

## Oral supplementation of aqueous moringa and ginkgo leaf extracts abates oxidative stress and testicular injury associated with boldenone injection in rats

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**Abstract:** Because of their muscle-building and growth-enhancing properties, anabolic androgenic hormones are still illegally administered to food-producing animals. Boldenone is an anabolic androgenic synthetic commercial steroid; that is derived from testosterone which exhibits strong anabolic and moderately androgenic properties. The present study was designed to investigate the possible effect of moringa leaf extract (MLE) and ginkgo leaf extract (GLE) on the changes in the structure and functions of rat testes after boldenone injections. A total of 48 male albino rats weighing 170-200 g and of 10-12 weeks age were divided into 6 groups (8 animals each). 1<sup>st</sup> Control group includes rats that injected intramuscularly with olive oil. 2<sup>nd</sup> and 3<sup>rd</sup> groups were animals received MLE (intragastrically, 200 mg/kg body weight) and GLE (intragastrically, 200 mg/kg body weight) respectively. 4<sup>th</sup> experimental group include rats that received intramuscular injections of boldenone undecylenate at (5 mg/Kg body weight) for 12 weeks; 5<sup>th</sup> and 6<sup>th</sup> groups where animals intramuscular injected with boldenone and treated with MLE or GLE at the same time respectively. The obtained results indicate that testosterone, testicular MDA and nitric oxide were significantly increased in boldenone group when compared with control group, also, catalase, super oxide dismutase and total thiol in testicular tissue were significantly decreased in boldenone group when compared with control group. The current results indicate that boldenone causes oxidative tissue damage by increasing lipid peroxidation in the testicular tissues and decreasing the level of antioxidant enzymes. Moreover, increased testosterone levels with biochemical indicators of testicular damage, and histopathological and cytokeratine immunoreactivity alterations supported this conclusion. It has also been shown that the co-treatment with GLE or with MLE provided significant prophylactic treatment from the testicular damage of boldenone.

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**Key words:** Steroid hormones; Boldenone; *Moringa Oleifera*; *Ginkgo Biloba*; Testes; Oxidative stress; Antioxidants; Rats.

**Abbreviations:** AAS, Anabolic androgenic steroids; MLE, Moringa leaf extract; GLE, Ginkgo leaf extract; SOD, super oxide dismutase; MDA, Malondialdehyde; NO, Nitric oxide; TP, Total protein.

### I: Introduction

Synthetic derivatives and active metabolites of the male testosterone are called Anabolic androgenic steroids (AAS) which originally designed for therapeutic uses to provide enhanced anabolic potency with negligible androgenic effects.<sup>1,2</sup> Anabolic-androgenic steroids caused some adverse effects such as disturbance of the endocrine and immune function, alterations of sebaceous system and skin changes of hemostatic system and urogenital tract.<sup>3-5</sup> Anabolic androgenic steroids increase protein synthesis within cells, which results in the buildup of cellular tissue (anabolism), especially in muscles. Boldenone (1, 4-androstadiene-17-ol-3-one; BOL) is an anabolic androgenic synthetic commercial steroid; Boldenone is derived from testosterone which exhibits strong anabolic and moderately androgenic properties and it has a very long half-life and can show up on a steroid

test for up to 1.5 years.<sup>4,6,7,11</sup> 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 were treated with boldenone.<sup>10-12</sup> There is strong indication that the duration, dosage, and chemical structure of the anabolic steroids are important for the serum concentration gonadotropins<sup>9</sup>.

The *Ginkgo biloba* tree is the oldest tree on earth: more than 200 million years old which is called the "living fossil".<sup>13</sup> It is a mixture containing 240 mg/g Ginkgo flavone glycoside (quercetin, kaempferol, isorhamnetin) and 60 mg/g terpene lactones (ginkgolides, bilobalide), those are the most important active ingredients in the extract.<sup>14</sup> Ginkgo leaf extract (GLE) from natural sources may reduce the risk of toxicity and preserve the therapeutic effectiveness in clinical trials and in recent years.<sup>15</sup> Important proteins

with functional properties have been isolated from *Ginkgo biloba* seeds.<sup>16</sup>

*Moringa oleifera*, an edible tree found worldwide in the dry tropics which is increasingly being used for nutritional supplementation.<sup>17</sup> *Moringa* has long been recognized in traditional medicine worldwide as having value both as a preventative and treatment agent of several health conditions, including the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, haematological and hepatorenal disorders.<sup>18</sup> *Moringa* leaves can be a good source of natural antioxidants.<sup>19</sup> The crude extract of phenolic compounds was obtained from *Moringa oleifera* Lam. The content of total phenolics in the *Moringa oleifera* extract is 118mg/ g. The extract of *Moringa oleifera* also has strong antiradical activity; Phenolic acids (derivatives of caffeic, p-coumaric and ferulic acids) are the dominant phenolic constituents of *Moringa oleifera* leaves extract.<sup>20</sup> Tousson et al.<sup>9</sup> and Tousson<sup>11</sup> explain the common phenomena in athletics and bodybuilders who suffer from infertility as they injected with some drugs as steroids (boldenone) to build muscles. Therefore, the aim of the present study was to investigate the possible prophylactic effect of GLE and MLE on the toxicity of the rat testes by boldenone injections.

## II. Materials and methods

### II.1: Animals

The experiments were performed on 48 male albino rats weighing  $180 \pm 20$ g and of 10-12 week's age. They were obtained from animal house of National Research Center, Dokki, Giza, Egypt. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (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 water available *ad libitum*. The temperature in the animal room was maintained at  $23 \pm 2^\circ\text{C}$  with a relative humidity of  $55 \pm 5\%$ . Light was on a 12:12 hr light -dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research.

### 2.2 Animal Treatments

Animals were divided into 6 groups (8 animals each). 1<sup>st</sup> Control group includes rats that injected intramuscularly with olive oil for 12 weeks. 2<sup>nd</sup> and 3<sup>rd</sup> groups were animals received MLE (intragastrically, 200 mg/kg body weight) and GLE (intragastrically, 200 mg/kg body weight) respectively. 4<sup>th</sup> experimental group include rats that received intramuscular injections of boldenone undecylenate at (5 mg/Kg body weight) for 12 weeks; 5<sup>th</sup> and 6<sup>th</sup> groups where animals intramuscular injected with boldenone and

treated with MLE and GLE at the same time respectively.

At the end of the experiment, the animals were fasted for 10 hours and then euthanized with intraperitoneal injection of sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes. Blood serum was separated by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at  $-18^\circ\text{C}$ .

### II.2: Methods:

**II.2.1: Blood samples:** Blood serum was analyzed to determine the concentration of testosterone using commercial kit that was supplied by Diagnostic Systems Laboratories (DSL), from Texas, USA.<sup>21</sup>

**Preparation of testicular homogenates:** Specimens were separated, weighed and homogenized separately with a 3 Potter Elvehjem tissue homogenizer. One part was homogenized in phosphate buffer (pH 7.0) for estimation of protein content and CAT enzymes activities levels; the second was 10% w/v heart homogenate in ice-cold saline for estimation of MDA, Total thiol, GSH, SOD and NO activity. The crude tissue homogenate was centrifuged at 11,739 g, for 15 minutes in a cold centrifuge, and the resultant supernatant was used for different estimations.

**2.4. Total protein:** Total protein content in tissue homogenate was measured according to the method of Lowry et al.<sup>22</sup>

### 2.5. Enzymatic and non-enzymatic antioxidant assays

**MDA assay:** Malondialdehyde (MDA), a noxious product of lipid peroxidation, was detected by TBARS analysis and measured as reported by Saggu et al.<sup>23</sup> The MDA results were expressed as the nmol/mg protein.

**Total thiol assay:** Total thiol was performed by the method of Sedlak and Lindsay [24] that modified by Chattopadhyay.<sup>25</sup>

**Catalase:** The catalase (CAT) activity was measured by monitoring  $\text{H}_2\text{O}_2$  (The substrate of the enzyme) decomposition at 240 nm according to the method described by Aebi.<sup>26</sup>

**Super oxide dismutase (SOD):** SOD activity was determination according to the method of Nishikimi et al.<sup>27</sup>

**Nitric oxide:** The Nitric oxide (NO) activity was measured by colorimetric method, Kits supplied by Bio-diagnostic, Egypt at 540 nm. By colorimetric method, Kits supplied by Bio-diagnostic.<sup>28</sup> In acid medium and in the presence of nitrite the formed nitrous acid diazotize suphanilamide and the product are coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish-purple color. Nitric oxide is expressed in sample as ( $\mu\text{mo}/\text{L}$ ).

### II.3.3.1: Histological investigation:

Three rats from each group were used for the histopathological and immunohistochemical studies. Testes were immediately removed from the dissected rats and divided transversely into two parts and immediately one part fixed by immersion in 10% buffered formalin solution and left for 24–48 h. The specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5 mm thick were cut using mean of rotary microtome and stained with haematoxylin and eosin (H&E) according to the method of Bancroft and Cook.<sup>29</sup>

#### Immunohistochemical detection of cytokeratin expression:

Expression of cytokeratin immunoreactivity (CK-ir) was detected using avidin Biotin Complex (ABC) method.<sup>30</sup> Paraffin sections (5µm thick) of fixed rat testes that mounted on gelatin chromalum-coated glass slides were dewaxed and rehydrated sections were washed in distilled water for 5 min, rinsed in PBST for 10 min and incubated with 10% normal goat serum for 15 min to reduce non-specific background staining. Then, the sections were incubated with anti-mouse CK monoclonal antibody (DACO Corporation, Carpinteria, CA, USA) diluted up to 1:100 and kept for 12 h at 4°C. The sections after 5 baths in PBST were incubated with biotinylated goat antimouseimmuoglobulin (Nichirei, Tokyo, Japan). The sections after 5 baths in PBST were further incubated with Avidin Biotin Complex (ABC: Nichirei, Tokyo, Japan) for 1 hour at room temperature.

The reaction was developed by using 20 mg 3-3'-diaminobenzidine tetrahydrochloride (DAB, Wako pure chemical industries, Ltd) in 40 ml PBST, pH 7.2 containing 10 ml of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 7-9 min at a dark room followed by distilled water then dehydrated and mounted. The criterion for a positive reaction confirming the presence of CK is a dark, brownish, intra cytoplasmic precipitate. For the negative control, the primary antibody was omitted to guard against any false positive results which might develop from a non-specific reaction.

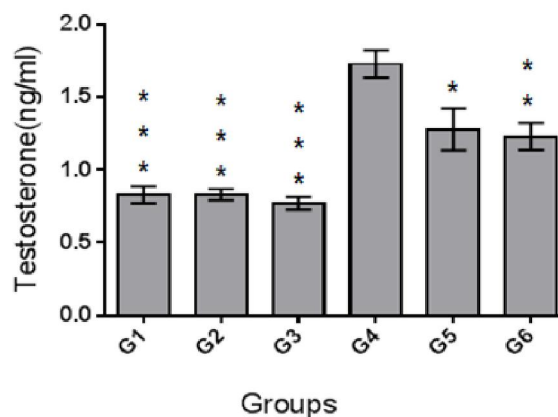
Brightness, contrast were adjusted using Adobe Photoshop software. Image analysis was adjusted using PAX-it image analysis software. The data was statistically analyzed using SPSS statistical version 16 software package (SPSS<sup>®</sup> Inc., USA).

#### II.4: Statistical Analysis:

Data were expressed as mean values+SE, and statistical analysis was performed using T test unpaired to assess the significant differences among the 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, Montreal, Canada).

#### III: Result:

The animals from practice appeared healthy and did not show any clinical signs of disease. Figure 1 showed the changes in serum testosterone concentration in different group under study. Serum testosterone concentration significantly increases in boldenone rat group when compared with control, MLE and GLE groups. On the other, treatment with MLE or GLE significantly decreases the testosterone levels when compared with boldenone group. Also Co-treatment of boldenone with GLE revealed in significant decrease in testosterone levels when compared with Co-treatment of boldenone with MLE.

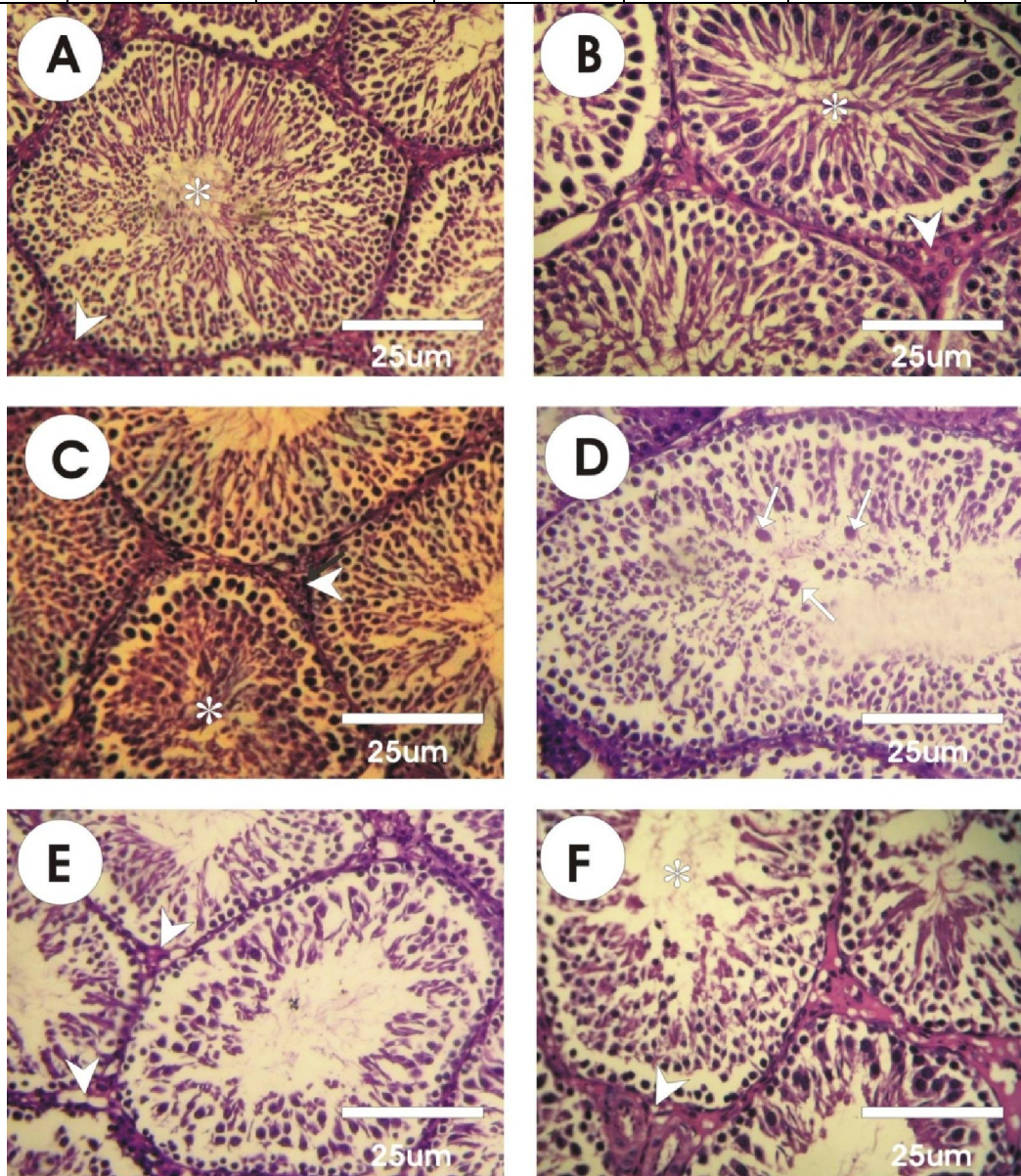


**Figure (1):** Changes in the concentration of serum testosterone in different groups under study. where G1, control group; G2, MLE group; G3, GLE group; G4, boldenone treated group; G5, Co-treated rats with MLE; G6, co-treated rats with GLE. Values are expressed as means  $\pm$  SE. T test was significant at  $P < 0.05$ . Dunnett test was significant from corresponding boldenone group value at \* $P < 0.05$ , \*\* $P < 0.01$ , and\*\*\* $P < 0.001$ .

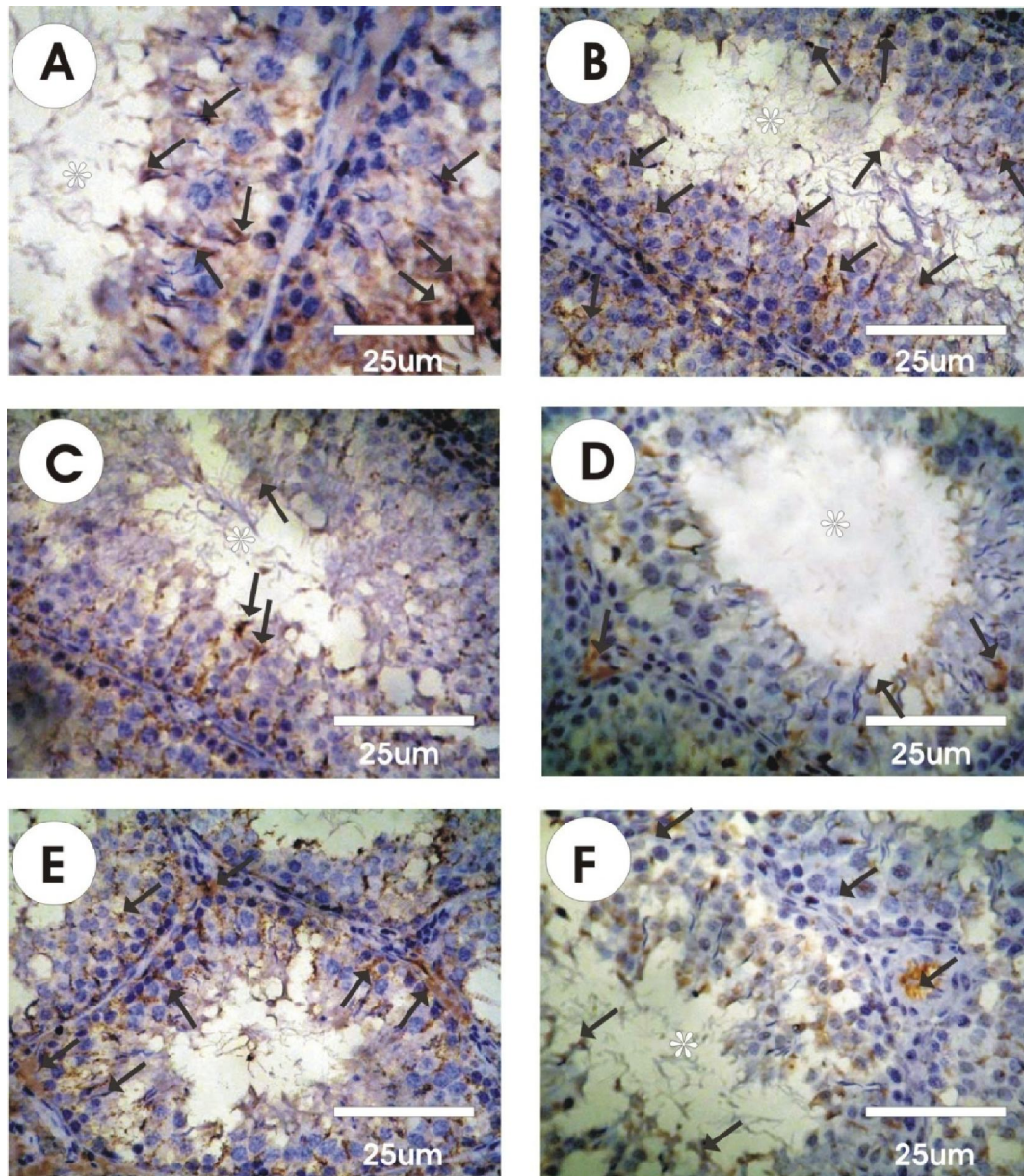
Table 1 showed that significantly increase in NO and MDA in boldenone group when compared with control, MLE, and GLE groups. On the other hand, NO and MDA showed decreased in co treated MLE or with GLE groups when compared with boldenone group. Catalase, SOD, total thiol, and total protein levels in boldenone group showed significantly decrease ( $p < 0.05$ ) when compared with control, MLE and GLE groups. On the other hand, catalase, SOD, total thiol, and total protein showed increased levels in co treated animals with MLE or with GLE groups when compared with boldenone group. Also, Table 1 showed that, Co-treatment of boldenone with GLE or with MLE revealed good improvement in enzymatic and non-enzymatic antioxidant results and there is no significant difference between their results.

**Table 1:** Changes in the NO (nmol/g), SOD (U/mg), catalase (U/mg), Total thiol (nmol/mg), total protein (g/dl) and MDA (nmol/mg) levels in testicular tissues in different groups under study.

Item	G1	G2	G3	G4	G5	G6
NO	115.22± 2.74**	121.01±2.7**	112.52± 4.73**	145.64± 2.95	132.0±1.91*	133.41± 2.96*
SOD	5.49± 0.42*	5.43± 0.33**	5.93± 0.57*	1.75± 0.18	3.34± 0.22*	3.37± 0.17 *
Catalase	64.50± 3.78 *	67.33± 6.12*	64.67± 3.93*	43.51± 4.04	55.83± 1.65*	55.70± 1.43*
Total thiol	2047±9.02 **	2055± 8.67**	2012± 4.40**	157± 89.40	1896±61.233*	186±31.67 *
Total protein	5.630± 0.37*	5.57± 0.24*	5.97± 0.29**	3.96± 0.31	5.11± 0.23*	5.17±0.25*
MDA	1.35± 0.15**	1.08± 0.08***	1.45± 0.15 **	2.20±0.11	1.847±0.022*	1.83± 0.023 *



**Figures 2:** Photomicrographs of rat testes sections stained by HE. **A-C:** Rat testes in control, MLE and GLE groups revealed structure of seminiferous tubules with regular cycle of spermatogenesis and the lumen of seminiferous tubules were fully packed with sperms (stars). **D:** Testes sections in boldenone rats group showed abnormal distribution of spermatocytes in the lumina of the seminiferous tubules, severe necrosis, a significant decrease in the number of spermatogenic cells with the presence of many syncytial cells in the seminiferous tubules and an increased in the seminiferous tubules lumen with lack of sperms. **E:** Testes sections in Co-treated boldenone with MLE showed mild improvement with mild normal regular distribution in spermatogenesis cycles with increased in the sperm. **F:** Testes sections in Co-treated boldenone with GLE showed moderate improvement with normal regular distribution in spermatogenesis cycles with increased in the sperm numbers and Leydig cells.



**Figures 3:** Photomicrographs of cyokeratine immunoreactivity (ck-ir) in the cross sections of rat testes. **A-C:** Moderate positive reaction for ck-ir (arrows) in the seminiferous tubules of control (A), MLE (B) and GLE (C) groups respectively). **D:** A mild positive reactions for ck-ir (arrows) were detected in the testis sections in boldenone group. **E&F:** Moderate positive reaction for ck-ir (arrows) in the testes in co-treated boldenone with GLE. **F:** Mild positive reaction for ck-ir (arrows) in the testes in co-treated boldenone with MLE.

The significance of difference was analyzed by T test and Dunnett test (compare all vs. boldenone group) using computer program. Values are expressed as means  $\pm$  SE. T test was significant at  $P < 0.05$ . Dunnett test was significant from corresponding boldenone group value at  $*P < 0.05$ ,  $**P < 0.01$ , and  $***P < 0.001$ , where G1, control group; G2, MLE group; G3, GLE group; G4, boldenone treated group; G5, Co-treated rats with MLE; G6, co-treated rats with GLE.

#### Histological investigation:

Histopathological study in control, MLE and GLE groups revealed regular cycle of spermatogenesis (Figs.1A-1C). The structural components of the testis are the seminiferous tubules and interstitial tissues (Leydig cells). Two types of cells are identified in rat seminiferous tubules, the Sertoli cells and the spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms). The Sertoli cells, rest on the thin basal

lamina (basement membrane) while the spermatogenic cells are arranged in many layers, namely, the spermatogonia, primary and secondary spermatocytes; spermatoids and finally mature spermatozoa (Fig. 1A).

Figure (1D) revealed various histopathological changes in the testes sections in boldenone group as disturbance and abnormal distribution of spermatocytes in the lumina of the seminiferous tubules, severe necrosis, severe marked degeneration in most of seminiferous tubules; a significant decrease in the number of sperms with the presence of many syncytial cells, a significant decrease in the numbers of Leydig cells (Fig. 1D).

On the other hand; testes sections in Co-treated boldenone with MLE showed mild improvement with mild normal regular distribution in spermatogenesis cycles, mild atrophy and an increased in the sperm numbers were observed (Fig. 1E). Testes sections in Co-treated boldenone with GLE showed moderate improvement with normal regular distribution in spermatogenesis cycles with an increased in the sperm and Leydig cells (Fig. 1F).

#### **Effect of MLE and GLE on testis cytokeratine immunoreactivity:**

The detection and distribution of cytokeratine immunoreactivity (CK-ir) in testis sections in the different groups under study were revealed in Figure 3. Testis sections in control, MLE and GLE groups showed moderate positive reaction for ck-ir (grade 3, 3 & 3 respectively) were detected (Figs. 3A-3C) while a mild to negative reactions for ck-ir (grade 1) were detected in the testis sections in boldenone group (Fig. 3D). On the other hand; moderate positive reaction for ck-ir (grade 3) were detected in the testes sections in co-treated boldenone with GLE (Fig. 3E). while a mild positive reaction for ck-ir (grade 2) were detected in the testes sections in co-treated boldenone with MLE (Fig. 3F).

#### **IV. Discussion:**

The obtained results indicate that; intramuscular injection of rats with boldenone adversely affects spermatogenesis, suggesting that anabolic-androgenic steroid hormone might play an important role not only in controlling normal testicular development, but also in maintaining normal testicular function and spermatogenesis.

Our results showed that, a significant increase in the concentration of plasma testosterone after boldenone injections comparing with the control. In agreement with our findings, serum testosterone levels in treated groups with anabolic-androgenic steroid were significantly higher than that in control group.<sup>1,9,31,32,33</sup> In contrast, the administration of testosterone alone did not induce any variation in plasma testosterone.<sup>34</sup> Also, Shimomura et al.<sup>35</sup>

showed that the treatment of rats with ethinyl estradiol alone significantly decreased testosterone levels in serum and the testis.

The present results showed that intramuscular injection of rats with boldenone has marked adverse effect on the testes as well as the effect of GLE and MLE therapy which the testosterone had a significant decreased in control, MLE, and GLE groups when compared with its value in boldenone treated group. Also, testosterone in boldenone group showed increased level when compared with co treated groups with MLE and GLE; there is strong indication that the duration, dosage, and chemical structure of the anabolic steroids are important for the serum concentration gonadotropins. A moderate decrease of gonadotropin secretion causes atrophy of the testes, as well as a decrease of sperm cell production.<sup>10</sup> Oligo, azoospermia and an increased number of abnormal sperm cells have been reported in athletes using anabolic steroids use, the gonadal functions will restore within some months.

Our results are in agreement with Tousson et al.<sup>9</sup> who reported that intramuscular injection in rabbits with boldenone adversely affects spermatogenesis, suggesting that anabolic-androgenic steroid hormones might play an important role not only in controlling normal testicular development.

Spermatozoa, like any other aerobic cell, are constantly facing the "oxygen-paradox"; the excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and contaminating leukocytes has been defined as one of the few etiologies for male infertility.<sup>36</sup> There are two main mechanisms by which reactive oxygen species (ROS) cause infertility. First, ROS damage the sperm membrane, which in turn, reduces sperm motility and their ability to fuse with the oocyte. Second, ROS directly damage sperm DNA, compromising the paternal genomic contribution to the embryo.<sup>37</sup>

Oxidative stress or oxidative cellular damage with its dual of free radical generation and profound lipid peroxidation are hallmarks of boldenone toxicity.<sup>2</sup>

The current results indicate that boldenone causes oxidative tissue damage by increasing lipid peroxidation in the testicular tissues and decreasing the level of antioxidant enzymes. Moreover, increased testosterone levels with biochemical indicators of testicular damage, and histopathological and cytokeratine immunoreactivity alterations supported this conclusion. It has also been shown that the co-treatment with GLE or with MLE provided significant prophylactic role from the testicular damage of boldenone.

The obtained results in agreement with Grigorov<sup>38</sup> and Ali et al.<sup>2</sup> who reported that although the human

body continuously produces free radicals, it possesses several defense system, which are constitutes of enzymes and radical scavengers such as superoxide dismutase, catalase and glutathione peroxidase while non-enzymatic category contains vitamin C, E, A,  $\beta$ -carotenoids, uric acid and ubiquinone. These are called “first line antioxidant defense system” but are not completely efficient because almost all components of living bodies, tissues and cells undergo free radical destruction.

GSH is considered to be one of the most very important components of the antioxidant defense of living cells. The reduced tri-peptide GSH is a hydroxyl radical and singlet oxygen scavenger, and participates in a wide range of cellular functions.<sup>39</sup> In the current study, GSH were significantly decrease in the testicular tissues after boldenone injection when compared with control group, also co-treatment with GLE or with MLE exhibited anti-oxidant effects not only on the non enzymatic defense system (GSH), but also on the enzymatic one such as catalase.

Our results showed that GLE has been widely used in activation of the antioxidant enzymes together with the substances that are capable of either reducing reactive oxygen species or preventing their formation, form a powerful reducing buffer, thereby form the protective mechanisms, which maintain the lowest possible levels of reactive oxygen species inside the cell. Our results indicate that rats were received intragastrically with GLE have elevated levels of catalase and SOD activity comparing with the rats that were intramuscular injected with boldenone only.

Our results are in agreement with Furman [40] who said that GLE can inhibit membrane lipid peroxidation by its antioxidant activity which was observed in the decreasing of MDA in the co-treated groups with MLE and GLE comparing to boldenone group. The present results indicate that decreasing in NO in boldenone group comparing with GLE treated group which come in agreement with Sahoo et al.<sup>41</sup> who said that GLE has inhibitory effect on nitric oxide.

Catalase, SOD, and total thiol levels in boldenone group showed significantly decrease ( $p < 0.05$ ) when compared with control group. On the other hand, Catalase, SOD, and total thiol showed increased levels in co treated group with MLE when compared with boldenone treated group.

Our results are in agreement with Owusu-Ansah et al.<sup>42</sup> who find that, MLE contain high total phenolic content and they suggested that the high total phenolic contents are responsible for the corresponding high antioxidant activities of the dried leaf samples of the *Moringa* accessions and hence support the acclaimed medicinal value of the crop.

The obtained results indicate that intramuscular injection of rats with boldenone showing marked histological changes in the seminiferous tubules such as thickened in basement membrane together with focal areas of vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium, fibrosis, degeneration of germinal epithelium with abnormal distribution of spermatozoa, decrease in sperms and Leydig cells and presence of many syncytialcells in the seminiferous tubule lumen. Our results are in agreement with Tousson<sup>11</sup> and Groot and Biolatti<sup>43</sup> who reported that, boldenone induce similar lesions in the testes of rabbits and cattle respectively. The present results are in agreement with Silcox et al.<sup>44</sup> and Veeramachaneni et al.<sup>45</sup> who studied the effects of zeranol and trenbolone acetate on testis function, live weight gain and carcass traits of beef bulls and Rodriguez et al.<sup>46</sup> who studied the comparative morphological of lamb and calf Sertoli cells treated with anabolic agent.

Co-treatment of boldenone with GLE or with MLE showed moderate or mild (respectively) improvement with normal regular distribution in spermatogenesis cycles with increased in the sperm numbers Leydig cells when compared withboldenone rats group. Also, in the present study indicate that GLE more effective than MLE. So, GLE supplementation enhancement of testicular damage induced by boldenone, and will be of major interest to be used as an adjuvant therapy under these conditions. Cytokeratin Antibody provides an economical means to evaluate the presence and status of selected keratin proteins. Keratins (cytokeratins) are intermediate filament proteins that are mainly expressed in epithelial cells. Cytokeratin is characterized by remarkable biochemical diversity, represented in human epithelial tissues by at least 20 different polypeptides. Keratins heterodimers composed of an acidic keratin (or type I keratin, keratins 9 to 23) and a basic keratin (or type II keratin, keratins 1 to 8) assemble to form filaments.<sup>47, 48</sup> The intensity of ck-ir in boldenone rat testis was significantly decreased when compared with control, MLE and GLE rat groups. Moderate to mild positive reactions for ck-ir were observed in co-treated boldenone with GLE in rat testis sections. Co-treatment of boldenone with MLE or GLE significantly increases when compared with boldenone group. Our results disagree with Maymon et al.<sup>49</sup> and agreed with Franke et al.<sup>50</sup> and Moll et al.<sup>47</sup> who reported that; the expression of specific cytokeratins appears to depend on the type of tissue as well as on the state of differentiation or development and pathologic conditions.

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