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Effects of Short and Long Term Growth Promoter Boldenone Undecylenate Treatments on Antioxidant Enzyme Activities and Oxidative Stress Markers in Rabbit Muscles

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ABSTRACT

Background: Boldenone is developed for veterinary use and recently used by bodybuilders in both off-season and pre-contest where it is well known for increasing vascularity while preparing for a bodybuilding contest. So, the present study was designed to investigate the possible effect of growth promoter boldenone undecylenate on total antioxidant capacity and oxidative stress on muscular tissues (skeletal, cardiac and smooth muscles). **Methods:** Forty adult New Zealand rabbits were divided into four groups (10 animals each). Control group includes animals that injected intramuscularly with olive oil and dissected after 3 weeks. The experimental groups include animals that receive one, two and three intramuscular injections of 5 mg kg^{-1} body weight boldenone, respectively. The animals were dissected after 3, 6 and 9 weeks, respectively where the interval of each dose of boldenone was three weeks. **Results:** The animals from practice appeared healthy and did not show clinical signs of disease and no rabbit was dead during experiment. Total antioxidant capacity represented in superoxide dismutase (SOD) and reduced glutathione (GSH) in different muscle tissues showed a significant increased in the boldenone treated groups compared with their value in control group. And the oxidative stress parameters such as malondialdehyde (MDA), nitric oxide (NO), total lipid and total protein values showed a significant increased in the boldenone treated groups compared with their value in control group. These alternations were increased with the increase the boldenone dose injection from one dose to three doses, respectively. **Conclusion:** These findings explain the common phenomena in athletics and bodybuilders who suffer from muscles damage (rhabdomyolysis), atherosclerotic heart disease and myocardial hypertrophy as they injected with some drugs as steroids (boldenone) to build muscles.

Key words: Steroids, boldenone, rabbit, muscle tissues, total antioxidant capacity, oxidative stress

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INTRODUCTION

Anabolic androgenic steroids are used to enhance strength and endurance in canine, equine and human athletes through increasing muscle protein production (Schanzer, 1996). Certain veterinary products fall under this act and have been reclassified as Schedule III drugs. These include boldenone, mibolerone, stanozolol, trenbolone and trenbolone and their esters and isomers (Soma *et al*., 2007; Tousson *et al*., 2011; 2012).

Boldenone undecylenate is on of anabolic steroids, (synthetic androgen hormone) that derived from testosterone (Yesalis *et al*., 1993; Sullivan *et al*., 1998; Cannizzo *et al*., 2007; Soma *et al*., 2007; Tousson *et al*., 2012, 2013). Boldenone is well known under the trade names Equipoise, Ganabol, Equigan and Ultragan. They were developed mainly for veterinary use, mostly for the horse treatment (Yesalis *et al*., 2000; Soma *et al*., 2007). Recently, it used by bodybuilders in both off-season and pre-contest where it is well known for increasing vascularity while preparing for a bodybuilding contest. Boldenone 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 (Hoffmann, 2002; Brookhouse, 2007). 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.

Oxidative stress has been defined as a disturbance in the balance between antioxidants and prooxidants (free radicals and other reactive species) with increased levels of prooxidants leading to potential damage. This imbalance can be an effect of depletion of endogenous

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antioxidants, low dietary intake of antioxidants and/or increased formation of free radicals and other reactive species (Halliwell, 1997). In human tissues, a condition of oxidative stress can be revealed through searching for specific biomarkers of oxidative damage to lipids, proteins and nucleic acids (Zhang and Li, 2006). El-Moghazy *et al*. (2012) reported that; intramuscular injection of rabbits with boldenone increased total protein, total lipid, Nitric oxide (NO), superoxide dismutase activity (SOD), glutathione (GSH) and malondialdehyde (MDA) in liver and kidney tissues comparing with the control group. Upon the previous information, the aim of the present study was to investigate the possible effect of growth promoter boldenone undecylenate on total antioxidant capacity and oxidative stress on muscular tissues (skeletal, cardiac and smooth muscles).

MATERIALS 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 Dakhahleia governorate and Zoology Department, Faculty of Science, Tanta University, Egypt, during winter 2011.

Animals: The experiment was performed on 40 adult New Zealand rabbits weighing (3.65±0.35 kg) and of 9 months age. The animals were fed *ad libitum* pellets standard rabbit ration and free access to water. Animals were divided into four groups (10 animals each). Control group (G_1) includes animals that injected intramuscularly with olive oil. Groups 2, 3 and 4 include animals that receive one, two and three intramuscular injections of 5 mg kg^{-1} body weight boldenone undecylenate, respectively. Boldenone undecylenate was dissolved in the olive oil. The animals were dissected after 3, 6 and 9 weeks, respectively (El-Moghazy *et al*., 2012; Tousson *et al*., 2013). At the end of the experiment, the rabbits were fasted for 10 h then euthanized with intravenous injection with sodium pentobarbital and subjected to a complete necropsy.

Muscles (Cardiac, skeletal and smooth) 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 (NO) according to Vodovotz (1996); total lipids according to Esher *et al*. (1973); superoxide dismutase activity (SOD) according to Oyanagui (1984); glutathione (GSH) according to Beutler *et al*. (1963) and lipid peroxidation (malondialdehyde; MDA) according to Lahouel *et al*. (2004).

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

The animals from practice appeared healthy and did not show clinical signs of disease and no rabbit was dead during experiment. As represented in Table 1, there was a significant increase in cardiac total protein, total lipid, superoxide dismutase (SOD), malondialdehyde (MDA), Nitric oxide (NO) and glutathione (GSH) in boldenone treated rabbit (G_2-G_3) as compared to control (G_1). These changes in total antioxidant capacity and the oxidative stress parameters were increased with the increase the boldenone dose injection from one dose to three doses (G_2 and G_4), respectively. On the other hand there was a significant increase in cardiac SOD, MDA and nitric oxide and a non significant change in cardiac total protein, total lipid and GSH as compared to G_2. In G_4, there was a significant increase in cardiac total protein, total lipid, SOD, MDA, NO and GSH as compared to control and G_2. On the other hand there was a significant increase in cardiac total lipid, MDA and GSH and a non significant change in cardiac total protein, SOD and NO as compared to G_3.

Table 2 showed that, a significant increase in smooth total protein, SOD, MDA, NO and GSH in one dose treated group (G_2-G_4) as compared to control group, meanwhile smooth total lipid showed non significant change as compared to G_1. These changes were increased with the increase the boldenone dose injection from G_2 and G_4, respectively.

As represented in Table 3, there was a significant increase in skeletal total protein; total lipid, SOD, MDA and GSH in G_2 as compared to control group, meanwhile skeletal NO showed non significant change as compared to G_1. In G_3, there was a significant increase in skeletal total protein, SOD, MDA, NO and GSH as compared to control group, meanwhile skeletal total lipid showed non significant change as compared to G_1. On the other hand there was a significant increase in skeletal total protein, SOD, MDA and GSH and a non significant change in skeletal total lipid and NO as compared to G_2. In G_4, there was a significant increase in skeletal total protein, total lipid, SOD, MDA and GSH as compared to G_1 and G_2 except for skeletal NO which show a non significant change as compared to G_1 and G_2. These changes in total antioxidant capacity and the oxidative stress parameters were increased with the increase the boldenone dose injection from one dose to three doses (G_2 and G_4), respectively.

Table 1: Changes in total protein, total lipid, superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and glutathione (GSH) levels in cardiac muscle homogenates in different groups under study

Groups	Total protein (mg g^{-1})	Total lipid (μg g^{-1})	SOD (U mg^{-1})	MDA (nmol g^{-1} tissue)	NO (μM)	GSH (mmol g^{-1} tissue)
G_1						
Control						
Range	201-219	0.109-0.119	33-35	41.8-44.3	51.2-54.1	0.037-0.044
Mean±SEM	210.8±3.4	0.114±0.002	34.14±0.37	43.02±0.48	52.86±0.52	0.041±0.001
G_2						
One dose						
Range	350-373	0.119-0.127	44.7-48.4	75-78.3	54.9-58.1	0.046-0.051
Mean±SEM	360.8±3.88	0.122±0.0015	46.56±0.7	76.54±0.67	56.1±0.59	0.048±0.0.001
p^a	<0.001	<0.05	<0.001	<0.001	<0.01	<0.01
G_3						
Two dose						
Range	375-478	0.118-0.125	51.9-54.6	104.5-112.4	58.7-61.1	0.048-0.057
Mean±SEM	400.2±19.58	0.1214±0.0013	53.2±0.45	107.9±1.42	60.12±0.4	0.051±0.002
p^a	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001
p^b	NS	NS	<0.001	<0.001	<0.001	NS
G_4						
Three dose						
Range	409-429	0.129-0.14	53.3-58.1	182.9-200.3	59.8-63	0.055-0.063
Mean±SEM	418.8±3.67	0.134±0.002	55.4±0.9	190.7±3.12	616.±0.54	0.059±0.001
p^a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
p^b	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001
p^c	NS	<0.001	NS	<0.001	NS	<0.01

pa: Value vs. control group (I), pb: Value vs. one dose group (II), pc: Value vs. two dose group (III)

Table 2: Changes in total protein, total lipid, superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and glutathione (GSH) levels in smooth muscle homogenates in different groups under study

Groups	Total protein (mg g^{-1})	Total lipid (μg g^{-1})	SOD (U mg^{-1})	MDA (nmol g^{-1} tissue)	NO (μM)	GSH (mmol g^{-1} tissue)
G_1						
Control						
Range	198-214	0.116-0.21	31-33.7	34.5-41.1	50.9-52.1	0.035-0.042
Mean±SEM	207.6±2.84	0.137±0.018	32.14±0.43	37.4±1.2	51.3±0.21	0.039±0.001
G_2						
One dose						
Range	345-359	0.12-0.15	43.9-45.9	67.3-72.3	52.9-55.4	0.042-0.044
Mean±SEM	352.6±2.5	0.132±0.005	44.9±0.33	69.9±0.86	54.1±0.46	0.043±0.0.0003
p^a	<0.001	NS	<0.001	<0.001	<0.001	<0.05
G_3						
Two dose						
Range	434-477	0.112-0.142	48.6-51	94.1-101	58.1-59.5	0.045-0.049
Mean±SEM	462.2±7.72	0.127±0.005	50.1±0.44	97.92±1.14	58.9±0.27	0.047±0.0007
p^a	<0.001	NS	<0.001	<0.001	<0.001	<0.001
p^b	<0.001	NS	<0.001	<0.001	<0.001	NS
G_4						
Three dose						
Range	400-430	0.136-0.148	50-51.2	168-185.2	58-60.1	0.047-0.052
Mean±SEM	412.4±5.1	0.142±0.002	50.6±0.23	177.2±3.59	59.2±0.42	0.049±0.001
p^a	<0.001	NS	<0.001	<0.001	<0.001	<0.001
p^b	<0.001	NS	<0.001	<0.001	<0.001	<0.001
p^c	<0.001	NS	NS	<0.001	NS	NS

pa: Value vs. control group (I), pb: Value vs. one dose group (II), pc: Value vs. two dose group (III)

Table 3: Changes in total protein, total lipid, superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and glutathione (GSH) levels in skeletal muscle homogenates in different groups under study

Groups	Total protein (mg g^{-1})	Total lipid (μg g^{-1})	SOD (U mg^{-1})	MDA (nmol g^{-1} tissue)	NO (μM)	GSH (mmol g^{-1} tissue)
G_1						
Control						
Range	213-221	0.106-0.115	28.7-33.2	24.1-29.7	45.8-48.9	0.03-0.035
Mean±SEM	217±1.4	0.11±0.002	30.8±0.83	26.7±1.0	47.2±0.55	0.033±0.001
G_2						
One dose						
Range	355-376	0.115-0.123	41.6-45.1	54.1-62.1	45.7-55.5	0.0367-0.039

Table 3: Continue

Groups	Total protein (mg g^{-1})	Total lipid (μg g^{-1})	SOD (U mg^{-1})	MDA (nmol g^{-1} tissue)	NO (μM)	GSH (mmol g^{-1} tissue)
Mean±SEM	366.6±4.0	0.12±0.001	43.1±0.63	58.9±1.4	51±1.6	0.038±0.0.0004
p[a]	<0.001	<0.01	<0.001	<0.001	NS	<0.001
G_3						
Two dose						
Range	466-480	0.112-0.119	47.3-52.6	84.3-90.3	46.7-59.7	0.042-0.045
Mean±SEM	473.6±2.7	0.116±0.001	49.96±0.9	87.9±1.1	55.9±2.35	0.0435±0.0005
p[a]	<0.001	NS	<0.001	<0.001	<0.05	<0.001
p[b]	<0.001	NS	<0.001	<0.001	NS	<0.001
G_4						
Three dose						
Range	413-432	0.124-0.132	47.5-50.7	138.3-167.4	55.9-57.8	0.043-0.0454
Mean±SEM	423.2±3.4	0.13±0.001	49.2±0.53	150.8±4.9	56.7±0.34	0.044±0.0004
p[a]	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001
p[b]	<0.001	<0.001	<0.001	<0.001	NS	<0.001
p[c]	<0.001	<0.001	NS	<0.001	NS	NS

pa: Value vs. control group (I), pb: Value vs. folic acid group (II), pc: Value vs. hypothyroid group (III)

DISCUSSION

The abuse of anabolic-androgenic steroids to enhance physical performance is widespread in sport communities despite their reported side effects. Biochemical analysis of the rabbit's muscles tissues after boldenone injection with different doses showed a significant increase in total protein, total lipid, NO, SOD, GSH and MDA comparing with the control group. In the present study, there is a significant increase in the total NO_x (NO metabolites) in rabbits that injected 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 rabbit vessels which react with nitric oxide to form peroxynitrite radicals, leading to low NO bioavailability and endothelial dysfunction (Ungvari *et al*., 2003). Fahey (1998) reported that the mechanism of action of boldenone undecylenate steroid hormones work by stimulation of receptors molecule in muscle cells, which activate specific genes to produce proteins. They also affect the activation rate of enzyme system involved in protein mechanism. Thus enhancing protein synthesis and inhibiting protein degradation. Effectiveness of anabolic steroids is dependent upon unbound receptor sites in muscle.

Injection of the anabolic steroid boldenone (5 mg kg^{-1} body weight) induced changes in oxidative stress biomarker levels and antioxidant defense systems in skeletal, cardiac and smooth muscles. Boldenone treatment can cause an oxidative stress situation in muscles as indicated by enhanced MDA, SOD and GSH extent. Our results showed that, significant increases in the activity of MDA, SOD and GSH in muscles tissues after boldenone injections in G_2, G_3 and G_4 comparing with the control ($p<0.05$). These changes in total antioxidant capacity and the oxidative stress parameters were increased with the increase the boldenone dose injection from one dose to three doses (G_2 and G_4) respectively. Mitochondria might be an important cellular target for oxidative damage since the mitochondrial membrane is rich in polyunsaturated fatty acids; alteration in the lipid environment of respiratory chain complexes may cause a decrease in their activity leading, in turn, to a perturbation of energetic metabolism. Our results are in agreement with Pey *et al*. (2003) who reported that the anabolic-androgenic steroids induced changes in oxidative stress. Our results are in agreement with Urhausen *et al*. (2003) in athletes abusing anabolic androgenic steroids and not agreed with Istasse *et al*. (1988) who, reported that, estradiol 17β increased nitrogen retention and decreased blood urea nitrogen concentrations. Our results are in agreement with Gabr *et al*. (2009) who reported that in addition to the growth promoting effects, anabolic steroids have been shown to adversely affect the cardiovascular and endocrine systems. Also, Gabr *et al*. (2009) and Tousson *et al*. (2013) reported that the total protein concentrations in male lambs were significantly increased after boldenone injections. Oxidative stress has been also recently implicated in hormone-induced prostate carcinogenesis (Tam *et al*., 2003). Thus, it is tempting to speculate with the possibility that the observed changes in prooxidant/antioxidant status could be causally related with the adverse effects of Anabolic-androgenic steroids on muscle tissues.

CONCLUSION

Using boldenone while preparing for a bodybuilding contest may cause an alteration in the biochemical parameters of the rabbit muscles. These findings explain the common phenomena in athletics and bodybuilders who suffer from muscles damage

(rhabdomyolysis), atherosclerotic heart disease and myocardial hypertrophy as they injected with some drugs as steroids (boldenone) to build muscles.

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