Changes in the hepatic and renal structure and function after a growth promoter boldenone injection in rabbits

Mostafa El-Moghazy¹, Ehab Tousson²∗ and Mohamed I. Sakeran³

¹ Animal Production Department, Faculty of Agriculture, Minoufiya University, Egypt
² Department of Zoology, Faculty of Science, Tanta University, Egypt
³ Biochemistry Section, Department of Chemistry, Faculty of Science, Tanta University, Egypt

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Abstract

Boldenone is an androgenic steroid that improves the growth and food conversion in food producing animals. In most countries worldwide, this anabolic steroid is forbidden for meat production. Recently, it is used by bodybuilders in both off-season and pre-contest, where it is well known for increasing vascularity while preparing for a bodybuilding contest. Therefore, our study was designed to investigate the possible effect of using growth promoter boldenone undecylenate on the rabbit liver and kidney structure and functions. Thirty-two adult New Zealand rabbits were divided into four groups. Control group includes animals that injected intramuscularly with olive oil and dissected after 3 weeks. Three experimental groups include animals that receive one, two and three intramuscular injections of 5 mg/kg body weight boldenone, respectively and dissected after 3, 6 and 9 weeks respectively, where the interval of each dose of boldenone was three weeks. Intramuscular injection of rabbits with boldenone increased the serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), urea and creatinine compared with the control group. We also found significant increases in the total protein, total lipid, nitric oxide (NO), superoxide dismutase activity (SOD), glutathione (GSH) and malondialdehyde (MDA) in liver and kidney tissues compared with the control group. Intramuscular injection of rabbits with boldenone exhibited mild to severe histopathological lesions in liver tissue as hepatocellular vacuolation in the centrilobular region and sinusoids and in the kidney, the renal glomerulei had completely lost their typical shape with the appearance of some vacuoles of different shapes and sizes with markedly congested sinusoidal and dilated blood vessels. These findings suggest that misuse of growth promoter boldenone undecylenate may contribute to continuous damage of the hepatic and renal function and structure that may lead to a progressive hepatic and renal diseases, so people should be careful if they want to use such steroids to enhance their strength and endurance.


∗) Corresponding author; e-mail: toussonehab@yahoo.com

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Steroids; boldenone; rabbit; liver; kidney

Introduction
The food gap is increasing in the third world countries especially with animal protein products, which are the most important source of food. They cover man’s needs of essential amino acids that he cannot gain metabolically but obtains then through feeding on meat and other animal protein sources that poor countries lack because they are very expensive. There are various reasons for that, the first are the remarkable price of animal feeds and the second is the weakness of genetic structure of farm animals. Therefore, it was necessary to search for new methods by which we can boost growth rate and feed efficiency of fattening animals. The most important of which are the anabolic steroids and other types of estrogenic agents, such as estradiol and diethylstilbestrol or estrogen-like such as zeranol, as well as androgenic growth promoters such as trenbolone acetate and boldenone undecyleenate.

Anabolic-androgenic steroids are used to enhance strength and endurance in canine, equine and human athletes through increasing muscle protein production and are synthetic substances related to the primary male sex hormone, testosterone (Schänzer, 1996; Sundløf, 2001; Soma et al., 2007; Kicman, 2008; Guan et al., 2010). Boldenone (1,4-androstadiene-17β-ol-3-one; BOL) is an androgenic steroid that improves the growth and food conversion in food producing animals. In most countries worldwide, this anabolic steroid is forbidden for meat production (Kuhn, 2002; Cannizzo et al., 2007; Soma et al., 2007). They were developed mainly for veterinary use, mostly for horse treatment and well known under the trade names Equipoise, Ganabol, Equigan and Ultragran. Until recently, the control of its illegal use was based either on 17β-boldenone or 17α-boldenone (its main metabolite in cattle) identification in edible tissues, hair, faeces or urine. Recent observations and data tend to demonstrate the natural occurrence (but not ubiquitous) in cattle of these steroids, making the analytical strategy of the control more complicated (Le Bizec et al., 2006). It has been demonstrated that precursors of 17α-boldenone can be detected in the faeces of rats fed with phytosterols (Song et al., 2000). Pompa et al. (2006) proved the biotransformation under aerobic conditions of certain precursors to α-boldenone in dried faeces samples. They were not able to identify these precursors but excluded a simple isomerisation between β- and α-boldenone and proposed that the same biotransformation in anaerobic conditions in the gut may lead to β-boldenone. Further they suggested that this de novo production probably involves microbial activity and catabolism products and consequently would also be possible in urine. The identity of this precursor is still unknown but it is often linked with the substitution of animal fat by vegetable fat in feedstuffs due to bovine spongiform encephalopathy (De Brabander et al., 2004). Vegetable fat is rich in phytosterols a group of compounds structurally resembling steroids. These plant
sterols would only need a minor transformation to be converted into a compound such as β-boldenone (Poucke et al., 2008). Boldenone has a very long half-life and can show up on a steroid test for up to 1.5 years. This is because of the long undecylenate ester attached to the parent steroid. Trace amounts of the drug can be easily detected for months after discontinued use (Hoffmann, 2002; Brookhouse, 2007). Recently, it is used by bodybuilders in both off-season and pre-contest periods, where it is well known for increasing vascularity while preparing for a bodybuilding contest. However, the action of these steroids on the liver and kidney functions and structure is still unclear. Therefore, our study was designed to investigate the possible effect of using growth promoter boldenone undecylenate on the rabbit liver and kidney structure and functions.

Material and methods

The experiment adhered to the guidelines of the ethical committee of the national research center, Egypt. The present study was conducted at a rabbit private farm in Dakhahlea governorate and Zoology Department, Faculty of Science, Tanta University, Egypt, during spring and summer 2010.

Animals

The experiment was performed on 32 adult New Zealand rabbits weighing \(3.25 \text{ kg} \pm 0.2 \text{ kg}\) and of 8-9 months age. The animals were fed ad libitum pellets standard rabbit ration (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch; Egyptian Company of Oils and Soap Kafr-Elzayat Egypt and free access to water. Animals were divided into four groups (8 animals each). Control group (G1) includes animals that injected intramuscularly with olive oil. Groups 2, 3 and 4 (G2, G3 and G4) include animals that receive one, two and three intramuscular injections of 5 mg/kg body weight boldenone undecylenate dissected after 3, 6 and 9 weeks respectively (Gabr et al., 2009). At the end of the experiment, the rabbits were fasted for 10 hr and then euthanized with intravenous injection with sodium pentobarbital and subjected to a complete necropsy.

Biochemical investigation

Blood samples were individually collected from the inferior vena cava of each rabbit in non heparinized glass tubes to estimate biochemical parameters. Blood serum was separated by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at \(-18^\circ\text{C}\). Blood serum was analyzed to determine the concentration of total bilirubin (ST bilirubin), direct billirubin (SD billirubin), serum glutamate oxaloacetate transaminase (SGOT; AST), serum glutamate pyruvate transaminase (SGPT; ALT), creatinine and urea spectrophotometrically using commercial diagnostic kits (AMS, Italy). Liver and kidney homogenate (10%; w/v) was prepared in ice-cold 0.067 M phosphate buffer (pH = 7) then, the homogenate was centrifuged
at 3000 rpm for 10 min. at 4°C. The resulting supernatant was used to determine the total protein by comassie blue according to Bradford (1976); Nitric oxide according to Vodovotz (1996), total lipids according to Esher et al. (1973); superoxide dismutase activity (SOD) according to Arthur and Boynel (1985); glutathione (GSH) according to Ellman (1959) and Lipid peroxidation (malondialdehyde; MDA) according to Buege and Aust (1978).

Histological preparation

Livers and kidneys were immediately removed from three dissected rabbits and divided into small pieces. Liver and kidney tissues were taken and immediately fixed by immersion in 10% buffered formalin solution and left for 24-48 hours. The specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5 μm thick were cut by mean of rotary microtome (Litz) and stained with haematoxylin and eosin (Bancroft and Cook, 1994).

Statistical analysis

Data were expressed as mean values ± SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

Results

Blood parameters analysis

Table 1 showed that, rabbit’s blood serum measures after boldenone injections were significantly increase ($P < 0.05$) in the SGPT, SGOT, urea and creatinine concentrations in G2, G3 and G4 compared with the control group. No clear differences

<table>
<thead>
<tr>
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<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
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<tbody>
<tr>
<td>ST bilirubin (mg/dL)</td>
<td>0.7 ± 0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.8 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.58 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.52 ± 0.03&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
<td>SD bilirubin (mg/dL)</td>
<td>0.158 ± 0.01&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.15 ± 0.007&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.12 ± 0.004&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.11 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>15.8 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.5 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.5 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>14 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.75 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.25 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.25 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>59.3 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.3 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.6 ± 1.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.3 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.84 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a, b, c</sup> Superscripts of different letters differ significantly ($P < 0.05$) from each other.
Table 2.
Changes in the concentrations (mean ± SE) of total protein, total lipid, nitric oxide (NO), superoxide dismutase activity (SOD), glutathione (GSH), and malondialdehyde (MDA) levels in different groups under study.

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Protein</td>
<td>222 ± 0.011ab</td>
<td>378 ± 0.006b</td>
<td>494 ± 0.052ab</td>
<td>435 ± 0.007a</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.119 ± 0.003b</td>
<td>0.128 ± 0.002ac</td>
<td>0.127 ± 0.001ac</td>
<td>0.145 ± 0.007ab</td>
</tr>
<tr>
<td>NO</td>
<td>56.95 ± 0.671ab</td>
<td>68.25 ± 0.749ab</td>
<td>70.25 ± 2.53a</td>
<td>76.03 ± 0.859ab</td>
</tr>
<tr>
<td>SOD</td>
<td>39.50 ± 1.32ab</td>
<td>56.25 ± 1.49ab</td>
<td>61 ± 1.08ab</td>
<td>60 ± 1.29a</td>
</tr>
<tr>
<td>GSH</td>
<td>0.049 ± 0.004abc</td>
<td>0.065 ± 0.002ab</td>
<td>0.061 ± 0.002ac</td>
<td>0.0733 ± 0.002abc</td>
</tr>
<tr>
<td>MDA</td>
<td>518.5 ± 10.2abc</td>
<td>522.7 ± 28.5ab</td>
<td>553 ± 8.78ac</td>
<td>530 ± 16.6bc</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Protein</td>
<td>195 ± 0.011a</td>
<td>31 ± 0.005a</td>
<td>368 ± 0.026a</td>
<td>418 ± 0.005a</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.122 ± 0.004</td>
<td>0.142 ± 0.016ac</td>
<td>0.139 ± 0.007bc</td>
<td>0.147 ± 0.004abc</td>
</tr>
<tr>
<td>NO</td>
<td>62.5 ± 1.5ab</td>
<td>69 ± 0.70abc</td>
<td>74 ± 1.82ac</td>
<td>74.5 ± 1.55ab</td>
</tr>
<tr>
<td>SOD</td>
<td>35.75 ± 1.548abc</td>
<td>55 ± 3.79ab</td>
<td>56.5 ± 4.69ac</td>
<td>46.75 ± 2.53a</td>
</tr>
<tr>
<td>GSH</td>
<td>0.045 ± 0.007abc</td>
<td>0.068 ± 0.005ab</td>
<td>0.068 ± 0.003ac</td>
<td>0.071 ± 0.002a</td>
</tr>
<tr>
<td>MDA</td>
<td>48.73 ± 3.58abc</td>
<td>151.33 ± 4.70ab</td>
<td>184.37 ± 13ac</td>
<td>327.03 ± 16.1abc</td>
</tr>
</tbody>
</table>

\(^a, \(^b, \(^c\) Superscripts of different letters differ significantly \((P < 0.05)\) from each other.

were observed regarding total and direct serum bilirubin concentrations in rabbit blood serum of different groups (table 1). The activity of ST billirubin after boldenone injections tended to increase in G2 and decrease in G3 and G4 compared with the control group, while of SD billirubin in rabbits blood serum tended to decrease in G2, G3 and G4 compared with the control group (table 1).

Table 2 showed that rabbit’s homogenate analysis were significantly increased \((P < 0.05)\) in the total protein and total lipid concentration in both liver and kidney after boldenone injections in G2, G3 and G4 compared with the control. Also, the NO, SOD and GSH concentrations in both liver and kidney tissues were significantly increased \((P < 0.05; \) table 2) after boldenone injections in G2, G3 and G4 compared with the control.

Histological examination

Histological examination of the liver of control rabbit revealed entirely normal hepatocytes structure bounded by portal tracts and having a central vein (fig. 1). This Histopathological alternations in rabbit liver and kidney were increased with the increase in the boldenone dose injection. The histopathological changes were gradual severe hydropic and vascular degeneration in the hepatocytes (figs. 2-4). A clear dilatation and congestion of the portal veins, in addition perportal necrosis of the hepatocytes that surround the portal areas, and the inflammatory infiltration was seen (figs. 2 and 3). Histological examination of the kidney of control rabbits revealed entirely normal structures of the renal cortex which comprised renal corpuscles, proximal and distal convoluted tubules (fig. 5). Kidney from treated rabbits
Figures 1-4. Haematoxylin and eosin stained liver sections. (1) Control liver group showing normal hepatocytes (arrow), central vein (CV), blood sinusoids and portal tract (PT). (2) One injection boldenone-treated group (G2) showing central vein, congested blood sinusoids (arrowheads), cytoplasmic vacuoles and cell infiltration (white arrows). (3) Two doses boldenone-treated group (G3) showing congested blood sinusoids (arrowheads), cytoplasmic vacuoles (arrowheads) and cell infiltration. (4) Three injections boldenone treated group (G4) showing congested blood capillary, severe cytoplasmic vacuoles (arrowheads) and fibrillar vacuolar content. This figure is published in colour in the online version.

with boldenone showed variable pathological changes in glomeruli and some parts of the urinary tubules (figs. 6-8). The most severe changes were in the Malpighian corpuscles lost their characteristic configuration and the renal tubules appeared with degenerated epithelium and wide lumen (figs. 7, 8). Some glomeruli seemed to have lost their attachments, mesangial stroma with leucocytic infiltrations were observed in the interstitium (fig. 6) and markedly congested sinusoidal and dilated blood vessels were detected. (figs. 7 and 8).

Discussion

Our results showed that boldenone have marked adverse effect on the liver and kidney, where it showed a marked alteration in the liver and kidney functions and structures after injection with different doses. Biochemical analysis of the rabbit’s blood serum showed a significant increase in SGOT, SGPT, urea and creatinine comparing with the control group. Our results are in agreement with Gabr et al. (2009) who
Figures 5-8. Haematoxylin and eosin stained kidney sections. (5) Control rabbits group showing a normal structure of the glomerulei (arrows) with a normal appearance of mesangial area and normal renal tubules (arrowheads). (6) One injection boldenone treated group (G2) showing mild cytoplasmic vacuoles and glomeruli with dilatation of subcapsular space (arrows). (7) Two injections boldenone treated group (G3) showing glomeruli with dilatation of subcapsular space (arrows) and congested and dilated blood vessels (star) and congested leucocytic infiltration. (8) Three injections boldenone treated group (G4) showing irregular glomerulei with expanded mesangial areas (arrows), congested and dilated blood vessels (star) and some glomerular vacuoles. This figure is published in colour in the online version.

reported that the liver and kidney function significantly increased after intramuscular boldenone undecylenate injection on weaned male lambs. Our results are not in agreement with Istasse et al. (1988) who reported that, $17\beta$-estradiol increased nitrogen retention and decreased blood urea nitrogen concentrations. Boldenone treatment can cause an oxidative stress situation in liver and kidney as indicated by enhanced MDA, SOD and GSH. Injection of the anabolic steroid boldenone induced changes in oxidative stress biomarker levels and antioxidant defense systems in rabbit liver and kidney. Our results showed that, significant increase in the activity of MDA, SOD and GSH in both liver and kidney after boldenone injections in G2, G3 and G4 comparing with the control ($P < 0.05$). Our results are in agreement with Pey et al. (2003) who reported that the anabolic-androgenic steroids induced changes in oxidative stress. Interestingly, reactive oxygen species (ROS) and MDA have been implicated in the pathogenesis of many types of liver injury and especially in the hepatic damage induced by several toxic drugs (Uchida et al., 1999).
Oxidative stress has also recently been implicated in hormone-induced prostate carcinogenesis (Tam et al., 2003). The present study showed a significant increase in the total NO in rabbits after injection with boldenone when compared to control group. This finding may be due to increased vascular oxidative burden associated with homocysteinaemia that induces NADPH oxidase and inducible nitric oxide synthase activity, contributing to increased superoxide radicals production in rat vessels which react with nitric oxide to form peroxynitrite radicals, leading to low NO bioavailability and endothelial dysfunction (Ungvari et al., 2003).

Our results showed that rabbits that receive one to three doses of boldenone revealed gradual disturbances of the hepatocytes radially arranged cords with sinusoidal congestion in liver and a multifocal glomerular injury with markedly congested sinusoidal and dilated blood vessels in kidneys. Also, this histopathological alternation in rabbit liver and kidney were increased with the increase the boldenone dose injection. Our results are in agreement with Dickerman et al. (1999) who reported that the anabolic steroid-induced hepatotoxicity and with Welder et al. (1995) who reported that the anabolic-androgenic steroids have toxic effects in primary rat hepatic cultures. Yesalis et al. (1993) reported that in spite of the growth promoting effects, anabolic steroids have been shown adverse effects in cardiovascular, hepatic, and endocrine systems. Stanozolol has been shown to cause inflammatory or degenerative lesions in centrilobular hepatocytes, ultrastructural alterations in the canaliculi and degenerative changes in mitochondria and lysosomes (Boada et al., 1999; Pey et al., 2003). Prolonged stanozolol administration provokes an increase in the activities of liver lysosomal hydrolases and a decrease in some components of the microsomal drug-metabolizing system and in the activity of the mitochondrial respiratory chain complexes without modifying classical serum indicators of hepatic function (Saborido et al., 1993; Molano et al., 1999). Many other adverse effects associated with anabolic androgenic steroids misuse were recorded to occur such as the disturbance of the endocrine and immune function, alterations of the sebaceous system and skin, changes of the haemostatic system and urogenital tract (Hughes et al., 1995; Sullivan et al., 1998). Boldenone increases nitrogen retention, protein synthesis, appetite and stimulates the release of erythropoietin in the kidneys and reducing protein destruction. These findings suggested that misuse of growth promoter boldenone undecylenate may contribute to a continuous damage of the hepatic and renal function and structure that may lead to a progressive hepatic and renal diseases.

References


