاث مجموعات تحتوي كل مجموعة على عشرة جرذان. المجموعة الأولى (المجموعة الضابطة): قسمت عشرة جرذان إلى ضابطة سالبة ولم تعالج وحُقنت الضابطة الإيجابية بزيت الخروع مرتين أسبوعياً / ٨ أسابيع. المجموعة الثانية (مجموعة NA): تم حقن عشرة جرذان بـ 5 NA مجم / كجم مرتين / أسبوع / ٨ أسابيع. المجموعة الثالثة (مجموعة الانسحاب): تم حقن عشرة جرذان بـ 5 NA مجم / كجم مرتين / أسبوع / ٨ أسابيع ، ثم تركت ١٢ أسبوعًا لتقييم التعافي. في النهاية ، تم تشريح العقدة الجيبية الأذينية وإخضاعها للفحص الهستولوجي والهستوكيميائي المناعي.

النتائج: أظهر فحص أقسام الأذين الأيمن للجرذان المعالجة بالناندرولون ديكانوات (مجموعة NA) الفصل الواسع في بنية العقدة الجيبية الأذينية وترتيب الخلية المضطرب. لوحظ تنكس الخلايا P مع زيادة فجوات السيتوبلازم. أظهرت بعض خلايا عضلة القلب تغيرات تنكسية بما في ذلك النوى المتحدبة المجزأة المتموجة. بعد التوقف عن NA ، لم تستعد العقدة الجيبية الأذينية بنيتها الطبيعية. تم زيادة متوسط النسبة المئوية للمساحة٪ من ترسب ألياف الكولاجين ، SYN و iNOS المناعي في المجموعة NA بشكل ملحوظ مقارنة بالمجموعة الضابطة التي لم تتعافى بعد سحب الدواء.

الخلاصة: تسبب استخدام الناندرولون ديكانوات في حدوث تغييرات خطيرة في أنسجة العقدة الجيبية الأذينية والتي ستهدد حياة مستخدميها وخاصة الرياضيين وبالتالي ، يجب أن تدار هذه المركبات فقط تحت إشراف طبي.