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Histological Studies on the Effect of Anabolic Androgenic Steroids (AAS) on the Liver of Male Albino Rats

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Abstract: Anabolic androgenic steroids (AAS) are the largest category of performance-enhancing drugs (PEDs) and are widely used to improve body image and sports performance in a basic and practical manner. Anabolic steroids are testosterone derivatives synthesized synthetically that increase muscle mass and strength. Sustanon®250 is one of the anabolic androgenic steroids (AAS) that normally contains four distinct testosterone esters that allow sustained release of testosterone into the blood and produce a long-lasting stable level. AAS use is seen as a threat to public health because these substances have adverse effects on all organs, tissues and bodily processes. Aims: This study was conducted to determine the effect of Sustanon® 250 on the histology of the liver. Materials and methods: Forty male albino rats were used in the current study; they were randomly divided into four groups. The control group (n=10) did not receive any treatment. Experimental groups II (n=10), III (n=10) and IV (n=10) are treated with Sustanon®250 in 50, 100 and 150 mg/kg intramuscularly weekly respectively for 8 weeks. Results: The IM injection of Sustanon®250 (50, 100 and 150 mg/kg) for four and eight weeks in male rats resulted in a significant ($p< 0.05$) elevation in the serum TH levels when compared to their respective control subgroups. Histological examination of the Sustanon®250 treated livers across all experimental subgroups showed extensive histopathological effects that were time and dose-dependent including mild deposition of collagen fibers around the central vein, in addition to congestion in the central vein, the sinusoids and the portal area. Hepatic necrosis was only observed at the dose of 50 mg/kg after four weeks. Furthermore, enlarged hepatic cells were observed after the treatment with a dose of 100, 150 mg/kg for eight weeks. Conclusion: This research examined the effects of various dosages of Sustanon®250 (50, 100, or 150 mg/kg/week) on body weight and liver histopathology in male albino rats. Therapeutic levels of TH were consistently elevated in response to both the dosage and duration of the treatment. Pathological lesions were seen in the liver of the male rats, which received treatment, both after four and eight weeks.

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Key words: Anabolic-Androgenic Steroids (AAS) • Sustanon®250 • PED • Liver • Testosterone • Health • Athletics • Abuse

INTRODUCTION

In any competitive setting, especially a sporting one, individuals will strive to achieve supremacy and obtain an advantage over their opponent. During the ancient games in 668 B.C., contestants investigated the effect of specific diets on their performance and this was the earliest method employed by athletes to get an advantage. For instance, ancient Greek Olympians and Roman gladiators both utilized performance-enhancing drugs (PEDs) to enhance their athletic talents [1].

Doping, or the use of (PEDs), has rapidly proliferated in the sporting and medical communities. the term "PED" In the context of performance enhancement in sports, refers to any synthetic or naturally occurring substance that is used or modified to improve athletic performance [2].

The market for PEDs is one of the industries that is expanding at the quickest rate today [3], some examples of common PEDs include growth hormones (GH), erythropoietin and blood doping, creatine, stimulants, diuretics and masking agents, anabolic androgenic steroids (AASs) [4].

AASs belong to the largest class of PEDs and are widely used to improve body image and athletic performance practically and straightforwardly [5].

Anabolic steroids AASs are a class of synthetic testosterone derivatives used to gain muscle mass and strength [6] and it is widely used to achieve body image and sport performance objectives [5].

Low testosterone-related medical disorders are treated using anabolic androgenic steroids (AAS). Clinically, testosterone and AAS are also used to treat renal failure, anemia and hypothyroidism. In children, growth retarded puberty and in the case of some chronic weaknesses such as AIDS and cancer [7]. In addition, it is used professionally to treat several instances of osteoporosis, male hypogonadism, infertility and lack of male libido [8].

Supraphysiologic and long-term use of AASs affects all organs, leading to cardiovascular, neurological, endocrine, gastrointestinal, renal and hematologic disorders [9].

AAS use is seen as a threat to public health since these medicines have adverse effects that affect all organs, tissues and functions of the body, including long-term consequences on the cardiovascular and reproductive systems [10]. AAS usage is also associated with severe liver disease [11], endocrine abnormalities, psychiatric disorders and neurologic consequences [12]. Nonetheless, they are frequently purchased illegally and abused for their anabolic, muscle-building and performance-enhancing properties [9].

Sustanon®250 is one of the androgenic anabolic steroids often misused by athletes, young ladies and young boys to gain muscle mass and enhance athletic performance [13]. It was designed to maximize the synergistic effect of using four blends of testosterone [14]. It is classified as a long-acting anabolic androgenic medicine and it is used therapeutically to treat osteoporosis, male hypogonadism and infertility cases. Sustanon®250 is favored by many bodybuilders for its ability to deliver quick effects [15].

In our previous study using the same doses of and time periods of Sustanon® 250, the histological results of all treated subgroups revealed severe degeneration of the seminiferous tubules, sloughing of spermatogenic cells with necrosis and apoptosis [16]. This study was conducted to determine the effect of Sustanon® 250 on the histology of the liver of male albino rats.

MATERIALS AND METHODS

Materials

The Drug Used: Sustanon®250 ampoules manufactured by N.V. Organon (London) have been obtained from the local pharmacies in Jeddah cities, Saudi Arabia. Each ampoule contains 1mL of an oily solution of Sustanon®250. Eash 1.0 mL of Sustanon ®250 consists of four testosterone ester compounds which include testosterone propionate (30 mg), testosterone phenylpropionate (60 mg), testosterone isocaproate (60 mg) and testosterone decanoate (100 mg). In the current study, three doses of Sustanon®250 have been selected which were 50,100 and 150 mg/Kg of the animal body weight (BW) [17].

Animals Model: Forty adult male albino rats weighing between 200 and 250 grams were acquired from King Abdulaziz University's Animal House of Pharmacy College in Jeddah, Saudi Arabia. In an animal roomtemprature at 23-25°C and 12h dark/ light cycle and free access to water, rats were divided at random into four equal groups each one including ten male rats and were housed Prior to the experiment starts, animals

were left for two weeks to acclimatize (Al-Tayib 2004). After two weeks, animals were divided into four groups, under the umbrella of the ethical approval accomplished by the King Abdulaziz National Committee of ethical approval (HA-02-J-00B).

Methods:

Animals Grouping: Rats were divided into four groups, ten male rats each. For Group I. each rat was injected once a week with arachis (peanut) oil intramuscularly (IM). For groups II Sustanon®250 (50 mg/kg BW), III Sustanon®250 (100 mg/kg BW) and IV Sustanon®250 (150 mg/kg BW) in oil suspension, each rat was injected once a week (IM) for four (subgroup A) and eight weeks (subgroup B) [16, 18].

Histological Preparation: At the time of sacrifice, the rats were killed by cervical dislocation under ether anesthesia and then the livers of all animals were isolated for further experimental evaluation. The livers were extracted, weighed and put in ormalin fixative. The tissue was then prepared through the paraffin technique [19]. For light microscopic examination, liver samples were fixed in 10% buffered formalin, dehydrated, cleared and embedded in paraffin. Serial 5 ìm sections of the liver were stained with Masson trichrome stain for collagen fibers [20].

Statistical Analysis: Statistical analysis was carried out using GraphPad Prism, a software program, version 5.0. (2007). Inc., CA, USA. All values in the results were expressed as means ± SE. The statistical difference among groups was determined using one-way analysis of variance; ANOVA followed by Tukey's multiple comparisons test. P values <0.05 were considered statistically significant.

RESULTS

Body Weight Results: In the current study, the four groups' body weights (BW) were determined. ANOVA one-way test was used to analyze the data.

The BW results revealed a significant reduction (P< 0.05) of male rats due to sustanon®250 injections (50, 100 and 150 mg /kg BW) after four weeks. While non-significant reduction was observed in the BW after eight weeks compared to their control subgroups (Table 1).

Testosterone Hormone (TH) Results: The differences in the mean testosterone hormone blood level among the different groups were analyzed using ANOVA one-way test.

Table 1: The Mean+SEM body weights (g) of the albino male rats after Sustanon®250 injections (50, 100 and 150 mg/kg) for four and eight weeks compared to control, (mean ± SE)

Groups	4 weeks	2nd duration (8 weeks)
Group I	273+9.38	225.4+16.48
Group II	210.6+ 15.67*I	221.2+ 23.51
Group III	200.5+6.37*I	198+19.13
Group IV	218.4+6.88*I	200.8+21.02

* Significant (P<0.05) compared to group I (control group).

Table 2: The mean ± SE testosterone blood levels (ng/ml) of the albino male rats after Sustanon®250 injections (50, 100 and 150 mg/kg) for four and eight weeks compared to control

Groups	4 weeks	8 weeks
Group I	131+10.29	132.2+10.17
Group II	100.2+ 12.7*	134.4+ 5.9*
	IA, IIIA, IIB	IIA, IVB
Group III	155+7.07*	146.4+2.69*
	IIA	IVB
Group IV	173.2+8.61*	180+10.95*
	IA, IIA	IB, IIB, IIIB

* Significant (P<0.05) compared to the mentioned subgroup (I, IIA,...etc.).

The difference in TH levels among groups (I, II, III and IV): The TH level results revealed a significant reduction (P< 0.05) due to 50 mg/kg sustanon®250 injection after four weeks (IIA subgroup) compared to its control (IA subgroup). A non-significant increase (P < 0.05) due to 50 mg/kg sustanon®250 injection was recorded after eight weeks (IIB subgroup) compared to its control (IB subgroup). The other two doses 100 and 150 mg/kg after four weeks (IIIA and IVA subgroups) revealed a significant increase in TH levels compared to their control and compared to each other. In group III, the only significant increase (P<0.05) in TH level was observed between the subgroup IIIA and IIA subgroup. In group IV, a significant increase (P <0.05) in TH level compared to IA and IIA subgroups.

A non-significant increase was reported (P < 0.05) due to 100 mg/kg sustanon®250 injection after eight weeks (IIIB) compared to control, while a significant increase (P < 0.05) was observed compared to IVB. The subgroup IVB revealed a significant increase in TH levels compared to IB, IIB and IIIB (Table 2).

The difference in TH levels within the groups (I, II, III and IV): In group II, a significant increase in TH levels was observed within the same group i.e. between its subgroups (IIA and IIB). No significant difference was observed within it (between IIIA and IIIB). A slight increase in TH level was observed in IVB compared to IVA (within IV group) (Table 2).

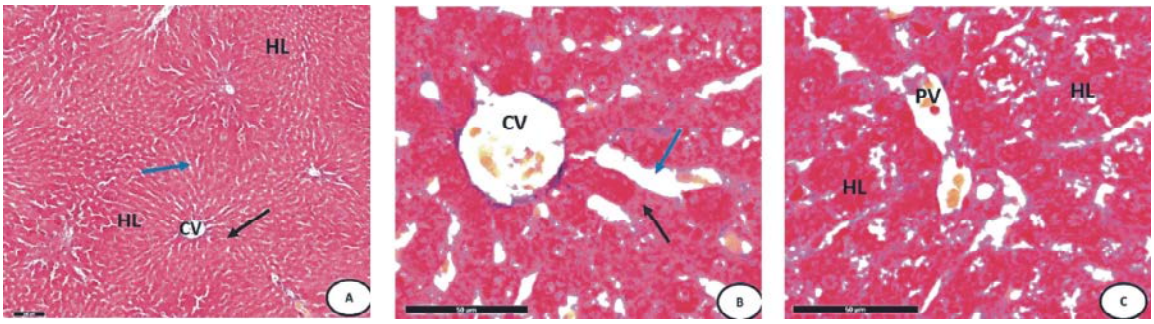

Fig. 1: A photomicrograph of a section of control group I of rat liver showing: (A): the parenchyma that is divided into roughly hepatic lobules (HL) liver cells (hepatocytes) are polyhedral and contain finely granular basophilic cytoplasm(Black arrow). The sinusoids are lined with squamous endothelial cells (Blue arrow) in the central vein (CV)(100x). (B): Shows the normal distribution of collagen fibers, around the central vein. Central vein lining by endothelial cells (CV) (400x) (C): Portal areas(PV) consist of branches of the hepatic artery, branches of the hepatic portal vein and branches of the bile duct. Group I Masson trichrome stain. (x 100, 400, 400 respectively).

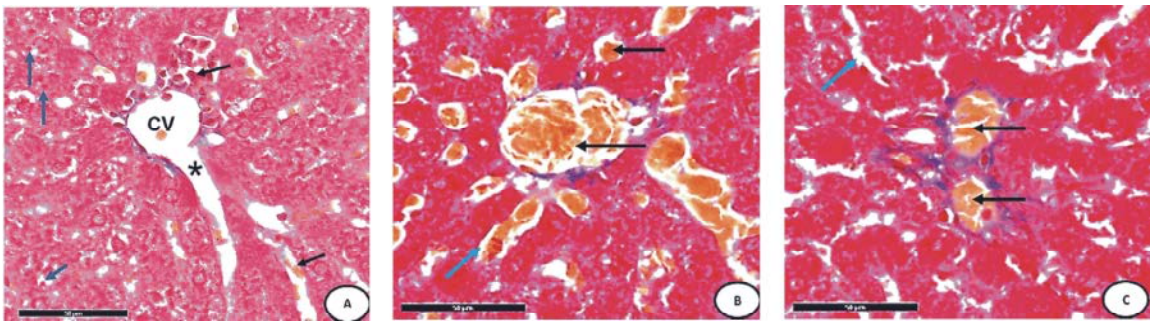

Fig. 2: A photomicrograph of a section of rat liver of group II treated with 50 mg/kg for (4weeks A) (8weeks B and C) showing: (A) not significant increase in collagen fibers around central vein (CV) and a portal tract (PV) Congestion of central vein and sinusoids (Black arrow). Hepatic necrosis (Blue arrow), dilation central vein(*). (B,C) mild deposition of collagen fibers around the central vein and a portal tract and congestion around the portal area was observed (Black arrow). Group II Masson trichrome stain. (x400).

Histological study:

Group I (control): The liver's smallest structural unit, known as the hepatic lobule, is hexagon-shaped and is encircled by sparse connective tissue septa that run parallel to the liver's surface layer.

At the center of each lobule is the central vein into which the sinusoids. From the central vein radiate the plates of hepatic cells toward the lobule periphery. The hepatic cells are arranged in plates two cells thick (Fig. 1A & 1B). Located between the plate of hepatic cells are the blood channels called sinusoids. The septa contain blood vessels (portal vein, hepatic artery) and bile ducts and are especially prominent at certain angles of the lobules where they form portal areas (Fig. 1C). In control group sections (GI) stained by Masson trichrome stain, show a normal distribution of collagen fibers, around the central vein and a portal tract (Fig. 1B).

Group II: Depending on increases in the dosage and length of exposure to sustanon, the histological sections of the livers of male rats displayed subtle to striking alterations. In group II, which was treated with Sustanon@250 in a dose of 50 mg/kg of body weight for 4 weeks, Masson trichrome stain showed slight congestion in the central vein and the sinusoids, no significant increase in collagen fibers around the central vein, cellular swelling and dilation central vein (Fig. 2A). However, after eight weeks, the livers of rat treated with dose 50 were observed, through histological examination and compared to the control group, mild deposition of collagen fibers around the central vein and a portal tract, an increase in the severity of congestion for the central vein, portal area and sinusoids. In addition, greater dilation was observed in the sinusoids (Fig. 2B & 2C).

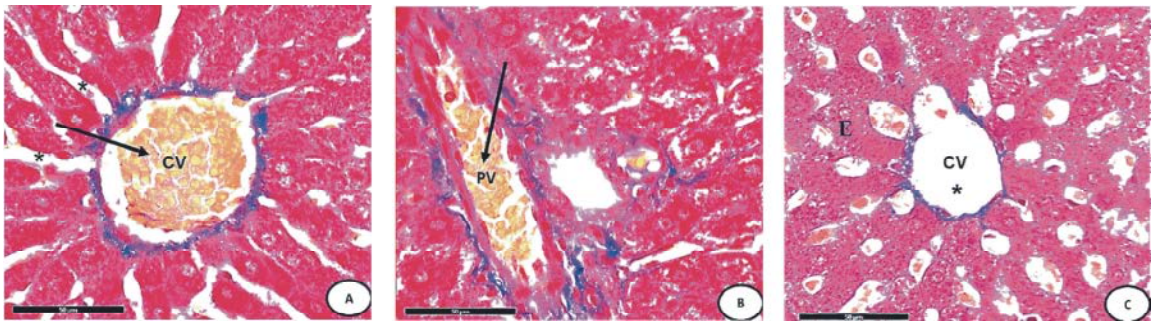

Fig. 3: A photomicrograph of a section of group III of rat liver treated with 100 mg/kg for (4weeks A and B) (8 weeks C) showing: (A): mild distribution of collagen fibers, around central vein and a portal tract, Increase of congestion of central vein (Black arrow) and dilation of sinusoids(*).(B): Increase of congestion in portal area (Black arrow) (C): Shows mild distribution of collagen fibers, around central vein. Dilation of central vein (*) enlarged centrilobular hepatocytes (E) Group III Masson trichrome stain. (x400).

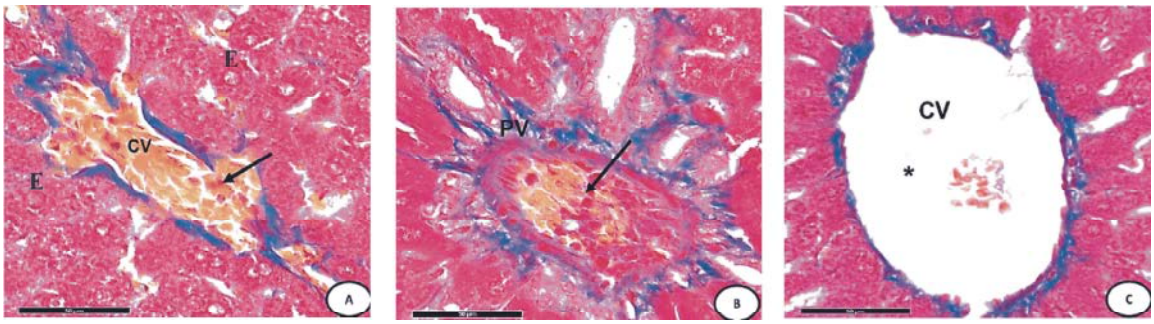

Fig. 4: A photomicrograph of a section of rat liver of group IV treated with 150 mg/kg for (4 weeks A and B) (8 weeks C) showing: (A): high increase in collagen fibers around the central vein. Enlarged centrilobular hepatocytes (E) and a noticeable increase of congestion with dilation of central vein(Black arrow)(B): Increased deposition of collagen fibers in the portal area(PV) with severe congestion (Black arrow) (C): Noticeable increase in central vein dilation (*) Group IV Masson trichrome stain. (x 400).

Group III: In group III which was treated by Sustanon@250 in a dose 100 mg/kg of body weight for 4 weeks, Masson trichrome stain showed marked dilation of sinusoids and diffuse vacuolation of hepatocytes with mild distribution of collagen fibers, around the central vein and a portal area. Additionally, an apparent increase in congestion in the central vein and portal area (Fig. 3 A, B, C). After 8 weeks of treatment with Sustanon@250, histological examination showed mild distribution of collagen fibers around the central vein and portal area. Dilation of the central vein and changes were also observed at the hepatocyte level, the most prominent of which was the enlargement of centrilobular hepatocytes (Fig. 3D).

Group IV: Using Masson trichrome stain in group IV, which was treated with Sustanon@250 in a dose of 150 mg/kg of body weight for 4 weeks, showed severe congestion in the central vein and portal area with a high increase in collagen fibers around the central vein and a portal tract. And some tissue abnormalities, the most prominent of which are Enlarged centrilobular hepatocytes. In addition congestion with dilation of central vein (Fig. 4A & B). After eight weeks of treatment, the expansion of the central vein was noticeable (Fig. 4C).

DISCUSSION

Testosterone is the primary androgen, which is the primary male sex hormone responsible for regulating male secondary sexual characteristics. Testosterone, in conjunction with follicle-stimulating hormone (FSH), promotes the generation of sperm [21]. The prevalence of usage of anabolic-androgenic steroids (AASs), particularly testosterone derivatives, among athletes has seen a significant and concerning escalation in several nations [22]. Sustanon®250 is often misused by athletes and young individuals, both male and female, to enhance

their physical appearance and improve athletic performance [17]. Physicians and professional bodybuilders should be aware of the increased susceptibility to hepatocellular carcinoma when anabolic androgenic drugs are misused at elevated doses [23].

In the present investigation, the administration of sustanon®250 (at doses of 50, 100 and 150 mg per kilogram of body weight) resulted in a substantial and statistically significant reduction in the body weight of male rats, as compared to the control group. Parallel results were provided by Al-Aubody and AL-Diwan [17], the study documented a significant decrease in body weight among both male and female rats after the administration of sustanon®250 injection at doses of 50, 100 and 150 mg/kg, as compared to the control group. Furthermore, their findings demonstrated that administering the maximum dosage of sustanon®250 (150 mg/kg) resulted in a reduction in body weight among the male subjects compared to the other male groups receiving treatment. However, no significant variations in body weight were seen among the female subjects receiving treatment. The decrease in body weight seen in the treated rats may be ascribed to the suppressive impact of elevated testosterone levels on growth and weight increase. The reason for this is a reduction in hunger, alteration of electrolyte levels and an elevation in lipid oxidation, all of which are a result of the heightened functioning of the enzyme carnitine palmitoyl transferase [24]. In a separate investigation carried out by Mutalip *et al*. [25], it was shown that androgens act as inhibitors for some fat cells' capacity to store lipids. This inhibition occurs by disrupting a signal transduction pathway that typically promotes the functioning of adipocytes. This might perhaps mitigate the increase in body weight. Additionally, they stated that AASs have the potential to reduce fat by elevating the basal metabolic rate (BMR).

The current research found a noteworthy decrease ($P<0.05$) in the blood levels of TH caused by the lowest dosage of Sustanon®250 (50 mg/kg) after four weeks (IIA subgroup), as compared to the control group (IA subgroup). However, the administration of the other two dosages, 100 and 150 mg/kg, after four weeks (in the IIIA and IVA subgroups) resulted in a noteworthy elevation in TH levels as compared to their respective control subgroups and to each other. These findings align with the study conducted by Rasul and Aziz [26], which reported that higher doses of Sustanon resulted in a significant rise in serum testosterone levels. Conversely, lower doses of Sustanon led to a non-significant increase in serum testosterone levels compared to the control group. These results align with earlier research [27, 28], that showed a correlation between the treatment of testosterone and its derivatives and changes in blood testosterone levels.

The effects of Sustanon@250 on organ histology are an important topic that should be explored further owing to its relevance to health. The potential for liver damage is a significant worry when it comes to the usage and misuse of anabolic-androgenic steroids (AAS). Physicians and professional bodybuilders should be aware of the increased risk of hepatocellular carcinoma when anabolic androgenic drugs are abused at high dosages [23].

Testosterone and its derivatives have mostly been shown to cause a particular kind of liver condition called cholestasis, as well as peliosis hepatis and both benign and malignant liver tumours. The present understanding is that the development of these illnesses involves the disruption of antioxidative agents, increased production of bile acids and stimulation of hepatocyte hyperplasia [9].

The histological sections of the liver in the present study revealed significant histopathological effects following treatment with Sustanon@250 at doses of 50, 100 and 150 mg/kg/week for four and eight weeks. These effects included severe blood vessel congestion, a substantial increase in collagen fibers surrounding the central vein and dilation in the central vein and sinusoids. These results are consistent with a previous study that observed severe blood vessel congestion, hepatocyte degeneration and an inflammatory response involving macrophages, lymphocytes and neutrophils (mononuclear cells). Additionally, necrosis and fatty liver were observed at a concentration of 150 mg/kg/week for eight weeks, compared to the negative and positive controls, as well as the concentrations of 50 and 100 mg/kg/week [18].

A separate study demonstrated that the liver samples revealed cellular swelling and vacuolar degeneration in the cytoplasm of hepatocytes, along with fatty change and programmed cell death in all groups for 15, 30 and 60 days. These changes persisted even after the treatment was discontinued for 30 days, but portal fibrosis was also observed. The study has determined that Sustanon, at concentrations of 5, 10 and 20 mg/kg of body weight, administered for periods of 15, 30 and 60 days, can cause hepatotoxic effects on the liver of male rats. These effects are both irreversible and progressive, persisting for 30 days after discontinuing the drug administration [29].

The histological changes in another study showed the severity of the high concentrations (150 mg/ kg/week) for four and eight weeks, which means that Sustanon can cause advanced hepatocyte injury. due to the liver being represented as a primary structure answerable for the breakdown of the Sustanon drug [30].

CONCLUSIONS

This study demonstrated the impact of different doses of Sustanon® 250 (50, 100, or 150 mg/kg/week) on the overall reduction of rats' body weight and liver of albino male rats. In almost all doses and periods, the TH levels were increased depending on the dose and the time of the treatments. The liver of the treated male rats revealed several pathological lesions after four and eight weeks.

REFERENCES

1. De Rose, E.H., 2008. Doping in athletes-an update. Clinics in sports Medicine, 27: 107-130.
2. Momaya, A., M. Fawal and R. Estes, 2015. Performance-enhancing substances in sports: a review of the literature. Sports Medicine, 45: 517-531.
3. Badr el Dine, F.M.M. and M.H. Attia, 2022. Assessment of knowledge, perception, attitude and use of performance-enhancing substances among students of Faculty of Medicine, Alexandria University, Egypt: a pilot study. Egyptian Journal of Forensic Sciences, 12: 1-16.
4. Heuberger, J.A. and A.F. Cohen, 2019. Review of WADA prohibited substances: limited evidence for performance-enhancing effects. Sports Medicine, 49: 525-539.
5. Mullen, C., B.J. Whalley, F. Schifano and J.S. Baker, 2020. Anabolic androgenic steroid abuse in the United Kingdom: An update. British Journal of Pharmacology, 177: 2180-2198.
6. Niedfeldt, M.W., 2018. Anabolic steroid effect on the liver. Current Sports Medicine Reports, 17: 97-102.
7. Marshall-Gradisnik, S., R. Green, E. Brenu and R. Weatherby, 2009. Anabolic androgenic steroids effects on the immune system: a review. Open Life Sciences, 4: 19-33.
8. Golds, G., D. Houdek and T. Arnason, 2017. Male hypogonadism and osteoporosis: the effects, clinical consequences and treatment of testosterone deficiency in bone health. International Journal of Endocrinology, 2017.
9. Petrovic, A., S. Vukadin, R. Sikora, K. Bojanic, R. Smolic, D. Plavec, G.Y. Wu and M. Smolic, 2022. Anabolic androgenic steroid-induced liver injury: An update. World Journal of Gastroenterology, 28: 3071.
10. Albano, G.D., F. Amico, G. Cocimano, A. Liberto, F. Maglietta, M. Esposito, G.L. Rosi, N. Di Nunno, M. Salerno and A. Montana, Year. Adverse effects of anabolic-androgenic steroids: A literature review. in *Healthcare*. of Conference.: MDPI.
11. Robles-Diaz, M., A. Gonzalez-Jimenez, I. Medina-Caliz, C. Stephens, M. García-Cortes, B. García-Muñoz, A. Ortega-Alonso, E. Blanco-Reina, R. Gonzalez-Grande and M. Jimenez-Perez, 2015. Distinct phenotype of hepatotoxicity associated with illicit use of anabolic androgenic steroids. Alimentary Pharmacology & Therapeutics, 41: 116-125.
12. Pope Jr, H.G., R.I. Wood, A. Rogol, F. Nyberg, L. Bowers and S. Bhasin, 2014. Adverse health consequences of performance-enhancing drugs: an Endocrine Society scientific statement. Endocrine Reviews, 35: 341-375.
13. Barroso, O., I. Mazzoni and O. Rabin, 2008. Hormone abuse in sports: the antidoping perspective. Asian Journal of Andrology, 10: 391-402.
14. Ejembi, J.P.I., 2016. Effects of Sustanon 250 on the Reproductive Performance of Albino Rats.
15. Al-abdaly, Y., E. Al-Kennany and E. Al-Hamdany, 2018. Concomitant occurrence of oxidative stress with sustanon in male rats. Basrah J. Vet. Res., 17: 137-147.
16. Ahmed, M.H., N.S. Al-Saud, A.M. Shaikh Omar, H. Fikry & S.M. Hassan, 2019. Histological Effects of Different Doses of Anabolic Androgenic Steroids (Sustanon® 250) on Testis of Male albino Rats. Australian Journal of Basic and Applied Sciences, 13: 72-86.
17. Al-Aubody, N.M. and M.A. AL-Diwan, 2014. Androgenic-anabolic steroids abusing effect on liver enzymes and lipid profile in male and female rats. Journal of College of Education for Pure Sciences, 4: 191-204.
18. Mahmood, H.K.J. and A.H. Abood, 2020. Impact of Anabolic Androgenic Steroid (Sustnon 250) on Histology of the Liver and Testes of Male Albion Rat. Biochemical & Cellular Archives, pp: 20.
19. Abd Hamza, E. and K.H. Rashid, 2017. Some hepatic and renal histological and physiological effects of the artificial testosterone (Sustanon) on female rats. Pakistan Journal of Biotechnology, 14: 369-372.
20. Foot, N.C., 1933. The Masson trichrome staining methods in routine laboratory use. Stain Technology, 8: 101-110.

21. Liljas, A., L. Liljas, G. Lindblom, P. Nissen, M. Kjeldgaard and M.R. Ash, 2016. Textbook of structural biology. Vol. 8. World Scientific.
22. Christou, M.A., P.A. Christou, G. Markozannes, A. Tsatsoulis, G. Mastorakos and S. Tigas, 2017. Effects of anabolic androgenic steroids on the reproductive system of athletes and recreational users: a systematic review and meta-analysis. Sports Medicine, 47: 1869-1883.
23. Solbach, P., A. Potthoff, H.J. Raatschen, B. Soudah, U. Lehmann, A. Schneider, M.J. Gebel, M.P. Manns and A. Vogel, 2015. Testosterone-receptor positive hepatocellular carcinoma in a 29-year old bodybuilder with a history of anabolic androgenic steroid abuse: a case report. BMC Gastroenterology, 15: 1-7.
24. Lee, D.M., T. Min, I. Choi, Y.P. Cheon, T. Chun, C.S. Park and K.H. Lee, 2010. Feeding effect of an anabolic steroid, nandrolone, on the male rat testis. Asian-Australasian Journal of Animal Sciences, 23: 1566-1577.
25. Mutalip, S.S.M., G.K.S. Singh, A.M. Shah, M. Mohamad, V. Mani and S.N. Hussin, 2013. Histological changes in testes of rats treated with testosterone, nandrolone and stanozolol. Iranian Journal of Reproductive Medicine, 11: 653.
26. Rasul, K.H. and F.M. Aziz, 2012. The Effect of Sustanon (Testosterone Derivatives) Taken by Athletes on the Testis of Rat. Jordan Journal of Biological Sciences, pp: 5.
27. Muraoka, K., 2001. Effects of testosterone replacement on renal function and apoptosis on mesangial and renal tubule cells in rats. Yonago Acta Medica, 41: 37-44.
28. Shiono, M., 2001. The effect of aging and exogenous testosterone replacement on nitric oxide concentration and activity of nitric oxide synthase in the rat corpus cavernosum. Yonago Acta Medica, 44: 45-53.
29. Al-Kennany, E. and E. Al-Hamdany, 2014. Pathological effects of anabolic steroid (Sustanon®) on liver of male rats. Iraqi Journal of Veterinary Sciences, 28: 31-39.
30. Stancampiano, M.R., A.K. Lucas-Herald, G. Russo, A.D. Rogol and S.F. Ahmed, 2020. Testosterone therapy in adolescent boys: the need for a structured approach. Hormone Research in Paediatrics, 92: 215-228.