



Histopathological and Morphometric Study of the Effect of Stanozolol on the Aorta of Male Rats

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Abstract:

The study aimed to find the histopathological and morphological effects of stanozolol, with different concentrations and periods on the aorta in the male rats. The study comprised 80 white males which set into four groups, with 20 rats for each group. First group set as control group and was left only on tap water and fed with pellets. Second group was injected with stanozolol 25 mg / kgbw and the third group was injected with stanozolol 25 mg / kgbw with vit.E 600 mg / kgbw while fourth group was treated with vit.E 600 mg / kgbw. The period of treatment was extend for 8 weeks, animals were euthanized at fourth, eighth and twelfth week after starting treatment. Histological sections of the aorta in the second group showed the presence of histological changes in the aorta represented by the placement of the fat droplets in all layers of the aortic wall accompanied by the enlarged nuclei of the smooth muscle cells. It was observed that the intensity of histopathological changes increased in week 8 and even at 12 weeks despite of the treatment was interrupted. The proliferation of smooth muscle cells toward the endothelium was observed with endothelial cell enlargement. In the third group, after four and eight weeks, thickening of the aorta lining was found with the deposition of the fat droplets as well as elastic fibers degeneration. The fibers replaced by fat droplets in the middle layer. In the fourth group, no histopathological changes were observed for all periods. The micromorphometric measurements of the all groups presented a significant increment in aortic diameter at a level $p \leq 0.05$ when compared with the control group.

Key words: Rat, stanozolol, vitamin E

دراسة مرضية نسجية وشكلية قياسية لتاثير الستانوزولول على الابهر في كور الجرذان

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(قدم للنشر في 2021/4/25 ، قبل للنشر في 2021/7/5)

ملخص البحث:

هدفت الدراسة لمعرفة التأثيرات المرضية النسجية والشكلية القياسية للستانوزولول وبتراكيز واولقات مختلفة لذكور الجرذ الامهق. ضمت الدراسة 80 ذكر من الجرذان المهقاء قسمت بصورة عشوائية إلى اربع مجموعات بواقع 20 جرذاً في كل مجموعة ، وشملت المجموعة الاولى السيطرة التي تركت بدون معاملة على الماء وحبيبات العلف فقط والمجموعة الثانية حقنت بالستانوزولول بتركيز 25ملغم/كغم من وزن الجسم اما المجموعة الثالثة فقد حقنت بالستانوزولول 25ملغم/كغم من وزن الجسم وجرعت بـ Vit. E 600 ملغم/كغم من وزن الجسم والمجموعة الرابعة جرعت بفيتامين E 600 ملغم/كغم من وزن الجسم فقط. حيث عوملت كل المجاميع لمدة ثمانية اسابيع، وتم القتل الرحيم عند الاسبوع الرابع والثامن والثاني عشر من بداية التجربة . شرحت الحيوانات واخذت نماذج من الابهر بواقع ثمانية عينات ولكل المجاميع. أظهرت المقاطع النسجية للأبهر في المجموعة الثانية وجود تغيرات نسجية لأبهر الحيوانات المعاملة بالستانوزولول 25 ملغم / كغم من وزن الجسم بعد اربعة وثمان اسابيع من بدء المعاملة تمثلت بتموضع قطرات الدهن في طبقات جدار الأبهر يصاحبها تضخم انوية الخلايا العضلية الملساء الوعائية ولاحظ زيادة شدة التغيرات النسجية بعد اربع اسابيع من توقف المعاملة (اي عند الاسبوع الثاني عشر) لوحظ تكاثر لخلايا العضلة الملساء الوعائية باتجاه البطانة مع تضخم خلايا البطانة وفي المجموعة الثالثة بعد اربع وثمان اسابيع وجد زيادة في سمك بطانة الابهر مع تموضع قطرات الدهن وتتكس الالياف المرنة . لوحظ ترسب الالياف محل تموضع قطرات الدهن في الطبقة الوسطى. وفي الرابعة لم يلاحظ وجود تغيرات مرضية نسجية عند التراكيزين و لكافة الفترات . وقد اظهرت القياسات الشكلية المجهرية للمجموعة الثانية على قطر الأبهر ارتفاع ملحوظ عند مستوى المعنوية $p \leq 0.05$ ولجميع الفترات عند اجراء مقارنة مع مجموعة السيطرة ، اما في المجموعة الثالثة لوحظ ارتفاع معنوي في كافة الفترات و عند مستوى معنوية $p \leq 0.05$ مقارنة مع مجموعة السيطرة. كما واظهرت المجموعة انخفاض معنوي لاقطار الجيبانيات عند كافة الفترات وبمستوى $p \leq 0.05$ عند مقارنتها مع المجموعة الثانية . اما المجموعة الرابعة اظهرت ارتفاعا معنوياً عند كافة الفترات الفترات.

الكلمات المفتاحية: الجرذ، ستانوزولول، فيتامين هـ



Introduction

Diseases of the cardiovascular system are the main cause of death in developed countries. (1). there are many side effects related to the misuse of stanozoloL, including the cardiovascular system (2). Prolonged use and high doses of these compounds can lead to irreversible damage to the cardiovascular system such as arteriosclerosis (3). The strongest effect of the misuse of anabolic androgenic steroids is cause of atherosclerosis (4). In this study, we have considered the following:

A- Histopathological changes in the aorta (reversible or irreversible)

B: morphological and morphometrical study of aortic layers and structures

Materials and methods

In this study, 80 albino male rats were used, aged 2-3 months, and weighing 125-200gm. They were raised in plastic cages in a room with a temperature of (22-28)C° with a duration of lighting and ventilation of 10 hours(5,6). The experimental animals were divided into four main groups, with 20 rats for each group. The first group set as control group, the second group was given stanozolol 25 mg / kg bw by intramuscular injection. The third group: were treated with stanozolol 25 mg / kg bw with 600 mg / kg of bw Vit E by oral dosing. The fourth group was treated with vitamin E, 600 mg / kg bw only. The second, third and fourth groups were treated for a period of eight weeks, euthanasia was performed after 4 and 8 week of treatment, and the fourth group left four more weeks without treatment and then the animals were euthanized at week 12.

The used vit. E in insurance product by premier health products ltd. UK at a concentration of IU400 / 286 mg, and the dose was determined according to the weight of the animal, where the dose was given at 600 mg per kilogram of body weight (7).

The structural androgenic compound stanozolol, brand name Venaject, was used in solution form manufactured by thaiger pharma / Thailand at a concentration of 50 mg / ml ,where the dose of 10 and 25 mg / kg was chosen depending on (8). The animals were weighed weekly to determine the dose.

To observe the histological lesions of the aorta, samples were taken and immersed in a 10% neutral buffer formalin solution, then processed by routine histological processing method to obtain sections of 5 micrometer thickness cut with a microtome device with a thickness of 5 - 6 micrometers and stained with Harris hematoxylin and eosin stain to clarify the features of the tissues included in the study and to achieve the



micromorphometric measurements which include thickness of aortic wall, luminal diameter, tunica media and tunica adventitia) depending on the method of (9). The tissue sections were photographed and micromorphometric measurement were achieved using a camera. USB 2.0 digital image camera. China equipped with scope image 9.0 image analysis software(10).

Statistical analysis :

Statistical analysis of the data for this study was performed by a two way analysis of variance (ANOVA) test, and the Duncan test was used in the results tables in the form of (rate \pm standard error). The tests are at a lower probability level ($P < 0.05$) (11).

Results

1- histopathological changes

No pathological lesions were observed in the aorta of control group rats, the tissue lesions of the aorta of animals treated with stanozolol 25 mg / kg of body weight after four and eight weeks of treatment represented by deposition of fat vacuoles or droplets in all layers of the aorta wall accompanied by enlarged vascular smooth muscle cell nuclei (Figure - 1) With thickened collagen fibers (Fig. 2), four weeks after the cessation of treatment (at the twelfth week), a proliferation of vascular smooth muscle cells towards the endothelium was observed (Fig. 3) with the enlargement of the endothelial cells (Fig. 4).

The group given vitamin E at a dose of 600 mg / kgbw after four and eight weeks of treatment presented thickens in the lining of the aorta with the deposition of the fat droplets and degeneration of the elastic fibers (Figure -5). Four weeks after the cessation of treatment (at the twelfth week), the fibers replaced the fat droplets in the middle layer (Figure -6).

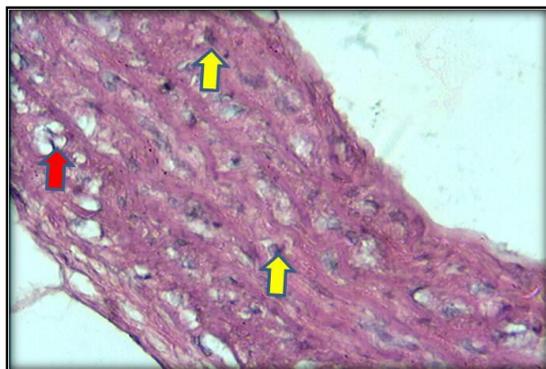


Fig 1: A histological section of a rat's aorta treated with stanozolol 25 mg / kgbw after eight weeks of treatment shows the deposition of fat vacuoles in the muscle fibers (red arrow) accompanied by enlarged vascular smooth muscle cell nuclei (yellow arrow). Hand E stain, 400X.

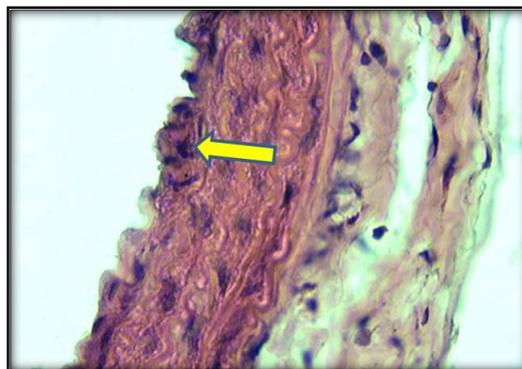


Fig. 2: Histological section of a rat's aorta treated with stanozolol 25 mg / kgbw after 8 weeks after treatment showing the hypertrophy in endothelial cells (yellow arrow) Masson's trichrome stain, 400X

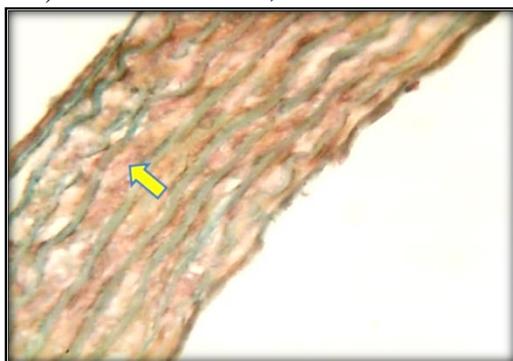


Fig. 3: A histological section of aorta in rats injected with stanozolol 25 mg / kgbw after four weeks of discontinuation (at the twelfth week) of treatment showing hypertrophy of smooth muscle fibers (yellow arrow) and collagen fibers Masson's trichrome stain, 400X

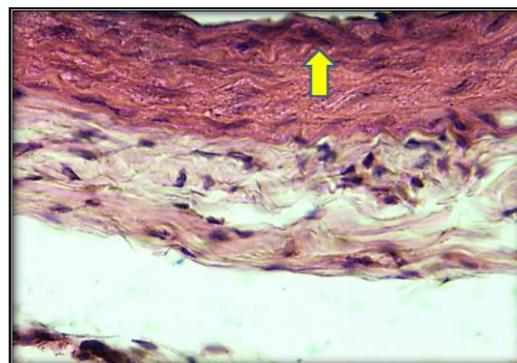


Figure 4: A histological section of the aorta of a rat injected by stanozolol of 25 mg / kgbw at 12th week shows endothelial hyperplasia (yellow arrow). Hand E, 400X.

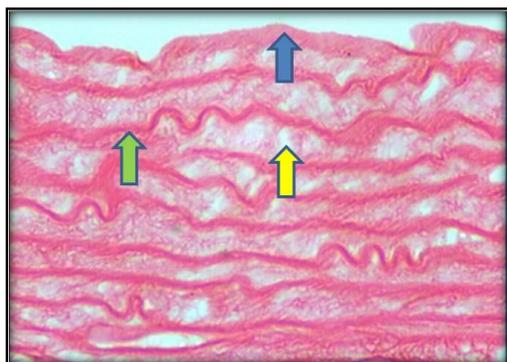


Figure 5: Histological section of a rat's aorta injected by stanozolol 25 mg / kgbw plus vit. E 600 mg / kgbw after four weeks of treatment showing the aortic lining thickening (blue arrow) with the positioning of the fat vacuoles (yellow arrow) and the degeneration of elastic fibers (Green arrow). H& E, 400

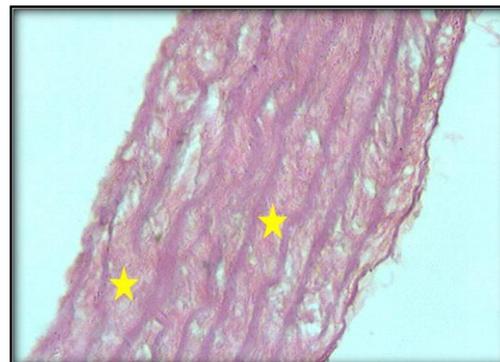


Figure 6: Histological section of a rat's aorta treated with stanozolol 25 mg / kg of body weight with vitamin E 600 mg / kg of body weight after four weeks of interruption (at the twelfth week). The treatment shows the substitution of the fibers for the location of the fat vacuoles in the middle layer (star). H& E, 400X

2- Aortic morphometric measurements

Tunica intima

Table -1 showed that the results of the micro-histological measurements of rats injected by stanozolol at a concentration of 25 mg / kgbw showed a significant increment in thickness of the tunica intima at a significant level of $p \leq 0.05$ for all groups compared with control group.

The group intubated with stanozolol 25mg / kgbw with vit.E 600mg / kgbw showed a significant rise in all periods, with a significance at $p \leq 0.05$ in comparison to rats of the control group, where the presence of the most significant increase was found at the eighth week and it was of 6.8 ± 0.11 . The group injected with stanozolol 25 mg / kgbw with vit.E only showed an insignificant decrease in the thickness of the tunica intima after four weeks of discontinuation of treatment (twelfth week). The group intubated with vit.E only showed a significant increase in the thickness of the inner tunica aorta at $p \leq 0.05$ and at all periods in comparison with the control group.



Table 1: shows the effect of vitamin E at a concentration of 600 mg / kg of body weight in male rats treated with stanozolol at concentrations of 10 and 25 mg / kg of body weight on the thickness of the tunica intima of the aorta, T. Intima / μm . mean \pm standard error

group	Treatment	4w	8w	12w
Group 1	control	3.8 \pm 0.12 a	3.9 \pm 0.11 a	3.8 \pm 0.11 a
Group 2	Stanozolol 25 mg / kg	6.4 \pm 0.18 b	5.1 \pm 0.12 b	6.6 \pm 0.13 b
Group 3	Stanozolol 25 mg / kg with vitamin 600 mg / kg E.	6.8 \pm 0.11 b	6.6 \pm 0.11 c	5.5 \pm 0.11 c
Group 4	Vitamin 600 mg / kg E.	5.1 \pm 0.14 b	5.8 \pm 0.11 b	5.3 \pm 0.29 b

Tunica media

Table -2 illustrated the results of the micro-morphometric measurements of tunica media in male rats injected with stanozolol 25 mg / kgbw, and the presence of a significant increase in the thickness of the middle tunica at the level of $p \leq 0.05$ for all periods when compared with the control group, and the presence of the most significant increase after four weeks of treatment When compared with the rest of the periods, it was of (133.6 \pm 6.1).

The group treated with stanozolol 25 mg / kgbw with vit.E 600 mg / kgbw exhibited a valuable increment in all periods, with a significance at $p \leq 0.05$, in comparison with the control group, where the most significant increase was found after four weeks of treatment, and it was of the value (128.4 \pm 4.5 b).

The rats in group injected with stanozolol 25mg/ kgbw with vit.E revealed an insignificant decrease in the thickness of the midsection after four weeks of treatment, while the increase was not significant at the eighth and twelfth weeks (four weeks after stopping treatment) at a level of $p \leq 0.05$ in comparison with the group injected with stanozolol 25mg / kgbw.



Table 2: shows the effect of vit.E 600 mg/ kgbw in rats injected with stanozolol 25 mg / kgbw on the thickness of the tunica media of the aorta, T. media / μm . mean \pm standard error

group	Treatment	4w	8w	12w
Group 1	control	75.8 \pm 0.7 a	73.3 \pm 0.6 a	78.2 \pm 0.6 a
Group 2	Stanozolol 25 mg / kg	87.4 \pm 1.8 b	109.4 \pm 6.5 b	133.6 \pm 6.1 b
Group 3	Stanozolol 25 mg / kg with vitamin 600 mg / kg E.	93.3 \pm 2.7 b	114.6 \pm 4.4 b	128.4 \pm 4.5 b
Group 4	Vitamin 600 mg / kg E.	97.7 \pm 2.6 b	79.4 \pm 3.4 a	82.9 \pm 2.4 a

Tunica adventitia

Table -3 showed significant increment in the group injected with stanozolol 25 mg / kgbw at a significant level $p \leq 0.05$ for the all periods in comparison with the control group. Most significant rise was found after four weeks of treatment when compared with the rest of the periods when the group treated with stanozolol 25mg /kgbw was recorded 65.8 ± 4.6 .

Rats injected with stanozolol 25 mg/kgbw with vit.E 600 mg/kgbw revealed a valuable increment in all periods at level of $p \leq 0.05$ in comparison with the control group, where the presence of the most significant increase was found after four weeks of treatment and it was a value of 68.8 ± 3.2 .

Whereas the rats injected with stanozolol 25 mg/kgbw with vit.E revealed an insignificant decrease in the eighth and four weeks after the treatment was discontinued in most periods



Table 3: shows the effect of vit.E 600 mg/kgbw in male rats treated with stanozolol 25 mg/kgbw on the thickness of the T. adventitia / μm . mean \pm standard error

group	Treatments	4w	8w	12w
Group 1	control	18.7 \pm 0.3 a	18.4 \pm 0.5 a	17.8 \pm 0.7 a
Group 2	Stanozolol 25 mg / kg	65.8 \pm 4.6 b	38.7 \pm 2.5 b	56.8 \pm 1.4 b
Group 3	Stanozolol 25 mg / kg with vitamin 600 mg / kg E.	68.8 \pm 3.2 b	38.1 \pm 1.1 b	31.8 \pm 0.7 c
Group 4	Vitamin 600 mg / kg E.	24.4 \pm 2.8 b	18.9 \pm 2.1 a	43.8 \pm 1.5 b

Wall thickness

Table -4 Showed the records of micromorphometric parameters for rats injected with stanozolol 25 mg/kgbw concerning thickness of aortic wall. Interval, as it was a value of 204 ± 11.6 .

In the group treated with stanozolol 25mg/kgbw with vit.E 600 mg/kgbw presented a significant increment in all periods at level of $p \leq 0.05$ in comparison with the control group, where the presence of the most significant increase was found after four weeks of treatment and it was a value of 204 ± 11.6 . Also, the group injected by stanozolol 25 mg/kgbw in addition to vit.E 600 mg/kgbw exhibited an insignificant decrease in the fourth week and 4 weeks after the injection was stopped , while the increase was not significant at the eighth week and at the level of Significant $p 0.05$ compared with the group injected with stanozolol 25 mg/kgbw.



Table 4: Shows the effect of vit.E 600 mg/kgbw in rats injected with stanozolol at 25 mg / kg of body weight on the thickness of the wall thickness / μm . mean \pm standard error

group	Treatment	4w	8w	12w
Group 1	control	100.7 \pm 0.8 a	95.6 \pm 0.7 a	97.4 \pm 1 a
Group 2	Stanozolol 25 mg / kg	205.8 \pm 8.3 b	153.2 \pm 3.4 b	134.8 \pm 3.1 b
Group 3	Stanozolol 25 mg / kg with vitamin 600 mg / kg E.	204 \pm 11.6 b	159.3 \pm 5 b	130.6 \pm 1.3 b
Group 4	Vitamin 600 mg / kg E.	112.4 \pm 5.4 a	104.1 \pm 4.2 a	148.8 \pm 2.5 b

The inner diameter of the aorta: the lumen of the aorta

Table -12 shows the results of microscopic measurements of males treated with stanozolol of 25 mg/kgbw for the inner diameter of aorta and the presence of a significant increment at $p \leq 0.05$ for all periods in comparison with the control group, and it was found that there was a high

The significant mean after eight weeks of treatment when compared with the rest of the periods in the group, as it was a value of 1303.6 ± 11.5 .

Also, the rats injected with stanozolol 25 mg/kgbw with vit.E had significant increment in all periods at $p \leq 0.05$ in comparison with the control group, where the presence of the most significant increase was found after eight weeks of treatment. With a value of 1426.8 ± 23.9 .

Also, the group treated with stanozolol 25 mg/kgbw with vit.E with concentration of 600 mg/kgbw indicated significant increment in the fourth and eighth weeks, while the decrease was significant after four weeks of stopping treatment (the twelfth week).

Rats injected with vit.E only revealed a significant increase in the most of the periods and for all combinations at a significant level of $p \leq 0.05$ compared with the control group.



Table 5: Shows the effect of vit.E 600 mg/kgbw in rats injected with stanozolol 25 mg /kgbw on the thickness of the aortic luminal diameter / μm . mean \pm standard error

group	Treatment	4w	8w	12w
Group 1	control	100.7 \pm 0.8 a	95.6 \pm 0.7 a	97.4 \pm 1 a
Group 2	Stanozolol 25 mg / kg	205.8 \pm 8.3 b	153.2 \pm 3.4 b	134.8 \pm 3.1 b
Group 3	Stanozolol 25 mg / kg with vitamin 600 mg / kg E.	204 \pm 11.6 b	159.3 \pm 5 b	130.6 \pm 1.3 b
Group 4	Vitamin 600 mg / kg E.	112.4 \pm 5.4 b	104.1 \pm 4.2 b	148.8 \pm 2.5 b

Discussion

The results showed the presence of histopathological changes of the aorta in rats injected with stanozolol 25 mg/kg bw after the eighth week of treatment. The location of fat vacuoles in all layers of the aorta wall was accompanied by enlarged nuclei of vascular smooth muscle cells.

The researchers (12) and (13). indicated that the use of steroid compounds is one of the causes of atherosclerosis. The appearance of these lesions is due to damage to the aortic lining resulting from oxidative stress by the production of steroids of superoxide, which causes a change in permeability. Endothelium cells and associated with the inflammatory response

There is consensus that atherosclerosis represents a statement increased oxidative stress with increasing dose, which is characterized by the oxidation of the fats and the proteins in wall of blood vessels, and hypothesis of oxidative modification indicates oxidation of low-density lipoprotein early in atherosclerosis. (14,15).

Studies have indicated the occurrence of arteriosclerosis due to the misuse of steroid compounds) due to the occurrence of some changes such as artery spsm, an increase in the generation of agglutination, increase atherogenesis, fibrinolysis, increased stiffness aorta and impaired vascular stimulation (16) and (17).

These lesions were repeated, but less severely, in rats injected with stanozolol 25 mg / kgbw with vitamin E 600 mg/kgbw at the fourth and eighth week and the histopathological changes regressed at the twelfth week of experiment where localization



and adhesion of the thrombus was observed. With the lining wall, fiber has replaced the positioning fat gaps in the middle layer. At the fourth week, while the two groups treated with Vit.E did not show any tissue lesions compared with the control group.

A study indicated that women who consumed vitamin E had a decreased risk of thrombus formation and venous adhesion (18). This is consistent with our current study. Some trials also showed that giving vitamin E gives positive signs regarding diseases of the cardiovascular system.

A diet rich in antioxidants results in an increase in the antioxidant capacity in the blood serum and works to reduce oxidative stress al. (19). This attributes the reason to the decline of lesions in the aortic wall.

The researcher (20). indicated in a study on a group of dogs that were given the compound stanozolol twice a week with the antioxidant Silymarin, a free radical scavenger such as superoxide, as this compound increased Sorbitol Dehydrogenase and reduced fat oxidation. This is consistent with our current study.

Microscopic measurements

Microscopic morphometric measurements are one of the important indicators in determining the changes in the tissues of the organism, as it gives results on the extent of the effect of a particular drug or substance on these tissues through the change in the dimensions of the morphometric measurements in the tissues.

The results of the current study showed that there was significant increment in thickness of the three layers (inner, middle and outer) of the aortic wall in rats injected with stanozolol 25 mg/kgbw with or without insurance E in all experiment groups except for the rats injected with stanozolol 25 mg with vit.E 600 mg/kgbw, where they showed a decrease in the thickness of these strains after four weeks of stopping treatment (the twelfth week) and in all trial groups.

These results were in agreement with the researcher (21) who indicated that the administration of testosterone (which is one of the types of anabolic steroids) led to an increment in the thickness of the tunica intima and media in the treated rats, and the administration of steroid compounds led to an rise in the thickness of the inner and middle tunica. In the thoracic aorta of treated rats, the lining of the aorta was damaged with morphological changes(22). The researcher (23) indicated that the use of steroid



compounds by bodybuilders led to an increase in the thickness of blood vessels, but the increase was not significant.

The results of measurements of the inner diameter of the aorta showed a significant increase in the groups of rats injected with stanozolol 25 mg/kgbw, as these results were in agreement with what was reported by (24) and (18). In mice, while our results were contrary to what was stated by the researcher (8). that administration of androgens did not lead to a change in aortic diameter or the amount of connective tissue in the treated groups.

Whereas the results of the current study showed insignificant increase in the total treatment with stanozolol 25 mg/kgbw with vitamin E 600 mg/Kgbw and the reason for this is that vitamin E led to an improvement in the work of the elastic fibers in the aortic wall, and thus an improvement in the process of contraction of the aortic wall. To improve the structure of the aortic wall.

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