

Histological Effect of Androgenic Anabolic Steroids on Liver

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Abstract: Anabolic androgenic steroids (AAS) they are the synthetic type of the natural male sex hormone (testosterone), they are widespread used amongst athletes to enhance performance. Abuse of AAS is common amongst players and it is usually accompanied by a many medically based complications. It was reported that hepatic problems include cholestasis, elevation of liver enzymes e.g. aminotransferases, as well as, benign hepatic adenomas, jaundice and to less extent of hepatocellular carcinoma was associated with their use.

A total of (12) adult New Zealand rabbits (*Oryctolagus cuniculus*) male, aged one year was selected. 2 control animals and 10 were treated with injections intramuscularly of the AAS, nandrolone decanoate (15 mg/kg) three times a week for 12 weeks. At the end of experiment the animals were sacrificed and the liver was dissected out and fixed using 10% buffered formalin-saline to prepare blocks for staining with haematoxylin and eosin for histological examination using the light microscope.

The liver of treated animals reveals that there are a mild to severe vascular congestion. There is swelling of hepatocytes, and inflammatory cell infiltration. Perivascular fibrosis, cellular necrosis was observed in other slides with sinusoidal congestions and extrabiliary bile pigments deposits.

The benefit of AAS comes with unwanted side effects. The total volume of the hepatocytes and sinusoids were increased in the studied animals. The total number of hepatocytes nuclei in experimental group also increased. The damage of the liver cells or at least increased permeability of the hepatocellular membrane which is noticed in the present work could explain the increment of plasma levels of liver enzymes which was studied by other researchers, as they notice an elevation liver enzymes activity such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT), and lactate dehydrogenase(LDH)..

Patients and physicians must kept in mind that the sequelae of AAS abuse are life threatening. So people should be aware about the complications of AAS use and a periodic examination of liver function and check out should be done to those treated groups.

Keywords: Androgenic anabolic steroid, histology, liver, liver enzymes.

Introduction

Anabolic androgenic steroids they are the synthetic type of the natural male sex hormone (testosterone), have been in commonly use among athletes to improve performance for many years. The hepatotoxicity risk is come to light as the dramatic increase in the use of AAS by athletes and young people. With the widespread use of AAS has come an associated increment of alertness of the adverse effects and sometimes serious sequelae of anabolic androgenic steroids use. Nonetheless, there

seems to be a constant increase in the use of AAS by players and for aesthetic functions. Recent evaluations put AAS use in the Sweden and USA at 1% of the population and it can rationally accepted that the amounts of usage in Canada are parallel¹. The online shopping using internet has elevates the illegal obtainability of these drugs without medical prescription and the buyers are usually unconscious of the dangers of administrating these drugs³. AAS abuse is common amongst sportsmen and is accompanied by a lot of medical problems.. documented

hepatic disease including cholestasis, benign hepatic adenomas, increment of aminotransferases, jaundice and in rare cases the development of hepatocellular carcinoma. High doses of AAS may lead to temporary sterility, and testicular atrophys. There are 3 common dosage forms in which AAS are administered: oral pills, skin patches and injectable steroids. Oral dosage forms are the most suitable. AAS administered by oral intake is rapidly absorbed, but it is converted largely to inactive metabolites, and only about one-sixth is existing in the active form. AAS can be administered by parenteral route, but it has irregular extended absorption time and greater activity in cypionate ester, enanthate, proionate or undecanoate form. parenteral steroids are typically administered intramuscularly, not intravenously; to avoid rapid changes in the concentration of the drug in the blood stream. Ciba was introduced Methandrostenolone (Dianabol) as an AAS by in the 1960s. Methandrostenolone was one of the AAS used to improve athletic act by the former East German Olympic programm. many adverse effects of AAS which include acne, gynecomastia, mood changes (aggressiveness) and testicular atrophy¹¹ were recorded to accompany the use of this AAS. Stanozolol, another carbon-17-alkylated AAS, has been reported previously to cause acute renal failure and severe cholestasis in a young athlete¹². the use of AAS and vitamin supplementation may associated with acute renal injury as it was reported in two male athletes¹³. Most of the synthetic androgens and anabolic agents are 17-alkyl-substituted steroids. Hepatic dysfunction is often associated with the administration of these drugs, e.g. increase in sulfobromophthalein retention and aspartate aminotransferase (AST) levels. Alkaline phosphatase values are also elevated. These changes occur early usually in the period of treatment, and the amount is proportionate to the dose. Bilirubin levels sometimes elevated until clinical jaundice is apparent¹⁴. cholestasis of the intrahepatic bile ducts has been reported to be caused by Methandrostenolone resulting in hyperbilirubinemia elevated aminotransferases, and clinical jaundice¹⁵. nandrolone decanoate has androgenic: anabolic activity 1:2.5-1:4 compared to testosterone.

Materials and Methods

A total of (12) adult New Zealand rabbits (*Oryctolagus cuniculus*) male, aged one year with body weight of (1.7-2.2) Kg was selected for the study. Animal were kept in steel cages with grid floor and fed by fresh trefoil diet and water supply ad libitum. They kept at room temperature (25 -27°C) and 12-hr light-dark cycles with good ventilation. Two animals served as control group. The remain 10 were the treated group which received injections of the anabolic-androgenic steroid, nandrolone decanoate (15 mg/kg) intramuscularly three times a week for 12 weeks. At the end of the experiment, the animals were sacrificed by exsanguinations after anesthesia using an intramuscular injection of ketamine (3.5 mg/ kg) and xylazine (5 mg/ kg). The liver was taken and dissected out and fixed using 10% buffered formalin-saline to prepare blocks for staining with haematoxylin and eosin for histological examination using the light microscope.

Results

For the study of the general histology of the liver, haematoxylin and eosin stains were used. The architecture of the liver slides of control group of animals, shows that the liver consist of hepatic lobule, each lobule consists of hepatocytes arranged in rows radiated from the central vein (figure 1). These hepatocytes are separated by hepatic sinusoids, between adjacent lobules there is the portal area (or triad) a branch of hepatic artery, branch of portal vein, and bile duct are the content of this triad. On examination of liver of treated animals; it reveals that there are a mild to moderate and some times sever vascular congestion. There is swelling of hepatocytes, and inflammatory cell infiltration (figure 2). Perivascular fibrosis, cellular necrosis was observed in other slides with sinusoidal congestions and extrabiliary bile pigments deposits (figure 3 and 4 a & b).

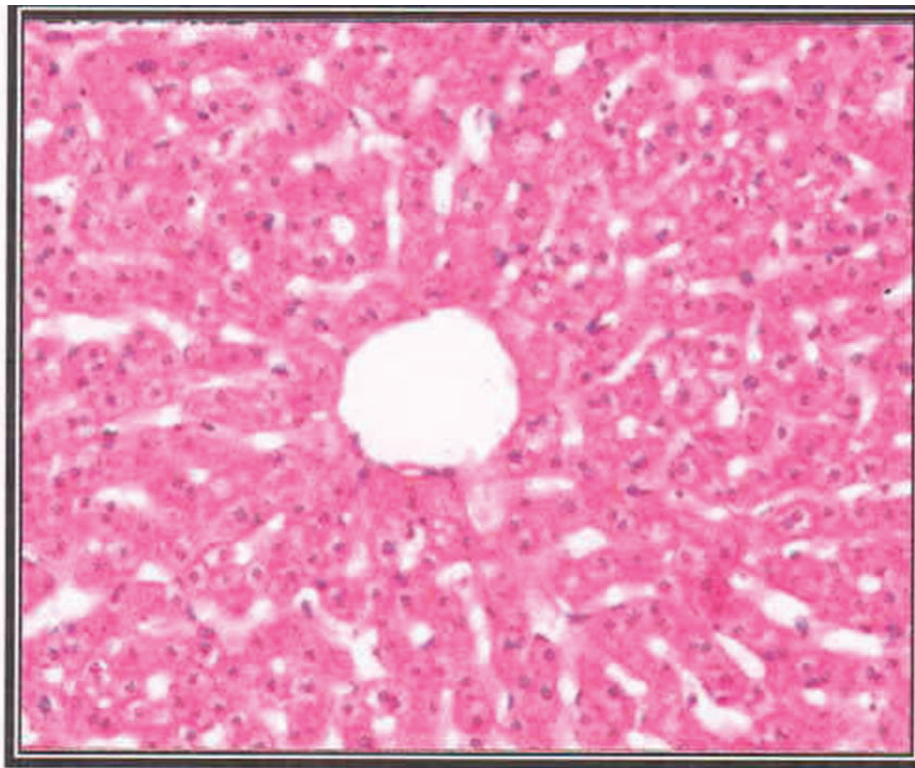


Figure.1: histological appearance of normal liver. See the central vein, the arrangement of hepatocytes and sinusoids. H&E stain. X400.

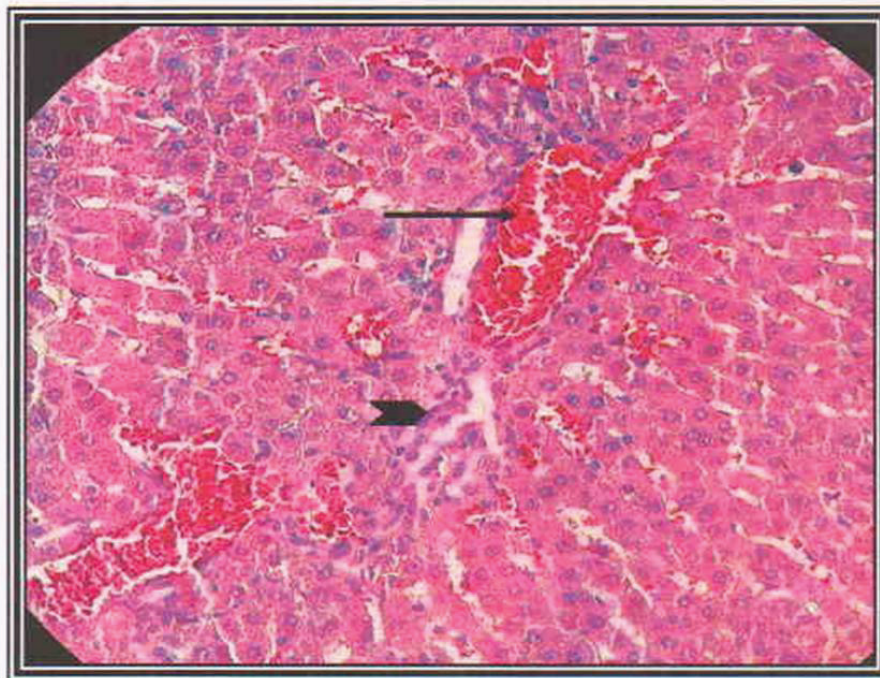


Figure.2: moderate to severe vascular congestion of the liver (arrow) with inflammatory cell infiltration (arrow head). H&E stain. X400.

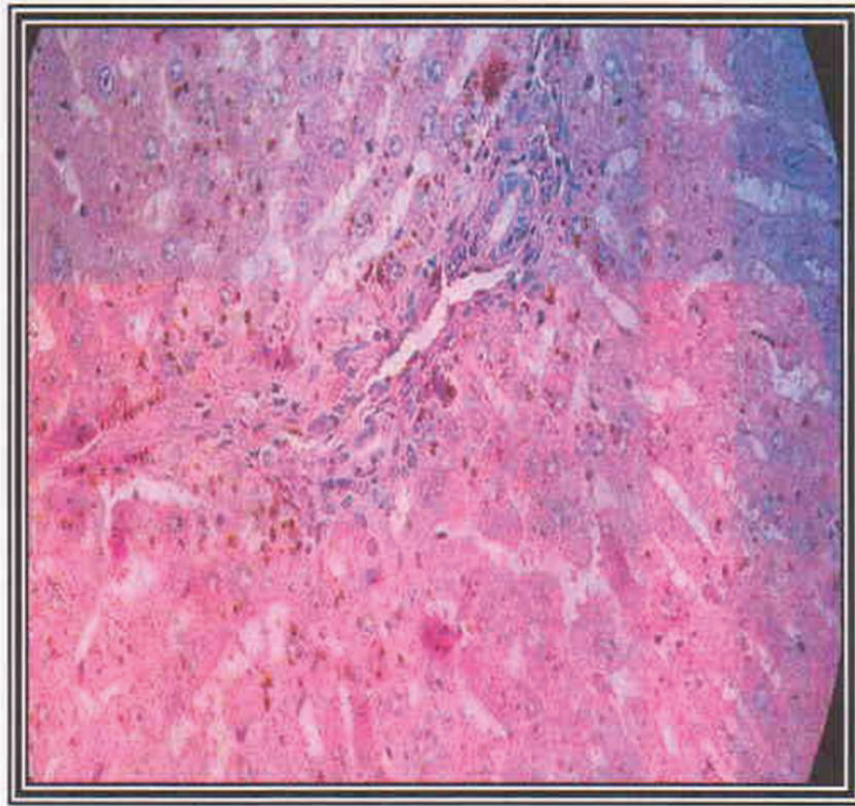
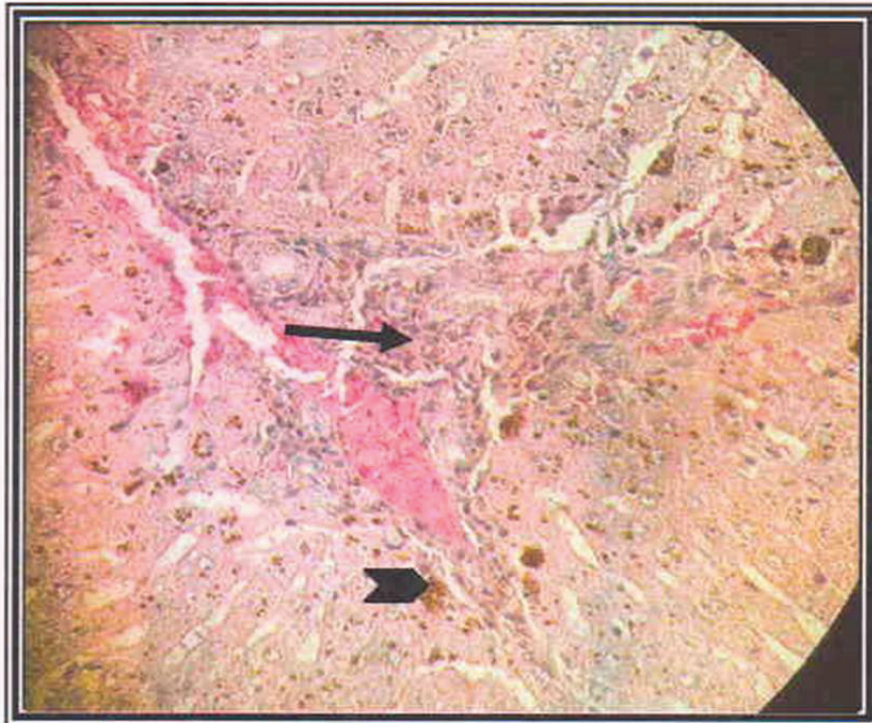


Figure.3: Perivascular fibrosis and cellular necrosis of hepatocytes. H&E stain. X400



A



B

Figure.4 A: moderate vascular congestion of the liver with inflammatory cell infiltration (arrow).

Fibrosis and bile pigment extravasation (arrow head).

B : sever vascular congestion with cellular necrosis. H&E stain. X400

Discussion

The advantages of anabolic steroids, unfortunately associated with unwanted side effects. Many organs demonstrate short and / or prolong side effects, and the use of AAS are accompanied by changes in the liver. The use of Anabolic androgenic steroid is not only by athletes who looking for better performance¹⁶. weight-lifters and bodybuilders, as well as prisoners are also show a high misuse levels ¹⁷. The availability of the AAS on the internet marketing and public gyms facilitate the ability of a person to get it in illegal ways, without medical prescription. As a consequence, the patients are able to take AAS without informing the physicians and in the same time, the doctors are less likely to be a

ware of the possibility of the use of AAS the non athlete population ³.

The present study clearly showed the effect of anabolic steroid use /or misuse on the liver histology. The histological changes on the liver which has been considered in the present work range from mild to sever vascular congestion with infiltration of chronic inflammatory cells, liver sinuses were congested, and collections of glycogen fibers and fatty degeneration appeared, some slides showed bile plugs in hepatocytes and canaliculi and also outside the cells and canaliculi. The effect of anabolic steroid had been studied previously on kidneys, pancreas from histological point of view. The side effect of AAS on the liver also studied

thoroughly but mainly concentrated on alterations on liver enzymes and the possibility of developing hepatic adenoma and carcinoma. The hepatic cellular damage caused by anabolic steroid occur also on ultrastructural level as shown by other studies, where the mitochondria showed increase in size and the appearance of crystalline inclusions, also there is increase in cytoplasmic volume. Most of the synthetic androgens and anabolic agents are 17-alkylsubstituted steroids. It is widely known that two chief mechanisms of liver injury induced by drugs; they are intrinsic and the idiosyncratic hepatotoxicity. Hepatocellular damage occurs by Intrinsic hepatotoxins in a way depending on the dose either in a direct way by the drug or indirectly by its metabolite. Some of the drugs, like acetaminophen, cause intrinsic hepatotoxicity, but most of this category agents are due to industrial toxins, household, or environmental toxins. The drugs that lead to idiosyncratic liver injury could be classified into metabolic and immunological categories.

In the first category, the drug is metabolized into metabolite which is toxic in susceptible persons, while in the later is similar to "drug allergy" or sensitivity following predisposition to the drug. Hepatocellular necrosis is the manifestation of intrinsic hepatotoxicity with little amount of inflammation, Meanwhile idiosyncratic drug responses usually appears with inflammation-dominant liver injury¹⁸. These facts explain the damage occurred to hepatocytes and the changes in liver structure of animals treated with anabolic steroids which were observed in previous studies and the present work. The mechanism of action of steroids on the cells, can be summarized by that as they are tetracyclic cyclopenta[a] phenanthrene formed compounds which are able to pass across cell membranes and to bind to the cytoplasmic receptors, to form a new- complex which binds to the DNA. After it bind to DNA, the steroid- receptor complex will starts a sequelae of process that finally leads to the making of cellular structures and proteins. positive nitrogen balance is the final result for cells. In spite of the wide range of reactions caused by the different androgenic anabolic steroids compounds, all androgenic anabolic steroids bind directly to one androgen receptor (AR)¹⁹. AAS were stimulate the synthesis of DNA in a potency between weak to strong effect and alteration to A- ring structure in a combination with non-polar substitution at 17alpha-positioned looks to be necessary for their activity²⁰. Previous studies by Dousta and Noorafshan

revealed that liver volume and weight in mice treated with anabolic steroids increased in comparison with that of the control group. the hepatocytes and sinusoids showed increment in their volume in the treated animals, too. The total number of hepatocytes nuclei in experimental group increased by 20%²¹. These findings are consistent with the results in this study as there were congestion of sinusoids and increase of hepatocytes size. The damage of the liver cells which is seen in the present work could explain the increment of plasma levels of liver enzymes which is studied to by other researchers, as they notice an elevation in plasma activity of enzymes of the liver e.g. aspartate aminotransferase (AST), alkaline phosphatase (AP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and gamma glutamyl transpeptidase (GGT). These enzymes are found in hepatocytes in high level and an increase in the plasma levels of these enzymes is a mark which reflect damage of liver cells or increased permeability of the hepatocellular membrane, at least²². The anabolic steroid, as consequence to their effect on the liver, could also affect free fatty acids metabolism by the liver and lead to the accumulation of triglycerides within the hepatocytes. This was appeared in the present work as fatty degeneration \ fatty changes of the liver which noticed in some slides, which called steatosis, and it is consistent with other studies that observed such hepatic changes due to anabolic steroids use²³.

The high doses of alkylated androgens can produce peliosis hepatica, cholestasis, and liver failure. They lower the level of plasma HDL2 and may elevate LDL. Hepatic carcinomas and adenomas also have been reported¹⁴. Gurakar et al. and another researchers studies showed the hepatotoxic effect of anabolic steroids especially in supraphysiologic doses, as that taken by bodybuild^{24,25,26}. Some studies reported development of hepatic tumor due to the use of anabolic steroids; this is confirmed by the regression of tumor size and return of liver function to normal by the cessation of steroid usage²⁷. Our study did not prolonged enough to elicit such changes in the studied animals. Ramachandran and Kakar study on the liver of patient use androgenic anabolic steroids didn't show inflammation nor hepatocellular injury¹⁸, their findings were inconsistent with the results of the present work. We agree with them in part in the occurrence of cholestasis in the anabolic steroids treated livers. Finally, Patients should be noted

that the sequelae of AAS abuse are life threatening. Meanwhile, the athletes and bodybuilders are aware of the legal and social ramifications of steroid abuse, they must be reminded about its medical risks.

Ethical Clearance: The animals were sacrificed under the ethical committee approval of college of medicine, university of Al-Qadisiyah.

Source of Funding:

The research is made entirely by self – funding.

Conflict of Interest: The authors declare that there is no conflict of interest with others.

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