Study the Effects of Anadrol Overdose on Liver Function in Male Rats

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Abstract

Anadrol (oxymetholone) is an active androgenic anabolic steroid that has been clinically studied in numerous diseases since the 1960s. It is used in the treatment of anemia and the replacement of male sex steroids. Unfortunately, in attempts to improve physical performance, anadrol could be misused by athletes, that can lead to poisoning and hepatotoxicity.

The aim of this study was to investigate the impact of anadrol on the liver function in rat model, via assessment of liver enzymes and histopathological study.

A forty male rats, weights about (200-300 gm), aged 8-12 weeks, after acclimatization, the rats were randomly divided into four groups (10 rats in each group) as follow: control group (in which all rats were administered normal saline (NS) via oral gavage), anadrol 10 mg/kg (Iran-Tehran Company) group (in which all rats were administered anadrol 10mg/kg via oral gavage), anadrol 20 mg/kg group (in which all rats were administered anadrol 20mg/kg via oral gavage), and anadrol 30 mg/kg group (in which all rats were administered anadrol 30mg/kg via oral gavage), the oral administration had continued for 8 weeks in single daily dose regimen. At the end of study liver function enzymes such as alanine aminotransferase & aspartate aminotransferase were measured via chemical analysis. Then histopathological study was done on the liver tissue in the four experimental groups.

Male rats that treated with anadrol displayed high level of liver enzymes, including as alanine aminotransferase & aspartate aminotransferase, as compared with control group. On the other hand, histopathological study exhibited significant injurious changes in the hepatic tissue in anadrol groups comparing with control.

When anadrol given in high doses results in hepatic injury, that can be cleared via elevated levels of hepatic enzymes and liver histopathological changes.

Keywords: Anadrol, Hepatic injury, ALT, AST, Anabolic Androgenic Steroid

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Introduction

Anadrol is an active androgenic anabolic steroid that has been clinically studied in numerous diseases since the 1960s. It is used in the treatment of anemia and the replacement of male sex steroids as a stimulator of bone marrow cells also it is used in some illnesses to improve general weakness. Unfortunately, in attempts to improve physical performance, anadrol could be misused by athletes and is therefore classified as ‘controlled substance schedule III.’ Anadrol poisoning contributes to hepatotoxicity, prostatic hypertrophy, azoospermia, and impotency (1).

As a testosterone 17-α derivative, anadrol demonstrate its anabolic effects via one of two mechanisms, either by direct activation of androgen receptors or indirectly by activation of specific estrogen receptors after its conversion to estradiol. The next step is that transportation of free testosterone into the cytosol of target cells and tissues, then either make binding with androgen receptors or undergo reduction, through the activity of 5α-reductase (cytoplasmic enzyme), into 5α-dihydrotestosterone (DHT). The latter mediator, 5α-dihydrotestosterone (DHT), will make stronger binding with androgen receptor (2.5 times) as compared with testosterone. After binding the drug-receptor complex will undergo conformational and structural changes, that result in entry of the drug molecules into the nucleus, followed by direct binding with hormone response elements (HREs), which include specific sequences of DNA nucleotides, then lead to gene expression and finally end with the required androgenic effects (2).

Anadrol, which had been approved as anabolic steroid by Food and Drug Administration (FDA), considered the potent one in body building as comparing to other anabolic steroids, in such condition body builder can get about 14.5 pounds/100 pounds of their weight (3). Furthermore, it is also cheaper, have higher activity, but mandatory monitoring of liver function should be done routinely (4, 5). Supraphysiologic-dose anabolic-androgenic steroid (AAS) use is associated with physiologic, cognitive, and brain abnormalities similar to those found in people at risk for developing Alzheimer’s disease (6).

Androgen’s treatment may result in liver dysfunction, adenosomas, and adenocarcinomas, and patients should have liver function tests performed every 3 to 6 months and hepatic lesions assessed by ultrasonography every 6 months (5). Long-term supraphysiologic-dose AAS exposures are associated with abnormalities in liver and kidney (7).

Liver toxicity associated with the use of anabolic steroids can be arranged from mild to life-threatening condition including liver transaminases level elevation, fatty liver, chronic vascular injury, and lipid profile changes hepatic cell carcinoma. Some of these injuries can be reversed by discontinuation of steroids, but other will be irreversible even drug cessation (9). It is well known that the illegal abuse of such anabolic steroids consider a growing factor to result in documented drug induced liver injury (DILI) that consequently result in severe liver dysfunction (9). Anadrol can result in significant increase in the level of liver transaminases like AST & ALT (10). When used, in double blind study in the treatment of HIV-wasting syndrome, anadrol result in elevate hepatic enzymes including AST & ALT into five folds (11, 12).

Materials and Methods

Animal grouping

Forty adult male rats weighted about (200-300 gm), aged 8-12 weeks, and were brought from the College of Science, university of Babylon. Animals were harbored in the animal house with a temperature controlled 20-25°C and 60-65% humidity with a fitted 12 hours light and 12 hours dark cycle for 14 days before the start of the experiment. Also, the rats were free to access food and water. In this study, the rats were divided randomly into 4 equal groups, 10 rats in each group, and as the following:

1. Control group: Rats in this group administered equivalent volume of normal saline (NS) via oral gavage route daily for 8 weeks (13).

2. Anadrol 10mg group: Rats in this group administered anadrol in a dose of 10 mg/kg via oral gavage route daily for 8 weeks (14, 15).

3. Anadrol 20mg group: Rats in this group administered anadrol in a dose of 20 mg/kg via oral gavage route daily for 8 weeks (16).

4. Anadrol 30mg group: Rats in this group administered anadrol in a dose of 30 mg/kg via oral gavage route daily for 8 weeks (17).

At the end of study animals were sacrificed via anesthesia, then blood and hepatic tissue samples collection had been done as below.

Preparation of drug

Anadrol 50 mg tablet (Iran-Tehran Company) was obtained and dissolved in normal saline as a vehicle to get anadrol solution, then given via oral gavage according to animal’s body weight (13).

Sample collection

At the end of study, animals were anesthetized with ketamine (50mg/kg) and xylazine (10 mg/kg) (5). Then blood sampling was done via direct cardiac puncture, furthermore, animals were sacrificed and hepatic tissues were obtained.

Blood sampling

Withdrawn blood was let to clot in gel tube then centrifuged at 4000 x g for 10 min to get serum, that directly sent for chemical analysis.
**Tissue sampling**

After animal scarification with anesthesia hepatic tissues were obtained and preserved in 10% formalin until histopathological study was done.

**Liver function analysis**

To get liver function parameters that include serum AST and serum ALT, chemical analysis was done measured with a fully automatic biochemical analyser (FUJI DRI-CHEM NX500). Briefly 10 μL of serum is deposited on a FUJI DRI-CHEM SLIDE TP-III. After depositing, the specimen spreads uniformly on the special spreading layer then reacts with reactive reagent that released from reagent layer to form color. The slide is incubated at 37 °C for a fixed time in the FUJI DRI-CHEM ANALYZER and the optical reflection density is measured at 540 nm. The optical reflection density is then converted into the total protein concentration using a calibration curve preinstalled in the analyzer (18).

**Histopathological analysis**

Histological specimens from the liver were prepared at the cancer Research Unit, faculty of Medicine, University of Kufa. Liver samples were fixed in 10% buffered formalin for at least 24 h before processing, as described previously (19). Briefly the fixed tissues were embedded into the paraffin wax followed by the dehydration process with a series of increasing concentrations of ethanol to remove the free or bound water. The embedded tissues were sliced using a microtome into the tiny section 5 μm. For histological assessment, the liver sections were mounted on plain glass slides and routinely stained with hematoxylin and eosin (HE) staining. HE-stained sections were observed for any abnormalities of histopathological features under a light microscope at 100×, 200×, and 400×.

**Hepatic histopathology scoring**

The degree of liver injury was scored based on the grading system done by the previous study (20), in which hepatocyte necrosis determined by the percentage of cell swelling, increase cytoplasmic eosinophilia, and nuclear changes including pyknosis (shrinkage), karyorrhexis (fragmentation), and karyolysis (nuclear loss). In addition to mild-moderate inflammatory changes, as shown via Table 1 below:

<table>
<thead>
<tr>
<th>score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (−)</td>
<td>Normal—no hepatocytes necrosis</td>
</tr>
<tr>
<td>1 (+)</td>
<td>Minimal–mild                  Focal, limited to centrilobular region               Less than 25% of affected lobules are necrotic</td>
</tr>
<tr>
<td>2 (++)</td>
<td>Mild-moderate                 Focal and multifocal                            Central to midzonal lobular region   50% affected lobules are necrotic</td>
</tr>
<tr>
<td>3 (+++)</td>
<td>Moderate to severe             Multifocal (centrilobular-portal region)               75%&gt;X&gt;50% affected lobules are necrotic</td>
</tr>
<tr>
<td>4 (++++)</td>
<td>Severe                        Multifocal                                    X&gt;75% affected lobules are necrotic</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Statistical analysis was performed using SPSS 26 (SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) with LSD post-hoc test was used to investigate differences between groups. While histological differences were confirmed using Kruskal-Wallis with Mann-Whitney U-test. Statistically, the present data significance was defined as $p \leq 0.05$ (21).

**Results**

**The effect of anadrol on liver function**

To investigate the effects of anadrol on liver function, liver function parameters including serum ALT and serum AST were carried out in experimental groups via chemical analysis.

**The effect of anadrol on the levels of ALT**

Anadrol 10 mg, 20 mg, and 30 mg groups demonstrated a significant ($p < 0.05$) higher levels of ALT as compared with that of control group. Furthermore, anadrol 20mg, 30mg groups showed a significant ($p < 0.05$) higher levels of ALT as compared with anadrol 10mg group. On the other hand, the study showed there is no significant elevated in ALT level in anadrol 30mg group when compared with anadrol 20mg. These findings as shown in Figure 1:

![Figure 1: Graph showing the effect of anadrol on the levels of ALT.](image-url)
Figure 1. The mean serum ALT level (U/L) in the four experimental groups: Data are expressed as mean ± SD; *P <0.05 versus corresponding control; # P <0.05 versus Anadrol 10 mg.

The effect of anadrol on the levels of AST

Anadrol 10 mg, 20 mg, and 30 mg groups showed a significant (p < 0.05) higher levels of AST as compared with AST level of control group. Additionally, Anadrol 20 mg, 30 mg groups exhibited a significant (p < 0.05) higher levels of AST when compared with anadrol 10mg group. Also, the current study showed there is no significant elevated in AST level in anadrol 30mg group when compared with anadrol 20 mg group. These results were summarized in figure 2:

Figure 2. The mean serum AST level (U/L) in the four experimental groups: Data are expressed as mean ± SD; *P <0.05 versus corresponding control; # P <0.05 versus anadrol 10 mg.

The histopathological effects of anadrol on hepatic tissue

According to used scoring system the histopathological results of hepatic tissue of rats of the four experimental groups are summarized by the following table 2 and figure 3.

Table 2. Hepatic histopathological damage percentage and score of the four experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Damage %</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anadrol 10 mg/kg</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Anadrol 20 mg/kg</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Anadrol 30 mg/kg</td>
<td>46</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 3. Mean rank of liver damage in the four experimental groups

Control group

Control hepatic tissue had normal architecture without hepatocytes necrosis with clear cell boundaries. According to the used scoring system, the severity of injury showed a zero degree of damaging (score mean = 0 and represent 0% of damage) all rats in this group show normal histopathological findings 100% as shown in figure 4:
Figure 4. Photomicrograph of rat liver section of control group shows liver normal histology, H&E stain 40 X.

**Anadrol 10 mg group**
Anadrol 10 mg group hepatic tissue had focal, limited to centrilobular region necrosis with mild changes in cell boundaries. In the term of histopathological grading from normal hepatic tissue, rats in this group showed up to 25% of affected lobules are necrotic as shown in figure 5.

Figure 5. Photomicrograph of rat liver section of anadrol 10mg/kg group shows mild centrilobular inflammation (blue arrow) surrounded by normal hepatocyte (yellow arrow), H&E stain 40 X.

**Anadrol 20 mg group**
Anadrol 20 mg group hepatic tissue focal and multifocal central to midzonal lobular region. In the term of histopathological grading from normal hepatic tissue, rats in this group showed up to 50% of affected lobules are necrotic as shown in figure 6.

Figure 6. Photomicrograph of rat liver section of anadrol 20mg / kg group shows moderate centrilobular inflammation (blue arrow) with increased cytoplasmic eosinophilia (yellow arrow) of surrounding hepatocyte, H&E stain 40 X.

**Anadrol 30 mg group**
Anadrol 30 mg group hepatic tissue moderate to severe multifocal (centrilobular-midzonal-portal region) are necrotic. In the term of histopathological grading from normal hepatic tissue, rats in this group showed up to 75% of affected lobules are necrotic as shown in figure 7.

Figure 7. Photomicrograph of rat liver section of anadrol 30mg / kg group shows severe portal inflammation (blue arrow), multifocal hepatocyte damage (green arrow), with increased cytoplasmic eosinophilia (yellow arrow) of surrounding hepatocyte, H&E stain 40 X.

**Discussion**

The effects of anadrol on liver
Present study showed significant changes in liver function among the four experimental groups, that included its effects on the liver markers such as ALT & AST enzymes, as clarify through the following sections.
The effect of anadrol on the level of ALT

The current study demonstrated a significant elevated ALT level in the three anadrol pretreated groups compared with the control group. These findings are consistent with previous studies (13, 22).

Such pathological changes, that indicated liver injury, can be attributed to major or minor changes in cell membrane integrity lead to significant changes in liver enzyme activities (23, 24).

The effect of anadrol on the level of AST

Additionally, present study showed a significant elevated AST level in the three anadrol pretreated groups compared with control group. These findings are similar with other studies (11, 12, 25), that showed elevated level of AST during androgenic anabolic steroids usage. These findings, that indicated liver injury, can be explained by increased metabolic rate of such xenobiotic by liver, in addition to increasing hepatocyte permeability and change in cellular integrity (23, 24, 26).

More interestingly the present study, depending on the microscopic examination of the liver of rats from four experimental groups and revealed variable histopathological findings showed that these anadrol pretreated groups significantly had hepatic tissue injury as compared with control group. The histopathological damage score in ranged from normal in control group, mild, moderate, and severe in anadrol pretreated groups.

Histopathological findings in the theses anadrol pretreated groups were associated with cellular swelling, increased cytoplasmic eosinophilia, RBCs extravasation, and nuclear changes (pyknosis, karyorrhexis, and karyolysis). In addition to inflammatory changes. Similar results also experienced by previous study (24), that found such injury occur due to the fact that the biotransformation of xenobiotic compounds is accumulated in the liver.

Conclusion

This work found that high doses of anadrol lead to liver injury. Further, it was found that this organ injury confirmed by the elevated level of hepatic specific injury markers, including ALT and AST, in addition to the histopathological changes that revealed the hepatic tissue injury.

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