ANABOLIC ANDROGENIC STEROIDS AND LIVER DYSFUNCTION IN MALE ADULT MICE

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ABSTRACT
Liver is one of the most important members of the body that play a fundamental role in the metabolism and secretion xenobiotics which makes it highly influences to their adverse and toxic effects. Liver infection or liver dysfunction caused by different drugs and toxic chemicals or their reactive metabolites (hepatotoxins) is known as hepatotoxicity. The current study is designed to know the effect anabolic androgenic steroids in liver function, histological structure and liver biomarkers. Experimental animals used in the study (30 Swiss mice) were allocated to three groups as control group, and test groups (N=10). The test groups received therapeutic dose 5 mg/kg of body weight and high dose 10 mg/kg of nandrolone decanato (ND) by subcutaneous injection, the dose was given each 48 hours, but control groups received the same volume of normal saline. The duration of the experiment was 4-weeks. The collection of blood samples, two days after the end of the period experimental from the control and treated groups. The serum samples were assayed for liver biomarkers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, alkaline phosphatase (ALP) and total bilirubin). It has been sacrificed animals in each group after the end of the experiment and the extirpation of liver for histopathological study. Results revealed that all ND-treated animals exhibited a significant increase of ALT, AST, ALP, total protein and total bilirubin. Also, our results of this study indicate that anabolic steroids at supraphysiological and high doses exerts histopathological changes in liver and might lead to toxic liver of males. The abuse of such drug by youth and adolescents requires taking steps represented by programs of awareness and guidance known health risk of these materials to public health to curb or at least minimize the spread of communities.

INTRODUCTION
Anabolic Androgenic steroids are steroid compounds similar work to the hormone testosterone, and either of these compounds are naturally produced within the body (endogenous), e.g. Testosterone,rostenediol and dihydroepiandrosterone) or exogenous synthetic chemically and pharmacologically related to testosterone (e.g. Anabol and nandrolonedecanoate) (Socas, 2005). In general there are over 60 types of these compound may to be able to use varying in chemical structure anabolic. It can be considered the effect of these hormones are generally associated with masculinization and the building of protein in muscles and bones. Exogenous synthetic anabolic Androgen considered relevant pharmacological and medicated for use in the treatment of many medical conditions, especially those related with disorders of sex hormones, such as breast cancer, reproductive system dysfunction, growth deficiency, certain blood disorders, osteoporosis, hypogonadal dysfunction and the commencement of late puberty in men and growth promotion ((Thiblin and Petersson, 2005; Jevdević et al., 2015). After taking testosterone absorbed in the small intestine and travels through the hepatic portal vein to the liver and there by the enzyme 17-OH-steroid dehydrogenase metabolizes almost entirely to 17-keto steroid (Kicman,2008). When taken in large quantities of testosterone liver enzymes become unable to metabolize large quantities of testosterone so some quantities remain unchanged testosterone. Therefore, many studies provide Anabolic steroid treatment may Stimulate occurrence hepatic structural changes (Maravelias et al., 2005; Chowdhury and Mahanta, 2014). There are proving verification of raising certain biochemical markers like alanine aminotransferases (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It can be considered a diagnostic guide for liver injury. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity whereas increases in both total and conjugated bilirubin levels are measures of overall liver function (Singh et al., 2011)

The effectiveness of enzymes serum Alanine aminotransferase (ALT) or serum glutamic pyruvic transaminase (SGPT) is based biomarkers to the poisoning of the liver where these enzymes play an important role in the metabolism of amino acids and gluconeogenesis. It catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to product glutamate and pyruvate. Increase the level of this enzyme in the event of damage to the liver due to increased liberation. Measure the level of this enzyme. The estimation of this enzyme is amore specific test for detecting liver abnormalities since it is primarily found in
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Effect of nandrolonedecanoate on serum biomarkers liver: The results represented in table-1 and illustrated in figure-1 revealed That (nandrolonedecanoate) at dose 5 mg/kg of body weight, resulted insignificant elevation (P<0.05) in Serum Transaminases (ALT & AST) and alkaline phosphate (ALP) activities recording percentage changes of 18.27%, 48.8% and 11.24% from the control level respectively.

Table -1: Effect of nandrolonedecanoate on serum ALT, AST and ALP activities in control and treated mice. Valued are expressed as mean ± S.E

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ALT (U/I)</th>
<th>AST (U/I)</th>
<th>ALP (U/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.56±0.23</td>
<td>1.34±0.25</td>
<td>22.7±1.67</td>
</tr>
<tr>
<td>Dose 5mg/kg % change of control</td>
<td>6.04±0.24</td>
<td>6.36±0.21</td>
<td>36.0±0.77</td>
<td></td>
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<tr>
<td>Dose 10mg/kg % change of control</td>
<td>18.27%</td>
<td>48.8%</td>
<td>11.24%</td>
<td></td>
</tr>
<tr>
<td>F Probability</td>
<td>P&lt; 0.05</td>
<td>P&lt; 0.05</td>
<td>P&lt; 0.05</td>
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</tbody>
</table>

Also, our data show significant increasing (p<0.05) in (ALT & AST) and ALP concentration at dose 10 mg/kg recording higher percentage changes than therapeutic dose 5mg/kg of 27.96% 63% and 21.49 % from the control level respectively. The results represented in table 2 and illustrated in figure 2 revealed that nandrolone decanoate, at dose 5 and 10mg/kg, resulted in a highly significant increase (P<0.01) in total protein recording percentage changes of 30.45% and 56.84% respectively compared to the control level. Static analysis showing significant increase in total bilirubin of animal treated with nandrolone at dose 5 and 10mg/kg when compared with control mice recording percentage changes of 59.76%, 90.01% respectively compared to control level (tab 2, fig 2).

Table -2: Effect of nandrolone decanoate on serum total protein and total bilirubin activities in control and treated mice. Values are expressed as mean ± S.E

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Total protein (U/I)</th>
<th>Total bilirubin (U/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>5.74±0.148</td>
<td>1.64±0.708</td>
</tr>
<tr>
<td>Dose 5mg/ kg % change of control</td>
<td>7.50±0.145**</td>
<td>30.45%</td>
</tr>
<tr>
<td>Dose 10mg/ kg % change of control</td>
<td>9.01±0.122**</td>
<td>56.84%</td>
</tr>
<tr>
<td>F probability</td>
<td>P&lt; 0.01</td>
<td>P&lt; 0.05</td>
</tr>
</tbody>
</table>

* are significantly different at level P<0.05 ** are significantly at level P< 0.01

Fig -2: Effect of nandrolonedecanoate on serum Total protein and Total bilirubin concentration in control and treated

Effect of nandrolonedecanoate on Histopathological alteration of liver: The results of this study reveal increase in central vein diameter in treated mice of ND at doses used in study 5 & 10 mg/kg. Fig.-3. Histopathological examination of the liver tissue is representative and shown in Figure 4A- C and the histological scoring of liver damage is presented in Table 3 at microsco-
pically examination, liver tissue from control mice group showed no histological changes and the normal histological structure of parenchyma cell, hepatic lobule and portal vein. They formed of hepatocytes radiating from central vein to the periphery of the lobules Fig. A1 and A2. Liver lobules of treated mice showed degeneration and coagulative necrosis in hepatocytes. However, at dose 5mg/kg treated mice liver showing vacuolation in some hepatocytes, congestion within central veins and some sinusoids 5 infiltration of inflammatory cells in the portal area with diffuse Kupffer cells proliferation in between the hepatocytes (Fig. B1, B2 & B3) Inflammatory cells infiltration in the portal area and fatty change in hepatocytes was shown in treated mice liver tissue at dose 10mg/kg (Fig. C1, C2 & C3). However, degeneration of hepatocytes, fatty change in hepatocytes, Existence coagulative necrosis in hepatocytes, diffuse kupffer cells proliferation in portal area, diffuse proliferation of kupffer cells between hepatocytes and dilatation in central vein are showed in Table-3.

Table-3: scoring of damage on histopathological examination of the mice livers in the different treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>control</th>
<th>5mg/kg</th>
<th>10mg/kg</th>
</tr>
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<tbody>
<tr>
<td>Degeneration of hepatocytes</td>
<td>-</td>
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<td>++</td>
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<tr>
<td>Dilated central vein</td>
<td>-</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Fatty change in hepatocytes</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Diffuse kupffer cells proliferation in between hepatocytes</td>
<td>-</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td>Diffuse kupffer cells proliferation in portal area</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coagulative necrosis in hepatocytes</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* are significantly different at P< 0.05

Fig- 3: Effect of nandrolonedecanoic groups on central vein diameter(µm) in control and treated mice

![Table-3: scoring of damage on histopathological examination of the mice livers in the different treatment groups.](image)

![Fig- 3: Effect of nandrolonedecanoic groups on central vein diameter(µm) in control and treated mice](image)
DISCUSSION

Liver is one of the largest and most important organs of the body, weighing in humans body around 1500 g (Naruse et al., 2007) and the more than 500 vital metabolic function, it contributes to the manufacturing clotting factors and urea that are liberated into the bloodstream, regulating the level of amino acids in blood. Moreover, parenchyma of liver serves as a member of storage for several important products including glycogen, fat and soluble vitamins in fat, but also included in the production of bile that is excreted in the intestinal canal and operate this article to remove toxic substances that acts as a filter for the body works to filter and separate the toxic substances from the bloodstream and disposal them (Saukkonen et al., 2006). The smooth endoplasmic reticulum for hepatocytes principal metabolic clearing house for both endogenous and exogenous chemicals like cholesterol, steroid hormones, fatty acids, proteins, drugs and alcohol but having a surplus of these chemicals reduces the production of bile and thus lead to a lack of ability the body to expel the chemicals out across the waste so when the liver leads its key role in the purification and conversion of these chemicals leads to the risk of exposure to toxic damage (Saukkonen et al., 2006). Hepatotoxicity is referring to the occurrence of liver damage or liver dysfunction, which is connected with an overloaded of drugs or xenobiotics (Navarroa and Senior, 2006). The compounds that cause liver damage are called hepatotoxins or heaptotoxicants. Hepatotoxicants are exogenous compounds of clinical relation and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett et al., 2004). Some proven medications may cause liver injury when introduced even within the therapeutic dose. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting liver cells (Saukkonen et al., 2006). Treatment caused by ND in mice an increase in the level of enzymes transaminases and alkaline phosphatase. Perhaps this increases to the drastic physiological effect resulting from the effects of ND either through interventional interacts with cell membranes of liver cells or through the action of free radicals resulting from the use of steroids (Georgiou et al., 1987). The damage that has occurred in the liver led to an increase permeability the plasma membrane of cells and this is associated with a higher effective enzymes transaminase and these results are agreement with the results of previous research by El-Halwagy et al., (2016), Chowdhury and Mahanta (2014). In the represent study rising biomarker liver enzymes of the ALT, AST and ALP from cytoplasm of damage hepatocytes after the rupture plasma membrane reflects radical mediated lipid peroxidation of liver cell membrane for hepatic cellular membranes, as several scientific investigations have shown that exposure to ND lead to increase the effectiveness of free radicals, the initial damage resulting from free radicals generated is combined with proteins and lipids of cellular membranes leading to the start of lipid peroxidation process and protein carbonylation and this results in the occurrence of structural changes of biomembranes and the loss of the safety of liver and decrease of metabolic efficiency (Pey et al., 2003 and this leads to a state of hypoxia of parenchyma for contracting fibrous tissue and the increased permeability of hepatocyte membrane due to irradiation exposure with release of ALT enzyme to bloodstream. The elevation of transaminase (ALT, AST) is indicator for liver injury because necrosis or destroyed of hepatic cells (Friedl, 1990). Also, the increases in ALP level due to obstruct cells (outside or inside)
for aggregated bile acid which due to dissolve the hepatic cells plasma membrane and release ALP (Seetharam et al., 1989). Serum proteins are synthesized and secreted by many cell types depending on the nature of the individual serum protein. An important function of serum protein is the maintenance of the normal distribution of body water by controlling the osmotic balance between the circulating blood and the plasma membrane of tissues, and the transport of lipids, hormones and inorganic materials (Harper et al., 1977). The results obtained in this study showed that, there is significant increase in serum total proteins suggested that the increase in protein ND-treated mice 2 might be the result of either damage of biological membranes or to changes in the permeability of the liver cells because increase necrosis liver cells. Our data showing elevated in total bilirubin level, that is explained as following When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing a buildup of bilirubin in the blood and extracellular (outside the cells) fluid. Serum bilirubin could be elevated if the serum albumin increases and the bilirubin shift from tissue sites to circulation. Elevated levels of bilirubin may also result due to decreased hepatic clearance and due to jaundice and other hepatotoxicity symptoms (Saukkonen et al., 2006) rise in bilirubin with few or no increase in Alkaline phosphate level indicate cholestasis. In acute human hepatic injury, total bilirubin can be a better indicator (Dufour et al., 2000). Histopathological results agreed with the measured activities of serum biomarker of liver's and provided supportive evidence for the biochemical analysis, in the current study. The histopathological alterations could be summarized as follows: degeneration and coagulative necrosis in the hepatic, inflammatory cells infiltration, congestion in portal vein, increase in central vein diameter and Kupffer cells proliferation were observed liver tissue of treated mice groups which related with the leakage of liver enzymes. These results are agreement with Haso et al., 2009 who reported vascular degeneration, lipid changes and dilating in central vein and hepatic venous at treatment rabbits with ND in muscle. In conclusion, the anabolic steroid and rogenic had side effect in liver causes liver injury and this happen in body builders athletic and young who used ND for increase muscle mass.

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