Effects of maturity on histopathological alteration after a growth promoter boldenone injection in rabbits

Ehab Tousson\textsuperscript{*}; Mohamed S. A. El-Gerbed\textsuperscript{2} and Somia Shaleby\textsuperscript{3}

\textsuperscript{1}Zoology Department, Faculty of Science, Tanta University; \textsuperscript{2}Zoology Department, Faculty of Science, Damenhour University; \textsuperscript{3}Zoology Department, Faculty of Science, Minoufiya University, Egypt
toussonhab@yahoo.com

Abstract: Boldenone is a derivative of the testosterone and it has dual effects on humans, directly and indirectly, directly as injection to build muscles and indirectly as through consuming meat of animals that were treated with boldenone. However, the action of these steroids on the liver, kidney and testes structure in immature animals still unclear, therefore, the aim of the present study was to investigate the effect of maturity on the intramuscular injection of boldenone undecylenate on the hepatic, renal and testicular structures. Thirty two New Zealand rabbits were divided into main groups (16 immature and 16 mature rabbits) and each main group is divided into four groups (4 animals each). Control group (G\textsubscript{1}) includes animals that injected intramuscularly with olive oil. Groups 2, 3 and 4 (G\textsubscript{2}, G\textsubscript{3} and G\textsubscript{4}) 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. The present results showed that intramuscular injection of rabbits with boldenone has a marked adverse effects on the liver, kidney and testes tissues and this effects were more observed in immature than in mature rabbits and this histopathological alternations were increased with the increase the boldenone dose injection. Our results showed that; immature rabbits that receive boldenone showed disturbances of the hepatocytes radially arranged cords with multifocal hepatocellular vacuolations in the liver, glomerulus mass reduction with multifocal glomerular injury in the kidney and disturbances of the cycle of spermatogenesis in the testes. These findings suggested that misuse of growth promoter boldenone undecylenate may contribute to a continuously damage of the hepatic, renal and testicular function and structure that may lead to a hepatic, renal and genital progressive diseases so young people especially should be careful if they want to use such steroids to enhance their strength and endurance.


Keywords: Steroids; Boldenone; Rabbit; Maturity; Liver; Kidney; Testes

1. Introduction

Anabolic-androgenic steroids caused some adverse effects such as disturbance of the endocrine and immune functions (Schänzer, 1996; Sullivan et al., 1998; Sundlof, 2001; Pey et al., 2003). Boldenone (1,4-androstadiene-17alpha-ol-3-one; BOL) is a derivative of the testosterone, which exhibits strong anabolic and moderately androgenic properties that improves the growth and food conversion in food producing animals (Yesalis et al., 1993; Sullivan et al., 1998; Kuhn, 2002; Soma et al., 2007; Kicman, 2008; Guan et al., 2010). It is well known under the trade names Equipoise, Ganabol, Equigan and Ultragan. They were developed mainly for veterinary use, mostly for the horse treatment. In most countries worldwide, this anabolic steroid is forbidden for meat production and human uses (Kuhn, 2002; Cannizzo et al., 2007; Soma et al., 2007). In US, it is not indicated for use in human and is only available through veterinary clinics (Hoffmann, 2002; De Brabander et al., 2004). Recently, boldenone used by bodybuilders in both off-season and pre-contest, where it is well known for increasing vascularity while preparing for a body building contest. It has a very long half-life and can show up on a steroid test for up to 1.5 years. Trace amounts of the drug can be easily detected for months after discontinued use (Brookhouse, 2007).

Anabolic androgenic steroids caused some adverse effects on many other adverse effects associated with anabolic androgenic steroids were recorded to be happened such as disturbance of the endocrine and immune function, alterations of sebaceous system and skin, changes of haemostatic system and urogenital tract (Pey et al., 2003). Alm-Eldeen and Tousson (2011) studies the histopathological alteration in the renal structure and functions after boldenone injection in adult male rabbits. Groot and Biolatti (2004) study the histopathological effects of boldenone in adult male cattle and reported that boldenone causes degeneration of the germinal epithelium of the testis and hypersecretion and cyst formation in the prostate. Boldenone has dual effects on humans, both directly and indirectly; directly as injection to build muscles and indirectly as through consuming meat of animals that where treated with boldenone. However, the action of these steroids on the liver, kidney and testes...
structure in immature animals still unclear, therefore, the aim of the present study was to investigate the effect of maturity on the intramuscular injection of boldenone undecylenate on the hepatic, renal and testicular structures.

2. 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 El-Gerbia governorate and Zoology Department, Faculty of Science, Tanta University, Egypt, during spring 2011.

Animals:

The experiment was performed on 32 New Zealand rabbits (16 immature weighing 1.25 kg ± 0.1kg; 6-8 weeks age and 16 mature rabbits weighing 2.25 kg ± 0.1kg; 14-16 weeks age). The animals were fed ad libitum pellets standard rabbit ration and free access to water. Animals were divided into two main groups (immature and mature rabbits) and each group was divided into four groups (4 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 (Alm-Eldeen and Tousson, 2011). At the end of the experiment, the rabbits were fasted for 10 hr and then euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

Histological investigation:

Livers, kidneys and testes were immediately removed from dissected rabbits and divided into small pieces. Small Species of the liver, kidney and testes 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 and stained with haematoxylin and eosin (Bancroft and Cook, 1994).

3. Results:

Hematoxylin and eosin stained sections of liver, kidneys and testes were evaluated under light microscopy.

Testis

Histopathologic evaluations of mature testicular tissue were examined in the sections dyed hematoxylin–eosin. Rabbits in control group (G1) showed normal testicular architecture with well-organized seminiferous tubules and regular course of spermatogenesis and Sertoli cells. All stages of transformation of the seminiferous epithelium from spermatogonia to mature spermatozoa could be seen in the tubules (Fig. 1A). The histopathological examination of mature and immature rabbit testes that treated with boldenone showed various histopathological changes, these alternations were increased with the increase the boldenone dose injection (Figs. 1B-D, 2B-D).

Mature rabbit testes sections in G2 that receive one intramuscular injection of boldenone and dissected after 3 weeks showed seriously damage in the integrity of spermatogenic cells of seminiferous tubules and also an increase of interstitial tissue. In addition, necrotic cells and debris were examined in seminiferous tubules (Fig. 1B). Mature rabbit testes sections in G3 that receive two intramuscular injections of boldenone and dissected after 6 weeks showed testicular lesions, irregular seminiferous tubules with edematous, a wide range of disorganization and undergoing degeneration (Fig. 1C). The spermatogenesis was almost absent, and characterized by a depletion of germ cells. Mature rabbit testes sections in G4 that receive three intramuscular injections of boldenone and dissected after 9 weeks showed severe necrosis and degeneration of seminiferous tubules was further enhanced (Fig. 1D). There was a reduced seminiferous epithelial layer and a few spermatogonia in nearly all the seminiferous tubules, diminished tubules containing a few germ cells also were seen. In addition to these changes, vacuoles were seen in the seminiferous epithelium, especially at the basal compartment indicating their location in the Sertoli cells.

The immature testicular structure in the control group (G1) was normal without any changes in the cell associations in the seminiferous epithelium or cell structure in the intertubular spaces (Fig. 2A). Structural effects on immature testis of boldenone indicated in (Figs.2B-D). Immature rabbit testes sections in G2 that receive one intramuscular injection of boldenone and dissected after 3 weeks showed seminiferous tubules with irregular basal lamina and are separated from each other. Tubules showed epithelial degeneration and atrophy (Fig. 2B). The initiation of tubular atrophy followed a particular pattern in which epithelial sloughing and epithelial cell degeneration. Immature rabbit testes sections in G3 that receive two intramuscular injection of boldenone and dissected after 6 weeks showed vacuoles in the epithelium, epithelial gaps, cellular degeneration, nuclear pyknosis preceding degeneration and multi nucleated germ cells (Fig. 2C). Immature rabbit testes sections in G4 that
receive three intramuscular injection of boldenone and dissected after 9 weeks showed severe necrosis, with partial loss of the spermatogenic cells (Fig. 2D). Intensified sloughing of immature cells was frequently seen and the germ cell arrangement within the seminiferous tubules was disrupted. Also, vacuolization in germinal epithelium and wrinkled basement membrane were seen.

Figure 1. Photomicrographs of testicular sections from control and boldenone -treated groups mature rabbit showing: In control (A, G-1) the testis surrounded by tunica albuginea with tunica vasculosa and subdivided into lobuli testes containing seminiferous tubules. Note: Sertoli cells with basal, clear oval nucleus, spermatogenic cells namely; spermatogonia; primary spermatocytes, spermatids and spermatozoa. Interstitium between seminiferous tubules housing interstitial cells of Leydig. B–D Photomicrographs of seminiferous tubules of mature rabbits treated with boldenone showing cellular alterations at different spermatogenic stages. (B, G-2) Mature rabbit treated group showing: intraepithelial vacuoles of variable sizes. Primary spermatocyte nuclei dividing normally with condensed chromatin. Tubular lumen contained necrotic tissue. A few nuclei were pyknotic. In 5 mg/Kg boldenone (C, G-3) mature rabbit treated group showing: Separated and irregularly outlined seminiferous tubules, intratubular vacuolizations, germ cell loss and abnormal cell associations and germ cell maturation arrest were evident. Multinucleated giant cells were frequently found. In 5 mg/Kg boldenone (D, G-4) mature rabbit treated group showing: Edema of the interstitial tissue with congested tunica vasculosa and thickened tunica albuginea. Notice the empty seminiferous tubules from the developing sperms. Abbrev: tunica albuginea (T), tunica vasculosa (V), seminiferous tubules (st), spermatogonia (g), primary spermatocytes (P), spermatids (d) and spermatozoa (Z). Leydig (L), vacuoles (V), Scale bar= 50 μm.

Figure 2. Photomicrographs of testicular sections from control and boldenone -treated groups immature rabbit showing: In control (A, G-1) Section of testes from immature rabbit. The seminiferous tubular cells and interstitial tissue were normal with active spermatogenesis and prominent interstitial cellularity. In Section of testes from immature rabbit treated with boldenone (B, G-2) showed interstitial tissues showed edema, hemorrhage and vacuolation. Many seminiferous tubules were edematous with intact germinal layer and undergoing degeneration. In Section of testes from immature rabbit treated with boldenone (C, G-3). Mild interstitial edema, and the seminiferous tubules showed cellular degeneration and atrophy. In Section of testes from immature rabbit treated with boldenone (D, G-4), all seminiferous tubules showed severe degeneration along with loss of spermatogenesis or atrophy. Tubules also show missing germ cells in the epithelium. Note: that degenerating germ cells show nuclear pyknosis, wide gaps between neighboring cells with enlargement of the Intercellular spaces, restoration of spermatogenesis in most of the seminiferous tubules, necrotic germ cell sloughed out into the lumen of seminiferous tubules were seen. Abbrev: seminiferous tubules (st), spermatogonia (g), primary spermatocytes (P), spermatids (d), vacuoles (V), Scale bar= 50 μm.

Liver
The light microscopy examination of liver section in control mature and immature rabbits clearly illustrates complete hepatic lobules with well formed hepatocytes with distinct portal triads. Hepatic cells were arranged in cord like fashion, which are separated by sinusoids and central vein was seen clear (Figs. 3A, 4A). The histopathological examination of rabbit liver sections that treated with boldenone showed various histopathological changes,
these alternations were increased with the increase the boldenone dose injection. Mature rabbit liver sections in G2 that receive one intramuscular injection of boldenone and dissected after 3 weeks showed mild degree of hepatocytes degeneration with sinusoidal dilatation and congestion at lower doses (Fig. 3B). On the contrary, mature rabbit liver sections in G3 that receive two intramuscular injection of boldenone and dissected after 6 weeks revealed remarkable degenerative changes represented by diffuse disorganization of the hepatic cords and cytoplasmic vacuolization. Also, there are inflammatory cells infiltration within the portal areas had been observed (Fig. 3C). Mature rabbit liver sections in G4 that receive three intramuscular injection of boldenone and dissected after 9 weeks showed extensive liver injuries characterized by extensive hepatocellular necrosis, degeneration in hepatic plates and loss of cellular boundaries and massive degradation of central vein (Fig. 3D). The histopathological examination of immature rabbit liver sections that treated with boldenone showed the same histopathological changes that found in mature rabbits (Fig. 4B-D). Only in G4, liver sections showed a severe sinusoidal congestion. Single and more frequently multiple vacuoles were detected and vacuolar contents sometimes appeared more fibrillar (Fig. 4D).

Figure 3. Photomicrograph of rabbit liver stained with Hematoxylin and eosin. (A) the histoarchitecture of the liver is intact in controls with clear central vein. In Section of liver from mature rabbit treated with boldenone (B, G-2& C, G-3) showed widespread necrosis, disorganization of hepatic cords, cytoplasmic vacuolization beside Congested blood capillary with inflammatory cells infiltration. In Section of liver from mature rabbit treated with boldenone (D, G-4), the hepatic parenchyma showing severe hepatocytes degeneration with cytoplasmic vacuolization and dilated congested sinusoids.

Figure 4. Light microscopy of immature rabbit hepatic tissue (H&E stained hepatic sections, scale bar= 50 μm). (A,G-1), normal hepatic tissue structure; In Section of liver from immature rabbit treated with boldenone (B,G-2& C,G-3), slight congestion and degeneration (arrow) were observed; In Section of liver from immature rabbit treated with boldenone (D,G-4) hepatic tissue congestion, cellular swelling, cytoplasmic vacuolization, severe degeneration and necrosis were evident.
Kidneys

Kidneys of control mature and immature rabbits exhibited normal renal tissue, where normal glomeruli, tubular epithelium tissue were observed (Figs. 5A, 6A). The histopathological examination of mature and immature rabbit kidney sections that treated with boldenone showed various histopathological changes (Figs.1B-D, 2B-D), these alternations were increased with the increase the boldenone dose injection. Mature rabbit kidney sections in G₂ that receive one intramuscular injection of boldenone and dissected after 3 weeks showed Karyomegaly with eosinophilic intranuclear inclusions was abundant, glomerular damage and tubular necrosis with invading, inflammatory cells were also characteristic lesions (Fig. 5B). While in G₃, kidney sections revealed congestion, hemorrhages, and tubular degeneration (Fig. 5C). Mature rabbit kidney sections in G₄ that receive three intramuscular injection of boldenone and dissected after 9 weeks showed hyper-cellular, swollen and degeneration glomerular structures. The proximal tubules showed the most prominent alterations marked necrosis, parenchyma degeneration of the tubular epithelial cells (Fig. 5D). The histopathological examination of immature rabbit kidney sections that treated with boldenone showed the some histopathological changes (Fig. 6B-D), where the renal glomerulei were completely lost their typical shape with the appearance of some vacuoles of different shapes and sizes with markedly congested sinusoidal and dilated blood vessels were detected. These alternations were increased with the increase the boldenone dose injection (Fig. 6B-D).

Figure. 5. Light microscopy of kidney tissues from mature rabbit (H&E stained kidney sections, scale bar= 50 μm). (A) Control kidney section of rabbit showing (normal histology) normal glomeruli and normal tubules; (B, G-2) and (C, G-3) kidney section of renal boldenone showed tubular cell swelling, cellular vacualization, congestion, and cellular necrosis. In Section of kidney from mature rabbit treated with boldenone (D, G-4) glomerular structures were found to be hyper-cellular and swollen. Also, karyomegaly with eosinophilic intranuclear inclusions was abundant, severe vacuolar degeneration of tubules. Abbrev: glomerular(g), proximal tubules(p).

Figure.6. Light microscopy of kidney tissues from immature rabbit (H&E stained kidney sections, scale bar= 50 μm). In control (A, G-1) Normal, kidneys tissues showed normal glomeruli and tubular epithelium. In (B, G-2) and (C, G-3) kidney section of renal boldenone. Glomerular damage and tubular necrosis with invading inflammatory cells. Lots of renal tubular epithelial cells destructure and amotic, also, tubular congestion and welling were seen. In Section of kidney from immature rabbit treated with boldenone (D, G-4) show degenerated glomeruli, cortical renal tubules show various degenerative changes with focal tubular necrosis invaded by inflammatory. Abbrev: Glomerular(g), proximal tubules(p).
4. Discussion

Anabolic and androgenic steroids are synthetic substances related to the primary male sex hormone, testosterone. Their biological actions include anabolic effect promoting muscle growth, behavioral effect causing aggressiveness among others, and hematopoietic effect making them attractive candidates for enhancement of athletic performance (Hughes et al., 1995; Schänzer, 1996; Yesalis et al., 2000; Pey et al., 2003; Kicman, 2008). Yesalis et al. (1993) and Gabr et al. (2009) reported that in spite of the growth promoting effects, anabolic steroids have been shown adverse effects in cardiovascular, hepatic, renal and endocrine systems. Anabolic-androgenic steroids therapy is associated with various adverse effects that are generally dose related; therefore, illicit use of the high doses taken by sportsmen carries substantial risks for health. A major side effect of anabolic-androgenic steroids therapy is hepatotoxicity, including elevated levels of liver enzymes, cholestatic jaundice, peliosis hepatis, and various neoplastic lesions (Shahidi, 2001). Boldenone is an androgenic steroid that improves the growth and food conversion in food producing animals. Now, in most countries worldwide, this anabolic steroid is forbidden for meat production and human uses (Kuhn, 2002; Cannizzo et al., 2007; Soma et al., 2007) where it has a very long half-life and can show up on a steroid test for up to 1.5 years (Hoffmann, 2002; Brookhouse, 2007).

This study is the first to show the effect of boldenone on immature animals. The present results showed that intramuscular injection of rabbits with boldenone has a marked adverse effects on the liver, kidney and testes tissues and this effects were more observed in immature than in mature rabbits and this histopathological alternations were increased with the increase the boldenone dose injection. Our results showed that; immature rabbits that receive boldenone showed disturbances of the hepatocytes radially arranged cords with multifocal hepatocellular vacuolations in the liver, glomerulus mass reduction with multifocal glomerular injury in the kidney and disturbances of the cycle of spermatogenesis in the testes. On the other hand; this marked histopathological alternations not remarked in adult rabbits. Our results are in agreement with a number of recent studies which provided evidence that anabolic steroid causes an adverse effect on the human health (Hughes et al., 1995; Sullivan et al., 1998; Bahrke et al., 2000). Yesalis et al. (1993) and Gabr et al. (2009) reported that in addition to the growth promoting effects, anabolic steroids have been shown to adversely affect the cardiovascular, hepatic, and endocrine systems in mature animals.

Anabolic-androgenic steroids therapy is associated with various adverse effects that are generally dose related; therefore, illicit use of the high doses taken by sportsmen carries substantial risks for health. 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. Our results are in agreement with Groot and Biolatti, (2004) who reported that Boldenone induce similar lesions in the testes of Cattle. These results are in agreement with Veeramachaneni et al. (1988) who reported that zeranol and estradiol induce similar lesions in the testes and epididymides of the prepubertal beef bull. These findings suggested that misuse of growth promoter boldenone undecylenate may contribute to a continuously damage of the testicular function and structure that may lead to infertility especially in immature animals. These findings explain the common phenomena in young athletics and bodybuilders who suffer from infertility after maturation as they injected with some drugs as steroids (boldenone) to build muscles. In conclusion, using boldenone while preparing for a young bodybuilding contest may cause an alteration in the histological structure of the liver, kidneys and testes. These findings suggested that misuse of growth promoter Boldenone undecylenate may contribute to a continuously damage of the hepatic, renal and testicular function and structure that may lead to a hepatic, renal and genital progressive diseases so young people especially should be careful if they want to use such steroids to enhance their strength and endurance.

Corresponding author
Ehab Tousson
Zoology Department, Faculty of Science, Tanta University
toussonhab@yahoo.com

References


