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EFFECTS OF NUTRITIONAL SUPPLEMENTS AND TESTOSTERONE HORMONE USED IN SPORT TRAINING CENTERS ON LIVERS IN MALE RATS

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ABSTRACT

The study aimed to evaluate the effects of some nutritional supplements (NSs) and testosterone hormone, commonly used in sports training centers, on liver function and creatine kinase (CK) enzyme activity. The experiment was conducted on 80 male white rats, divided into eight groups with 10 rats in each group. The groups included: a control group, a group of rats exercised using a treadmill, a group treated with NS, a group injected with a testosterone blend, a group dosed with NS and injected with a testosterone blend, a group treated with NS and exercised, a group injected with a testosterone blend and exercised, and a group treated with NS, injected with a testosterone blend and exercised. The results showed a significant increase in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) enzymes in the group treated with NS and testosterone blend compared to the other groups. Histological examinations of the liver revealed severe hepatocyte necrosis, disappearance of some nuclei, apoptosis in certain cells, fatty changes (steatosis) with loss of cellular boundaries, focal infiltration of Kupffer cells, and severe sinusoidal congestion in rats treated with NS and testosterone blend. In contrast, the other groups exhibited normal hepatic cells. In conclusion, treatment with NS and testosterone blend negatively impacts liver enzyme activity and CK levels, in addition to causing pathological changes in liver tissue.

Keywords: Nutritional Supplement, ALT, AST, Certain Kinase, Male Rats.

INTRODUCTION

Nutritional supplements (NSs) are commercially available dietary components, such as amino acids, calcium, caffeine, creatine, folic acid, herbs, iron, minerals, omega-3, and vitamins, consumed in addition to the diet (Alqrache *et al.,* 2021; FDA, 1994; FDA, 2015; Knapik *et al.,* 2016; Thomas *et al.,* 2016). The history of NS use dates back to 776 BC, when natural substances like dried figs, cheese, and wheat were consumed during training for the ancient Greek Olympic Games to enhance energy, although no scientific evidence supported their effectiveness at the time (Alfathi *et al.,* 2023; Al-Taee and Saeed, 2023).

Modern NSs are available in various forms, including powders (e.g., whey and rice protein), capsules, tablets, and drinks, making them accessible for widespread use (Al Nozha and Elshatarat, 2017; Alqrache *et al.,* 2021). While NS consumption is

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widespread and favoured by people, overusing them without reason has been associated with health issues ranging from minor inconveniences to severe life-threatening situations (Al Jammas et al., 2024; Kamangar and Emadi, 2012; Naqui *et al.,* 2018; Suzic *et al.,* 2011).

Athletes of all ages use performance-enhancing substances like NSs, with men and weightlifters being frequent users. However, the widespread use of these substances for body changes lacks backing on their safety and effectiveness even among casual exercisers (El-Khoury and Antoine Jonville, 2012; Heikkinen *et al.,* 2011). Excessive intake of supplements without medical oversight can carry substantial health dangers. This is especially true when incorporating steroids or testosterone into the mix. Such practices can result in disruptions and various health problems affecting the heart and reproductive system. Moreover, liver damage may also be a consequence of these actions (Al Abdaly, 2023; Harrison *et al.,* 2004; Saeed *et al.,* 2023). Misusing testosterone can lead to effects on the hypothalamic-pituitary-gonadal axis. This can result in issues such as infertility and sexual dysfunction. Men who abuse androgens are at a higher risk of infertility compared to those who do not use them (Grant *et al.,* 2023; Henriksen *et al.,* 2023; Horwitz *et al.,* 2019; Rahnema *et al.,* 2014).

This study aimed to investigate the physiological and biochemical changes induced by the combined intake of NSs and testosterone blend in individuals engaged in gym exercise, using White Male New Zealand Rats as an experimental model to explore the potential influencing factors and associated health effects.

MATERIALS AND METHODS

Study Area:

The study was conducted in the animal house of the College of Science, Department of Biology, University of Mosul, Iraq.

Nutrient Supplement

The nutritional supplement, "Mass Attack," was provided by the official agent in Iraq, Mirias Group Company, in a soluble powder form. Each rat received a daily oral dose of 2.4 g using a gavage needle. The NS composition included 70 g protein, 78 g carbohydrate, 646 kcal energy, 12.6 g BCAA, 9.4 g L-glutamine, 10 g creatine, and 3 g of Tribulus terrestris.

Testosterone blend

The testosterone blend, Sustanon, a multi-dose vial containing 250 mg/ml of testosterone blend, was used as the steroid hormone. Male rats were intramuscularly injected into the thigh with a dose of 1 mg/kg every 10 days for three months, following protocols by Frankenfeld *et al.* (2014) and Jwad and Mohammed (2017).

Animals

Eighty adult male albino rats (2–3 months old, 250–400 g) were sourced from the animal house of the College of Veterinary Medicine, Tikrit University. After confirming their health status, the rats were acclimatized for 10 days under controlled conditions (temperature: 25–28°C, 14-hour light cycle, and good ventilation) with a standard pellet diet and free access to water.

Biochemical analyses

Blood samples were collected from the corner of the animal's eye at different intervals during the experiment. For biochemical analyses, blood was divided into two parts: one in tubes containing EDTA for blood tests and the other in gel tubes for serum separation. Serum was obtained by centrifuging the samples at 4500 rpm for six minutes and stored at -20°C. Biochemical parameters, including AST, ALT, and CK enzyme levels, were measured using diagnostic kits.

Ethical Approval:
The study was approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul (Ref: UM.VET.2023.071, Date: 17-8-2023).

Study Design
The doses used in the experiment were based on preliminary experiments and also mimicked the doses used in sports halls (Hartgens and Kuipers, 2004).

The animals were randomly divided into eight groups, each consisting of ten rats, and treated as follows:

1. Control group (G1): given distilled water.
2. Exercise group (G2): trained on a treadmill /3 days per week for 3 months (Hartgens and Kuipers, 2004).
3. Supplement group (G3): given supplements at a daily dose of 2.4 g for 3 months.
4. Testosterone group (G4): given testosterone at a dose of 1 mg/kg every 10 days\3 months\IM.
5. Supplement and testosterone mixture group (G5): given the supplement at a dose of 2.4 g/orally and injected with testosterone 1 mg/kg/10 days for 3 months.
6. Supplement and exercise group (G6): rats received the supplement 2.4 g/orally/with treadmill training 3 times/week for 3 months.
7. Testosterone and exercise group (G7): Testosterone was injected (1 mg/kg/10 days) and treadmill trained 3 days/week for 3 months.
8. Supplement, testosterone and exercise group (G8): rats received the supplement orally, injected with testosterone (1 mg/kg/10 days) and treadmill training 3 times per week for 3 months.

Statistical Analysis
Data were analyzed using the SAS statistical program (SAS, 2001) in a completely randomized design. Group differences were determined using Duncan's Multiple Range Test at a significance level of P≤0.01 (Hinton, 2014).

RESULTS

Alanine aminotransferase (ALT):
Figure (1) illustrates the significant increase in ALT enzyme activity among the experimental groups. The highest mean value was observed in the group treated with both the nutritional supplement (NS) and testosterone blend (110.20±0.02 IU/L). The group treated with NS alone showed a mean of 98.13±0.03 IU/L, with no significant difference compared to the testosterone-treated group (98.15±0.03 IU/L).

The group treated with NS, testosterone blend, and exercise had a mean of 97.72± 0.04 IU/L, while the NS and exercise group showed a mean of 95.31± 0.02 IU/L, which was comparable to the testosterone blend and exercise group (95.32 ± 0.03 IU/L). The control group exhibited a lower mean ALT activity of 93.09±0.09 IU/L, whereas the lowest value was recorded in the exercise-only group (80.20±0.03 IU/L).

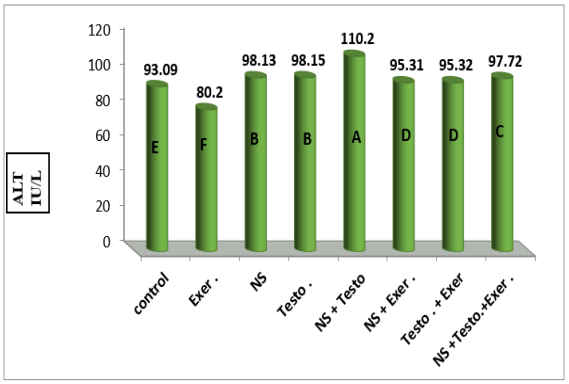

Figure 1: Effect of the NS and testosterone blend on the effectiveness of the ALT in the blood serum of male rats. Values are represented by mean ± SD. Rats/group = 10.

Aspartate transaminase (AST):
Figure (2) illustrates a significant increase in AST enzyme activity in the group of rats treated with the nutritional supplement (NS) and testosterone blend compared to the other groups (P≤0.01), with a mean value of (140.17±0.03) IU/L. Elevated AST levels were also observed in the groups treated with NS alone (133.08±1.05 IU/L) and

testosterone blend alone (133.06±1.37 IU/L). This suggests that both substances independently contribute to hepatic stress or damage, with their combination exacerbating this effect.

The group treated with NS and testosterone blend combined with exercise exhibited slightly lower AST activity (124.83 ± 0.36 IU/L) than the NS or testosterone-only groups but remained elevated compared to the control group. Notably, AST levels were reduced in the groups treated with NS and exercise (123.75±0.46 IU/L) and testosterone blend and exercise (124.08± 0.35 IU/L), though they did not reach the baseline level of the control group (123.19 ±0.05 IU/L). The lowest AST activity was observed in the exercise-only group, with a mean value of (110.19±0.06 IU/L), indicating the protective effect of physical activity on liver function.

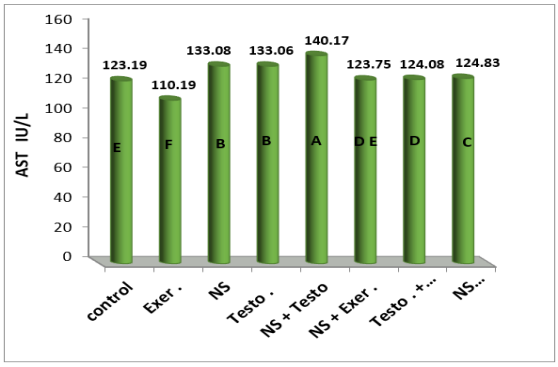

Figure 2: The effect of the NS and Testosterone Blend on the effectiveness of the AST in the blood serum of male rats. Values are represented by mean ± SD. Rats/group = 10.

Creatine kinase enzyme:

Figure (3) demonstrates a significant elevation in (CK) activity in the group treated with the (NS) and testosterone blend, with an arithmetic mean of (430.30 ± 0.05 IU/L) compared to the other groups at the probability level (P≤0.01). This was followed by the group receiving the NS alone, with a mean of (402.30 ± 0.10 IU/L), and the testosterone blend group, which exhibited a mean of (343.32 ± 0.10 IU/L). Interestingly, CK activity was decreased in the groups combining NS and testosterone blend with exercise (200.29 ± 0.03 IU/L), testosterone blend with exercise (190.95 ± 0.02 IU/L), and NS with exercise (190.34 ± 0.03 IU/L). The lowest CK activity was observed in the exercise-only group, which exhibited a mean of (186.59 ± 0.61 IU/L), approaching the control group's mean (186.18 ± 0.09 IU/L).

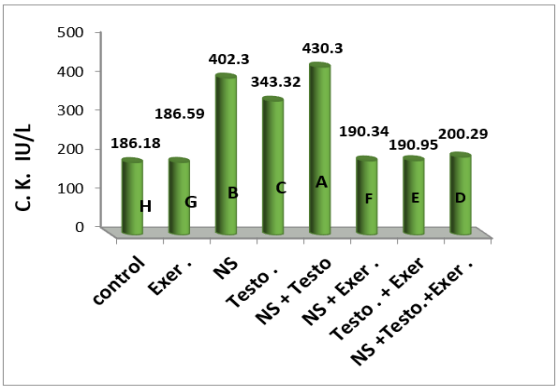

Figure 3: The effect of the NS and testosterone blend on the effectiveness of the enzyme creatine kinase in the blood serum of male rats. Values are represented as mean ± SD. Rats/group = 10.

Histological results

Histological examination of the liver in the control group revealed normal liver architecture, with intact central veins, hepatocytes, Kupffer cells, and sinusoids (Figure 4). Similarly, the liver of rats subjected to exercise training displayed normal hepatocytes, without signs of apoptosis or necrosis, and the number of Kupffer cells remained comparable to the control group, with healthy sinusoids (Figure 5). In the NS-treated group, histological sections revealed hepatocyte necrosis, loss of cellular boundaries (indicated by red arrows), focal infiltration of Kupffer cells (blue arrows), and sinusoidal dilation (Figure 6). In the testosterone blend-treated group, hepatocytes exhibited apoptosis, characterized by cytoplasmic shrinkage, chromatin condensation, and focal Kupffer cell infiltration, alongside sinusoidal congestion with red blood cells (Figure 7). The combination of NS and testosterone blend resulted in severe hepatocyte necrosis, disappearance of some nuclei, apoptosis in certain cells, fatty changes (steatosis), and pronounced sinusoidal congestion (Figure 8).

In the NS-treated rats with exercise, liver histology showed normal hepatic architecture, though some sinusoidal congestion was noted (Figure 9). Liver sections appeared largely normal with a few apoptotic cells in the testosterone blend with the exercise group (Figure 10). In the NS and testosterone blend-treated rats with exercise, the liver showed fewer normal hepatocytes, a few necrotic cells with hyperchromatic nuclei, and a reduction in the extent of necrosis compared to the NS and testosterone-only group (Figure 11). These findings suggest that exercise may partially mitigate liver damage caused by NS and testosterone, although some residual damage remains.

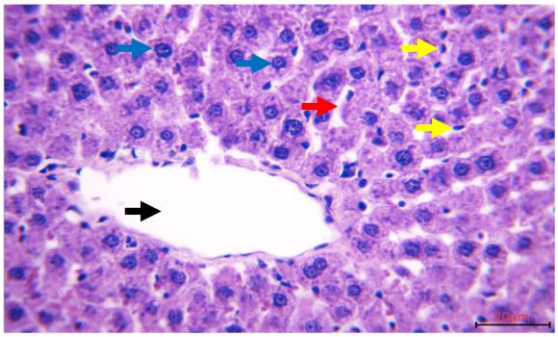

Figure 4: Histological section of the liver of the normal rats (control), showing normal appearance of central vein (black arrow), hepatocytes (blue arrows), Kupffer cells (yellow arrows) and sinusoids (red arrow). (20 µm H&E).

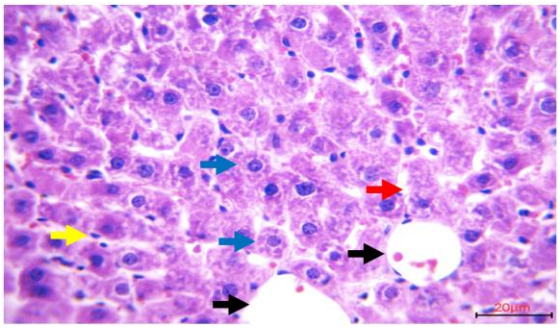

Figure 5: Histological section of the liver of the exercise-training rats, showing normal appearance of central vein (black arrows), normal hepatocytes without apoptosis/necrosis (blue arrows), the number of Kupffer cells remained the same as those of the control group (yellow arrows) and normal sinusoids (red arrow). (20 µm H&E stain).

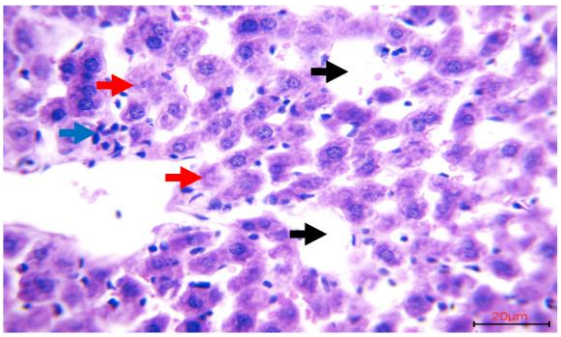

Figure 6: Histological section of the liver in the NS-treated rats, showing hepatocyte necrosis and loss of cellular boundaries (red arrows) with focal infiltration of Kupffer cells (blue arrows), and sinusoid dilation (black arrows). (20 µm H&E stain).

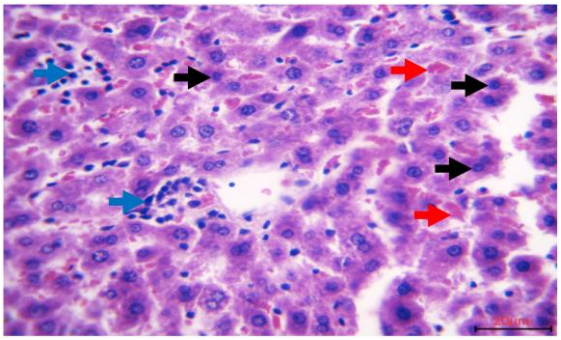

Figure 7: Histological section of liver in the testosterone blends treated rats, showing apoptosis of hepatocyte with shrunken cytoplasm with chromatin condensation (black arrows), and focal infiltration of Kupffer cells (blue arrows), and sinusoidal congestion with red blood cells (red arrows). (20 µm H&E stain).

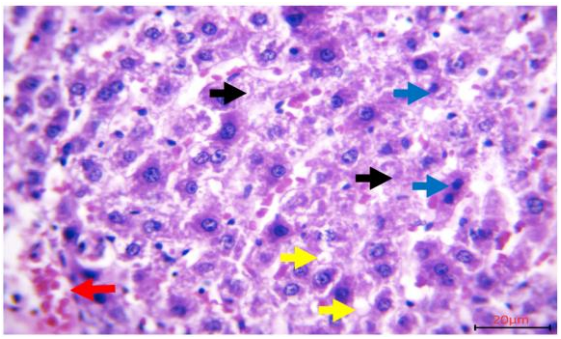

Figure 8: Histological section of the liver in the NS and testosterone blend-treated rats, showing severe necrosis of hepatocytes with the disappearance of some nuclei (black arrows), and apoptosis in some cells (blue arrows), and fatty changes (steatosis) (yellow arrows), and sever sinusoidal congestion (red arrow). (20 µm H&E stain).

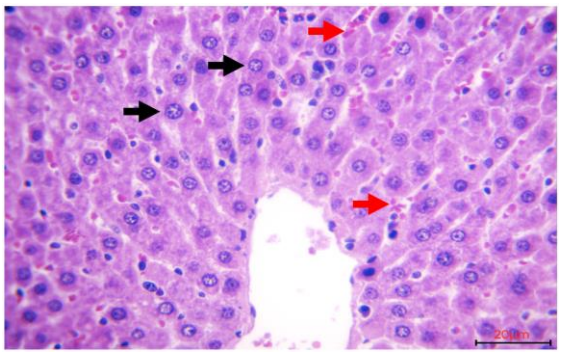

Figure 9: The Histological section of the liver in the NS-treated rats with exercise training, showing normal hepatic architecture (black arrows) with sinusoidal congestion (red arrows). (20µm H&E stain).

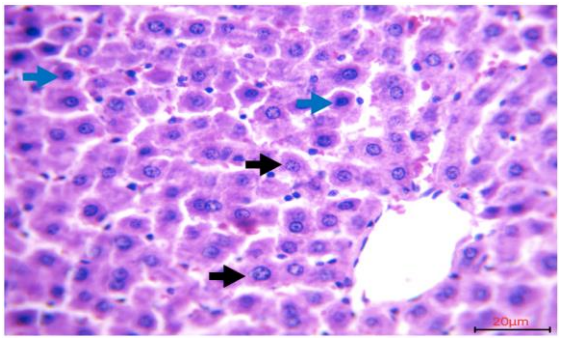

Figure 10: Histological section of the liver in the testosterone blends treated rats with exercise training, showing normal hepatic cells (black arrows) and some apoptotic cells (blue arrows). (20 µm H&E stain).

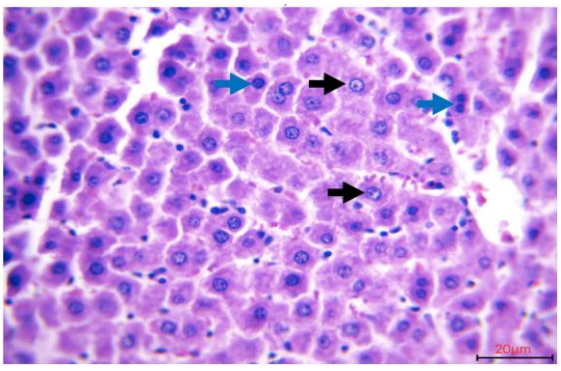

Figure 11: Histological section of the liver in the NS and testosterone blend-treated rats with exercise training, showing few normal hepatic cells (black arrows), and few necrotic cells with hyperchromatic nuclei (blue arrows), the level of necrosis decreased in these groups compared with the NS and testosterone-treated group. (20 µm H&E stain).

DISCUSSION

The results of this study reveal significant liver damage associated with the combined use of nutritional supplements (NS) and testosterone, as indicated by elevated ALT, AST, and CK activities, alongside histological alterations in the liver. The ALT enzyme exhibited its peak activity, in the group that received both NS and testosterone treatment which indicates an impairment of liver function caused by the combined effects of these substances. Elevated ALT levels were also noted in the groups that only received NS or testosterone supplements. This suggests that the liver may be under strain due to supplementation of steroids and other substances that could interfere with liver function and lead to oxidative harm (Karacor *et al*., 2014).

The rise in AST levels indicates that there may be liver damage because AST release from liver cells is often linked to stress (Almaiman, 2018). Research has demonstrated that testosterone can lead to Free Radicals production that harms liver cells and worsens liver damage (Wu *et al*., 2023). Additionally, the strain from increased protein metabolism caused by NS supplementation is likely linked to reduced detoxification and mitochondrial issues resulting in increasing AST levels (Alnuimi and Alabdaly, 2022; Guyton and Hall, 2023). CK levels, as a measure of muscle damage, showed an increase in the group that received both NS and testosterone. This indicates that muscle injury may be attributed to the impact of testosterone (Tasgin *et al.,* 2011). Consequently, this could result in cell damage and leakage of enzymes (Lok *et al*., 2010; Altememy and Saeed, 2023). Physical activity has been shown to lower CK levels in the groups receiving exercise treatments. This suggests that exercise may aid in muscle recovery and decrease stress (Tasgin *et al*., 2010).

The liver tissue examination revealed cell death and damage in hepatocytes and blood vessels in the groups that received both substances together. These effects suggest harmful synergy between NS and testosterone that interferes with the liver's functioning. Testosterone-triggered stress and mitochondrial issues play significant roles in these liver changes resulting in fat buildup and cell demise (Jwad and Mohammed, 2017).

Enough physical activity helped to reduce some of the liver injury in both the groups that were not treated with testosterone and those that were treated with it. Physical activity boosts the body's ability to fight against substances and supports the liver in healing itself by improving blood flow to remove toxins (Michalopoulos, 2007; Abdulrazaq & Alabdaly, 2023) Nevertheless, the benefits of activity weren't enough to offset the negative impact of NS and testosterone on the liver.

CONCLUSION

The research findings indicate that overconsumption of (NS) as well as testosterone use, in excess and their combination can have harmful impacts on the liver and muscles. High levels of liver enzymes (ALT and AST) along with elevated muscle damage markers (CK) were detected in the study participants. Furthermore, histological analysis revealed liver damage characterized by necrosis and apoptosis. Engaging in exercise offered some effects by promoting recovery in the liver and muscles. However, it was not able to completely offset the adverse consequences of nutritional supplements and testosterone consumption. The findings highlight the dangers of using these substances without caution and stress the importance of monitoring the health of the liver and muscles.

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تأثيرات بعض المكملات الغذائية المستخدمة في مراكز التدريب الرياضي على فعالية وظائف الكبد في ذكور الجرذان

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هدفت الدراسة إلى تقييم تأثير استخدام بعض المكملات الغذائية (NSs) وهرمون التستوستيرون المستخدمين في مراكز التدريب الرياضي على وظائف الكبد ونشاط إنزيم الكرياتين كيناز (CK) .تم إجراء التجربة على ٨٠ جرذ أبيض من الذكور، قُسّمت إلى ثماني مجاميع، تضم كل مجموعة ١٠ جرذان. شملت المجاميع: المجموعة الضابطة، مجموعة الجرذان التي مارست التمارين باستخدام جهاز المشي، مجموعة عُولجت بالمكملات الغذائية، مجموعة حُقنت بمزيج التستوستيرون، مجموعة عُولجت بالمكملات الغذائية وحُقنت بمزيج التستوستيرون، مجموعة عُولجت بالمكملات الغذائية مع التمارين، مجموعة حُقنت بالتستوستيرون مع التمارين، وأخيراً مجموعة عُولجت بالمكملات الغذائية وحُقنت بالتستوستيرون مع التمارين. أظهرت النتائج ارتفاعاً معنوياً ($P\leq0.01$) في نشاط إنزيمات الألنين أمينوترانسفيراز (ALT) والأسبارتات أمينوترانسفيراز (AST) وإنزيم الكرياتين كيناز (CK) في المجموعة التي عُولجت بالمكملات الغذائية ومزيج التستوستيرون مقارنةً بالمجاميع الأخرى. كما كشفت الفحوصات النسيجية للكبد عن حدوث نخر شديد في خلايا الكبد، واختفاء لبعض الأنوية، وظهور علامات موت الخلايا المبرمج (Apoptosis)، وتغيرات دهنية(Steatosis) مع فقدان الحدود الخلوية، وتجمع بؤري للخلايا الكبدية كوبفر (Kupffer cells)، واحتقان شديد في الجيوب الكبدية، وذلك في مجموعة الجرذان التي عُولجت بالمكملات الغذائية ومزيج التستوستيرون مقارنةً بالمجاميع الأخرى التي أظهرت خلايا كبدية طبيعية. نستنتج أن استخدام المكملات الغذائية مع التستوستيرون يؤدي إلى تأثيرات سلبية على وظائف الكبد ونشاط إنزيم الكرياتين كيناز، إلى جانب تغييرات نسيجية مرضية في أنسجة الكبد.

الكلمات المفتاحية: المكملات الغذائية, ALT, AST، الكرياتين كاينيز، ذكور الجرذان.