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Original Article

Impact of chronic androgenic steroid exposure on liver toxicity

Manal EA El-Halwagy[1], Sherif H Abd-Alrahman[2,3], Rasha Hamed Mahmoud[1], Fares K Khalifa[4], Nevine S Darwish[5], Abla A Attia[5], Amany S Mohamed[6]

[1]Department of Biochemistry, Faculty of Science for Girl's, King Abdulaziz University, P.O Box 51459, Jeddah 21453, Saudi Arabia; [2]Department of Biochemistry, College of Science, King Saud University, P.O Box 2455, Riyadh 11451, Kingdom of Saudi Arabia; [3]Department of Pesticides Residue and Environmental Pollution, Central Agricultural Pesticide Laboratory, Agricultural Research Center, Giza 12618, Egypt; [4]Department of Biochemistry, Faculty of Science, King Abdulaziz University, P.O Box 51459, Jeddah 21453, Saudi Arabia; [5]Department of Histology, Research Institute of Ophthalmology, Giza, Egypt; [6]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Cairo University, Egypt

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Abstract: Adverse effect of the chronic anabolic androgenic steroid (AAS) exposure on liver tissue and function of adult male albino rats were evaluated. Total 48 adult male albino rats were divided into four groups; (G1) controls, (G2) therapeutic (5 mg/Kg b.w), (G3) low dose (0.9 mg/Kg b.w), (G4) high dose (18 mg/Kg b.w), rats were treated orally with anabol for 3 months. Blood samples were taken from all rats once a month, half of the animals in each group were sacrificed, and organs were dissected for the histopathological examination. Results showed that elevation in plasma liver biomarkers alaninaminotransferase (ALT) aspartateaminotransferase (AST), total proteins and albumin was recorded. Enhancement in superoxide dismutase (SOD) and catalase. Reduction in total reduced glutathione (GSH) concomitant with elevation in lipid peroxidation biomarker (MDA). Histopathological findings encountered changes in the liver architecture. Stop giving anabol in recovery period drawback these findings. Chronic administration of high dose of anabol induced serious injury in liver tissue and remarkable changes in liver biomarkers. Liver recovery needs long time to nearly reach to normal after the chronic exposure to androgenic steroids.

Keywords: Anabolic androgenic steroid (AAS), anabol, liver biomarkers, oxidative stress

Introduction

Anabolic androgenic steroids (AAS) are either endogenous occurring naturally within the body (e.g. testosterone, and rostenediol, dihydroepiandrosterone) or exogenous synthetic derivatives of testosterone (e.g. anabol and nandrolone decanoate) [1]. Testosterone is a steroid hormone that exists both free and bound to plasma proteins. It is the natural male hormone which is produced primarily by the testes. It is also produced by females but in fewer amounts. It is responsible for the androgenic (masculinizing) and anabolic (tissue building) effects throughout male adolescence and adulthood [2]. When ingested, testosterone is absorbed in the small intestine and transported to the liver via the portal vein where it is nearly completely metabolized to 17-keto steroid by the enzyme 17-OH-steroid dehydrogenase [3] when large amounts of testosterone are ingested, the enzyme system gets saturated, allowing some testosterone to remain unchanged. Anabolic steroid treatment may induce hepatic structural changes [4]. Anabolic steroids are usually administered orally as well as by injection. They are used by athletes to enhance performance and by non-athletes to improve appearance. Although their use is illegal and banned by sport governing bodies, yet, survey and drug-testing data indicate continued use by competitive athletes at all levels [5]. The fact that the frequency of steroid use appears to have increased significantly over the past three decades among adolescents, women and recreational athletes is also of growing concern. Their abuse presents an interesting public health challenge, as they are associated with deleterious physical and psychological outcomes [6]. Strong evidence exists demonstrating that AAS result in in-

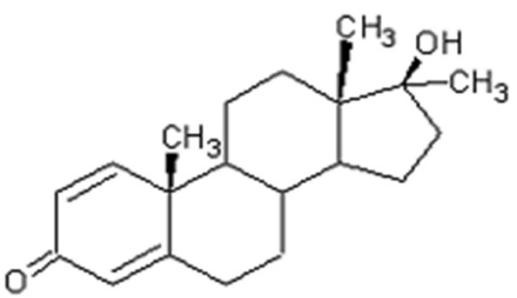
creasing body weight and muscular strength. However, there is also, increasing evidence that their abuse is associated with adverse effects on the liver, serum lipids and the reproductive system [7]. These effects are also associated with increased levels of irritability, aggression, personality disturbance and, dependence and psychiatric ailments [8]. Free radicals and other ROS are derived from normal essential metabolism or from external sources. Free radical formation occurs in cells due to both enzymatic and non-enzymatic reactions [9]. The balance between free radical production and antioxidant defense has important health implications. If there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops causing chronic or permanent damage [10]. If free radicals are not inactivated, their chemical activity can damage all cellular macromolecules (proteins, carbohydrates, lipids and nucleic acids). They can also change DNA structure and induce genotoxicity that may cause cancer [11].

This work assess the effects of therapeutic, low toxic and high toxic doses of anabol on oxidative stress and liver function and tissue archtechtiure in adult male albino rats treated for 3 months. The reversibility of anabol effects was also studied after one month post-administration.

Materials and methods

Test material

Anabol (Methandienone) 17β-OH-17α-CH3-1, 4-androstadien-3-one.



Chemical Structure of Anabol

It was imported from Thailand in the form of pink hexagonal tablets (5 mg each). It was dissolved in tap water and given to rats orally [12] by gastric intubation at a daily dose of 0.9, 5, and 18 mg/day. These doses correspond to the therapeutic, low toxic and high toxic doses respectively [13]. The dose was modified by Paget's Formula [14] to suite the rat body weight.

Animal protocols

Apparently 48 healthy adult male albino rats weighing 150+10 gm were maintained in the breeding animal house in the Faculty of Medicine, Cairo University. They were housed in hygienic metal cages and kept in clean well ventilated room. They were fed ad libitum with a standard laboratory diet and had free access to water. Rats were left for two weeks before commencement of the study to be acclimatized to lab conditions. All rats were weighed periodically in order to adjust the dosage according to the body weight.

Experimental design

Adult male albino rats were randomly divided into four equal groups: (G1) rats served as controls and given tap water. (G2) rats were treated orally with (5 mg/Kg body wet.) of anabol equal therapeutic (G3) rats were treated orally with (0.9 mg/Kg body wet.) of anabol equal low dose (G4) rats were treated daily orally with (18 mg/Kg b.w) of anabol equal high dose. The experiment was expanded for four months.

Sampling

Blood samples were obtained at the end of each experimental period (1st, 2nd 3rd & 4th months) from the retro-orbital plexus using capillary tubes [15] centrifuged at 2000 g. Plasma was separated and stored at (-20°C) for biochemical examination. At the end of the treatment period, half the animals in each group were sacrificed by decapitation to obtain the organs for histopathological examination and the rest were sacrificed after a recovery period of one month (the 4th experimental period). During this recovery period through the 4th month of this study, the tested drug was stopped to allow recovery from the toxicant effects.

Biochemical studies

Aspartate and Alanine transaminases (AST and ALT) activities were determined according to

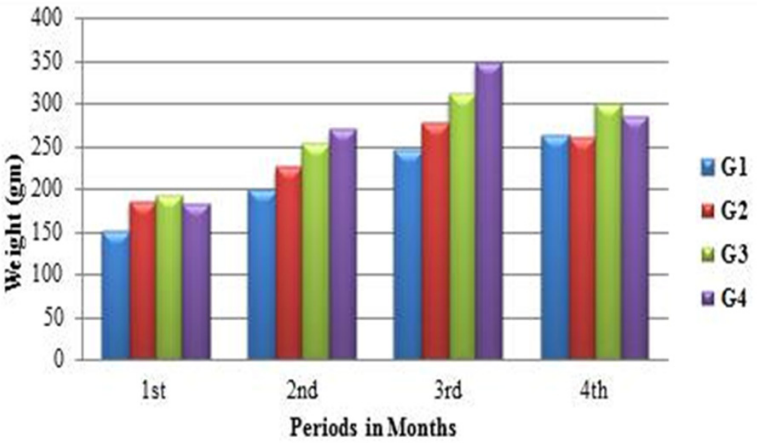


Figure 1. Weight changes in rats treated with anabol (G1: Control group. G2, G3 and G4: The experimental groups. 4th month = recovery period).

[16]. Total protein was determined by Biuret method according to [17]. Albumin in plasma was measured according to [18]. Total Glutathione Content (GSH) was determined in red blood cells according to [19]. Malondialdehyde (MDA) level was determined in plasma according to the method of [20]. Antioxidant enzymes activity of superoxide dismutase (SOD) and catalase were measured according [21, 22].

Histopathological study

By the end of the treatment period (3rd month) and recovery period (4th month), animals were sacrificed and tissue samples from liver, testis & epididymis were obtained and fixed in 10% formalin. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene and embedded in paraffin. Sections of 4-5 microns were cut and stained by haematoxylin & eosin (H&E) [23] and examined by light microscope.

Statistical analysis

Computer software package SPSS 15.0 was used in the analysis. One-way ANOVA were used to estimate the differences in quantitative variables. All data were expressed as mean ± S.D. at *P* value <0.05 significant [24].

Results

Biochemical results

Figure 1 showed progress increase in weight of rats in all groups administrated anabol with different doses all through the treatment periods. On the other hand, decline in rats weights were recorded at the end of the recovery period. The depicted data in **Table 1** revealed that plasma aspartate aminotransferase (AST) enzyme one of the liv-er biomarkers had gradual significant increase all through the experimental periods. The significance was between control at P<0.05. This increase in AST activity is dose and time dependent in all treated groups. Likewise, rats treated with 0.9, 5, and 18 mg/Kg bw doses level of anabol drug induced the same effect on plasma alanine aminotransferase (ALT) enzyme. Moreover, anabol treatment exhibited significant increase in total plasma proteins and albumin versus control in most of experimental groups at P<0.05 all through the experimental periods. It should be noted here that the above examined parameters recorded significant increase in recovery period; withdrawn of drug; throughout the experimental groups, but this increase showed draw back in comparison to the presence of drug. A slight elevation in malondialdehyde (MDA) lipid peroxidation biomarker was recorded in anabole treated groups with different doses, significant in the high dose treated group (18 mg/kg. bw) versus control and all other groups all through the treatment periods. Reduction in total glutathione content (GSH) was concomitant with the elevation in MDA. The reduction was pronounced in the high dose treated group significant versus control and other groups at P<0.05. On the other hand gradual elevation in each of antioxidant enzymes catalase and superoxide dismutase was recorded all through the experimental periods, the increase in the enzymes activities was dose and time dependent. A pronounced elevation was recorded in high dose treated animals significant versus other groups at P<0.05. Withdrawn of anabol in the 4th month did not affect the level of each of MDA, GSH, SOD and catalase as demonstrated in **Table 2**.

Histopathological examination liver

Liver of the control group (G1) showed normal liver architecture and cells (**Figure 2**). Whereas,

Table 1. Effect of chronic administration of anabol on plasma liver biomarkers of albino rats

		AST (mM/ml)	ALT (mM/ml)	Total Protein (gm/100 ml)	Albumin (gm/100 ml)
1st Month	G1	35.00±26.25	14.40±3.51	5.87±0.73	6.36±0.92
	G2	60.20±16.68	23.00±3.21	6.59±2.19	8.27±2.24
	G3	71.58±10.83a	26.00±2.24a	9.39±2.96	9.21±2.02
	G4	81.80±24.94a,b	28.40±4.88a	11.89±4.80a	11.52±4.11a
2nd Month	G1	35.00±26.25	14.80±2.39	5.38±2.24	5.90±2.36
	G2	70.62±16.68a	26.00±1.41a	10.40±3.61a	10.83±1.38
	G3	83.52±10.83a	28.20±4.78a	14.36±0.89a	15.65±3.75a
	G4	89.03±24.94a,b	32.40±2.88a	19.95±4.80a,b	17.63±3.61a,b
3rd Month	G1	35.00±26.25	14.20±3.12	5.18±1.02	6.36±0.92
	G2	84.24±43.17a	30.60±8.53a	16.38±3.69a	14.81±4.10a
	G3	93.84±21.51a	33.00±7.84a	20.68±5.48a	17.02±3.58a
	G4	100.9±34.02a,b	40.80±4.32a,b	26.52±3.27a,b	25.01±5.05a,b
4th Month recovery	G1	35.00±26.25	13.80±5.31	5.38±2.24	6.77±0.34
	G2	72.62±7.81a	28.40±4.34a	10.11±4.32a	10.47±1.09
	G3	80.72±20.12a	31.20±5.98a	11.40±3.61a	13.83±1.38a
	G4	85.89±21.26a	37.00±10.29a	12.03±5.05a	15.29±4.52a

G1: control group, G2 (therapeutic), G3 (low dose) and G4 (high dose). All data are expressed as Mean ± Standard Deviation (SD). Significant value is set at the level of $P<0.05$ (ANOVA test). (a) Significant difference versus control. (b) Significant difference versus G2.

Table 2. Effect of chronic administration of anabol on oxidative stress biomarkers in blood and plasma of treated albino rats

		GSH (mg/dl)	MDA (μMole/ml)	Catalase (U/mg protein)	SOD (U/mg protein)
1st Month	G1	28.83±1.85	10.12±2.07	5.89±0.21	12.28±0.11
	G2	25.34±0.07a	10.57±3.35	8.09±0.18a	14.01±0.21
	G3	22.24±0.91a	11.79±2.42	6.10±0.22	11.28±0.11
	G4	19.47±0.44a,b	12.00±4.79a,b	10.08±0.43	17.28±0.11a,b
2nd Month	G1	28.51±1.83	10.20±2.76	5.99±0.25	12.28±0.11
	G2	22.14±0.66a	12.52±1.41	9.89±0.21	16.01±0.41a
	G3	18.08±1.33a	12.29±3.65	5.69±0.27	12.01± 0.21
	G4	16.49±0.58a,b	13.06±3.11a	13.09±0.29a,b,c	19.41± 0.51a,b,c
3rd Month	G1	28.73±1.58	10.12±2.07	6.09±0.22	12.28±0.11
	G2	20.36±0.22a	11.79±2.42	9.09±0.19	13.01±0.41
	G3	14.72±0.46a	12.59±3.06	7.19±0.62	16.11±0.51a,b
	G4	8.57±0.73a,b,c	13.75±4.32a,b	15.09±0.29a,b,c	24.01±0.21a,b,c
4th Month recovery	G1	28.85±1.63	10.20±2.76	6.09 ± 0.22	12.28±0.11
	G2	22.82±0.91a	13.85±2.88	11.19 ± 0.62a	17.21±0.81a
	G3	17.56±0.61a,b	13.25±7.12	8.09±0.22a,b	14.01± 0.21a,b
	G4	12.14±0.78a,b,c	14.34±2.90a,c	19.09±0.29a,b,c	26.11±0.81a,b,c

G1: control group, G2 (therapeutic), G3 (low dose) and G4 (high dose). All data are expressed as Mean ± Standard Deviation (SD). Significant value is set at the level of $P<0.05$ (ANOVA test). (a) Significant difference versus control. (b) Significant difference versus G2. (c) Significant difference versus G3.

low dose treated group (G2) showed normal liver architecture with slight central vein congestion. Sinusoidal dilatation and congestion were seen. Many Von Kupffer cells were noticed as well (**Figure 3A**). During the 4th month (recovery), the cell cords and central vein were of normal architecture. Only mild dilatation of blood sinusoids was observed (**Figure 3B**). On

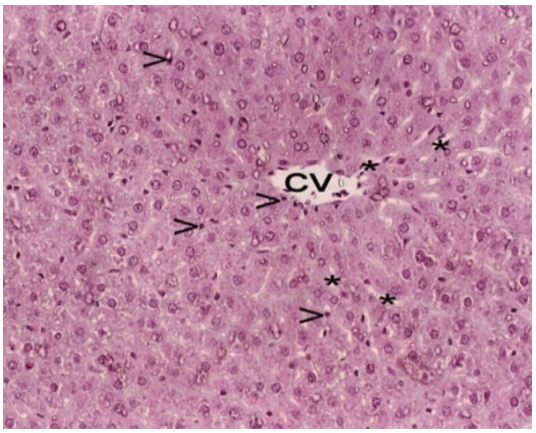


Figure 2. Normal liver tissue of control rats showing central vein (CV), blood sinusoids between liver cell cords (*) and scattered Von Kupffer cells (>) (H&E ×40).

the other hand, therapeutic dose treated group; G3: (5 mg/kg bw) central vein was dilated with Von Kupffer cells aggregating around its wall. The hepatocytes showed edema and marked vacuolation of their cytoplasm with the blood sinusoids being compressed between the hepatocytes. Few mitotic figures were also seen (**Figure 3C**). During the 4th month (recovery), the central vein was dilated and congested. Some hepatocytes showed degeneration and hyalinization with loss of nuclei. Some nuclei recorded mitotic division. The hepatocytes showed less edema and less vacuolation. The blood sinusoids disappeared among edematous cells (**Figure 3D**). G4 high dose treated group showed marked dilatation and congestion of the central vein and sinusoids. Degeneration of hepatocytes with loss of nuclei was observed. Necrosis and areas of hyalinization were noted with marked distortion of liver cords leading to total loss of liver architecture. Marked edema and vacuolation of hepatocytes were also noticed. Focal areas of blood accumulation were noted in the liver parenchyma (**Figure 4A**). During the 4th month (recovery), the central vein was still dilated and congested. Blood sinusoids were diminished and less congested. Some cells were degenerated with hyalinization. Other cells showed loss of nuclei or evidence of pyknosis. Edema and vacuolation of hepatocytes resided greatly (**Figure 4B**).

Discussion

Androgenic Anabolic steroids (AAS) are effective in enhancing physical performance but they have adverse side effects that can jeopardize health. Most athletes use more than one steroid at one single time. Protocols and regimens for AAS use are believed to enhance the effects of these drugs and lessen harm to the body [25-27]. In the present work, rats treated with anabol showed significant increase in both total protein and albumin levels throughout the three treatment months. The weights of experimental rats increased progressively through the experimental period. These results were consistent with those of [28, 29]. This could be explained by the fact that supraphysiologic doses of AAS result in significant increase in muscle mass and size, weight and strength, and acceleration of muscle and bone growth. These drugs stimulate receptors in muscle cells which activate specific genes to produce proteins thus increasing tissue mass helping muscle build-up. They also prevent tissue breakdown following intense workout and speed recovery after muscle trauma [29].

In the current study, both ALT and AST activates showed progressive increase throughout the experimental periods. Highest levels were recorded in high dose treated animals in all periods especially in the 3rd month. As for the recovery period a rise was still encountered as but to a less degree than the periods of treatment. These results are in agreement with those of other authors who stated that AST and ALT levels are commonly elevated in AAS use [30-32]. The above findings were confirmed by marked changes in liver tissues architecture especially in high dose treated group. These results run parallel with that reported by [33]. The number of Kupffer cells was increased in liver parenchyma and that the content of collagen was increased in the wall of the central lobular vein, in the hepatic parenchyma and within the portal triad. In adult male rats treated with different doses of AAS for 5 weeks, these results suggest that treatment with high doses of AAS is potentially deleterious to the liver leading to incipient fibrosis [30] reported that hepatotoxicity of AAS abuse was particularly true for orally administered AAS which produced marked liver affection more extensively than those parenteral administered. This difference in effect is explained by the fact that higher drug concentrations are presented to the liver on first pass metabolism and that liver cell damage occurs readily as the liver attempts to breakdown the oral agents. Glutathione is a low

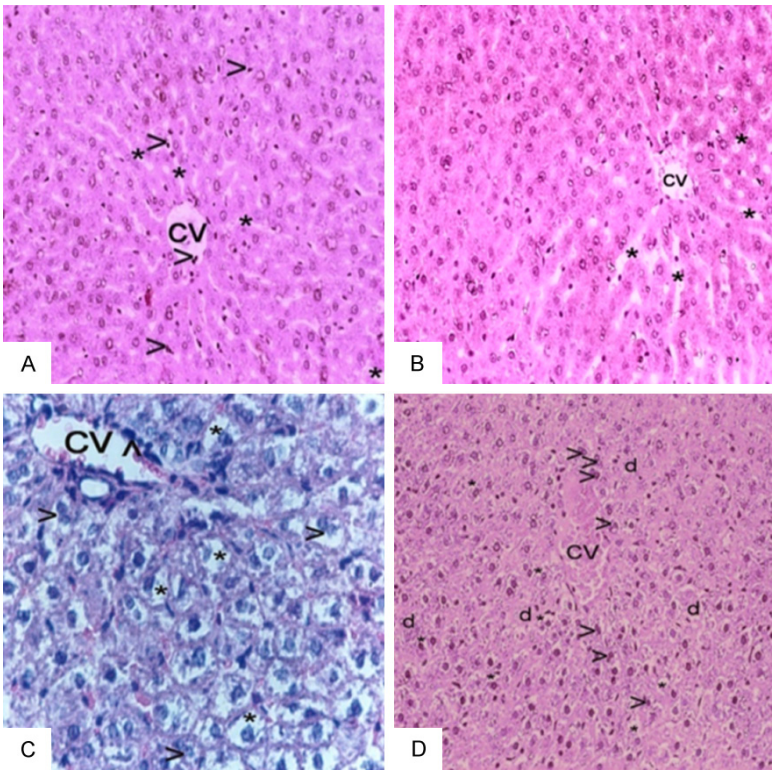


Figure 3. A. Liver tissue belonging to G2 of anabol-treated rats (3rd month) showing slightly congested central vein (CV) and sinusoids (*), note the scattered Von Kupffer cells (>) (H&E ×40). B. Liver tissue belonging to G2 of anabol-treated rats (4th month) showing no congested central vein (CV) and slightly dilated sinusoids (*) (H&E ×40). C. Liver tissue belonging to G3 of anabol-treated rats (3rd month) showing dilated, congested central vein (CV), mitotic divisions (>) and vacuolation of cell cytoplasm (*) (H&E ×40). D. Liver tissue belonging to G3 of anabol-treated rats (4th month) showing dilated, congested central vein (CV), mitotic divisions (>), vacuolation of cell cytoplasm (*) and areas of degeneration and hyalinization (d) (H&E ×40).

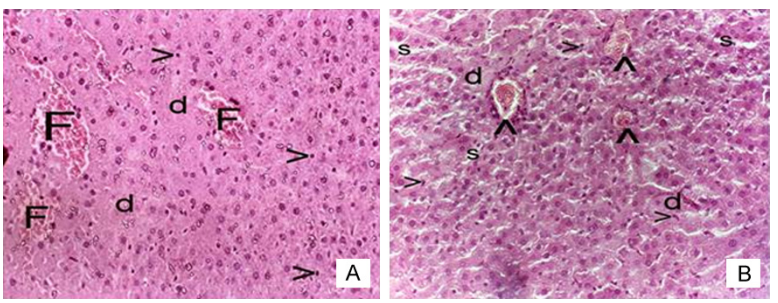


Figure 4. A. Liver tissue belonging to G4 of anabol-treated rats (3rd month) showing marked cell edema with loss of architecture, areas of degeneration and hyalinization (d), focal blood aggregations (F) and pyknotic nuclei (>) (H&E ×20). B. Liver tissue belonging to G4 of anabol-treated rats (4th month) showing dilated sinusoids (s), pyknotic nuclei (>) and degenerated cells (b) (H&E ×40).

molecular tripeptide that is present in every mammalian cell, with highest concentration in the liver, kidney and other parenchymal organs. Because of its multi-functions in redox and conjugation reactions, a reduction of glutathione levels in the liver may be expected to be critically linked to the organ function. Plasma concentrations of glutathione are considered to be directly related to the hepatic glutathione content [34]. Prolonged AAS intake induced significant decrease in GSH levels especially at the 3rd month. in high dose treated group; G4; These results are consistent with those of [35, 36] who reported that AAS-treated rats showed significantly decreased liver GSH levels in addition to increased levels of total superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activities detected in the liver of treated animals. Thus, they concluded that continuous and prolonged ingestion of AAS provoked a local and sustained oxidative stress state in the liver despite the up-regulation of enzymatic antioxidant activities.

Meanwhile, plasma MDA levels showed no significant changes within all doses of the used drugs throughout the experimental period of the present study. These results are in agreement with those reported by [34] who revealed that ingestion of high doses of AAS in rats showed no difference in plasma MDA found between treated and untreated groups which means that anabol does not induce lipid peroxidation.

Conclusion

Alteration in the structure and function of liver as a primary site of anabole clearance was recorded in this study. Alterations include changes in liver function biomarkers (ALT, AST,

albumin and total proteins) as well as, provoke of oxidative stress biomarker and enhancement of SOD and catalase enzymes and reduction in GSH. Changes in liver tissue architecture confirm these findings. With drown of anabole slightly drawback these findings.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Sherif H Abd-Alrahman, Department of Biochemistry, College of Science, King Saud University, P.O Box 2455, Riyadh 11451, Saudi Arabia. Tel: 00966-561776615; E-mail: drsherif_hussein@yahoo.com

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