Evaluation of the renal protection conferred by amygdalin against animal growth regulator Boldjan induced renal toxicity and injury in male rats.

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DOI: 10.21608/jbaar.2024.354033

Abstract:
Among the supplements that athletes utilize most frequently are anabolic-androgenic steroids (AAS). Boldjan is an AAS drug used by young adults who want to look better and feel better about themselves and used in veterinary medicine. Thus, the goal of the current study was to investigate if amygdalin extract could protect male rats from kidney damage, oxidative stress, and toxicity caused by the anabolic steroid Boldjan. Four groups (Gp1, Control; Gp2, Amygdalin; Gp3, Boldjan; Gp4, Boldjan+ Amygdalin) were created out of forty mature male Wistar rats. When compared to control Gp, Boldjan significantly increased relative body weights (RBW), relative weights of the kidney, serum urea, creatinine, potassium ions, calcium ions, and chloride ions, and caused renal damage. It also significantly decreased sodium ions. By contrast, in post-treated rats Gp (Boldjan+Amygdalin), Amygdalin markedly restored the kidney damage generated by Boldjan. Amygdalin may be a useful prophylactic supplement to reduce kidney toxicity caused by Boldjan, perhaps through regulating oxidative stress reactions.

Key Words: Anabolic-androgenic steroids, Boldjan, Amygdalin, kidney toxicity.

Introduction
A class of man-made compounds called AAS is produced from testosterone and its precursors [1]. Abuse of AAS has been linked to many illnesses, including kidney damage [2], cardiovascular disorders [3-5], liver dysfunction [6-8], and testicular problems [9,10]. Injectable AAS can help athletes build and grow more muscle directly [5,11]. Boldenone, Ultragan, Rexobol, Mibolerone, Ultima-Tren E, Neurabol, Stanozolol, Equigan, Trenbolone, Equipoise, Winsol, Rexogin, Ganabol, Trenorol and Boldjan are well-known injectable AAS that developed mainly for veterinary use by athletes to enlarge their muscles and by women in a variety of cosmetics to enhance the volume of their lips, cheeks, buttocks, and breasts [10,12]. Moreover, humans may be indirectly affected by consuming meat from animals that have received AAS treatment. Veterinarians and young people looking to put on muscle and gain power use a new AAS medication called Boldjan. AAS has two anabolic effects: it promotes cell proliferation in the first place, and it improves male characteristics by having androgenic effects [13,14]. AAS has been classed as a Schedule III medication and applies to several veterinary products [1,15]. Prunus armeniaca (apricot), Prunus persica (peach), and Prunus amygdalus var. amara are just a few of the Rosaceae fruit kernels that naturally contain cyanides, of which amygdalin, formerly known as
laetrile, is one of numerous nitrilosides [16,17]. The two glucose molecules that make up amygdalin are hydrocyanic acid, an anti-neoplastic substance, and benzaldehyde, which has an analgesic effect. The oil obtained from kernels of apricots is essentially rich in oleic, linoleic acids and α-, γ- and δ-tocopherols [21,22]. Vitamin B17 (VitB17) is extracted from the kernels of apricots, and it is one of many nitrilosides [23]. Vitamin B17 has appeared in several pharmacological properties including antimicrobial anticancer, anti-inflammatory activities, and antioxidant [24,25]. AAS has been utilized dramatically in recent years, particularly by young adults who want to improve their appearance and boost their self-esteem, therefore; the objective of the present investigation was to examine the preventative effects of amygdalin extract against AAS Boldjan-induced kidney toxicity in male rats.

**Materials and Methods**

**Chemical and reagent**

The Boldjan (50 mg/ml) vial was acquired from Laboratorios-Tornel Comp. located in Holland. Amygdalin was purchased from Amazon for a natural oils Company with a dose of 100 mg/kg body weight.

**Experimental design**

Forty male Wistar rats, a weight of approximately 150g, were used for the experiments and obtained from our university farm. The male rats were housed in the laboratory room for seven days before the start of the research and provided with a standardized diet ad libitum. Following two weeks of acclimation, rats were divided into four groups (Gps; each with ten animals).

First Gp: Control: This group of rats won't get any medicine.

Second Gp: The Amygdalin involved giving animals an intramuscular injection of Amygdalin (100 mg/kg body weight/day) twice a day for two weeks.

Third Gp: Boldjan; rats receive an intramuscular Boldjan injection (10 mg/Kg BW/week) for four weeks.

Fourth Gp: Injectable rats treated with Boldjan and subsequently with Amygdalin (Boldjan+Amygdalin); rats received intramuscular injections of Boldjan for four weeks, followed by two weeks of Amygdalin treatment.

Following a 10- to 12-hour fast, rats from each group were fully necropsied and put to sleep using sodium pentobarbital IP after the experiment. The rats’ inferior vena-cava was used to draw blood samples, which were then placed in non-heparinized glass tubes. The blood was then incubated for ten minutes at room temperature, allowed to clot, and then centrifuged for ten minutes at 3000 r.p.m. to extract the serum. The serum was then separated and stored in a clean plastic vial with a stopper at –80°C until the serum parameters were estimated. Each group’s kidney was divided in half and preserved at -80 C while the other half was fixed in 10% buffer formalin for histological analyses.

**Kidney functions and Electrolyte estimation:**

Following Patton and Crouch, urea and creatinine levels were measured [26]. Using commercial kits of Indian Sensa core electrolyte, the method developed by Abd Eldaim et al. [27] was surveyed to measure the amounts of potassium (K +), calcium (Ca ++), sodium (Na +), and chloride (Cl - ) ions.

**Histopathological investigation:**

The kidney was promptly removed from each group, cleaned in 0.9 saline solutions, and preserved in 10% neutral buffered formalin. As per Tousson’s standard protocol, sections were stained with Ehrlich’s hematoxylin and counter-stained with eosin [28].

**Statistical analysis:**

Using one-way ANOVA for statistical analysis and data expressed as mean values ± SE, it was possible to ascertain whether there were any significant differences among the groups.
differences between the experimental groups. For biochemical data, statistical significance was determined and denoted by \( p < 0.05 \). All statistical analyses were performed using SPSS software.

**Results:**
As represented in Table 1 revealed that treatments of rats with Boldjan induced a significant elevation in relative body weight (RBW) and relative weights of kidney (RKW) as compared to control Gp. Meanwhile, post-treatment of Boldjan with Amygdalin (Boldjan+Amygdalin) induced a significant depletion in relative body weight (RBW) and relative weights of kidney (RKW) as compared to Boldjan Gp.

**Effect of Boldjan and Amygdalin on Kidney Functions:**
As represented in Table 2 revealed that treatments of rats with Boldjan induced a significant elevation in serum creatinine and urea as compared to control Gp. On the other hand, post-treatment of Boldjan with Amygdalin (Boldjan+Amygdalin) induced a significant depletion in serum creatinine and urea as compared to Boldjan Gp.

**Effect of Boldjan and Amygdalin on electrolytes levels**
As represented in Table 3 revealed that; treatments of rats with Boldjan induced a significant elevation in serum potassium ions, calcium ions, chloride ions, and a significant depletion in serum sodium ions as compared to control Gp. On the other hand, post-treatments of Boldjan with Amygdalin (Boldjan+Amygdalin) induced a significant depletion in serum potassium ions, calcium ions, and chloride ions and a significant elevation in serum sodium ions as compared to Boldjan Gp.

<table>
<thead>
<tr>
<th>Table 1: Changes in RBW and RKW in different groups.</th>
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<tbody>
<tr>
<td><strong>RBW (g/100 g)</strong></td>
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<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td><strong>Amygdalin</strong></td>
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<tr>
<td><strong>Boldjan</strong></td>
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<tr>
<td><strong>Boldjan+Amygdalin</strong></td>
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Data are expressed as mean ± SE of 10 observations. *Significant difference from the control at \( p < 0.05 \), #Significant difference from the Boldjan at \( p < 0.05 \).

Relative organ weight = \( \frac{\text{Organ weight}}{\text{Body weight}} \times 100 \)

<table>
<thead>
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<th>Table 2: Variations in kidney function levels in different Gps.</th>
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<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
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<tr>
<td><strong>Control</strong></td>
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<tr>
<td><strong>Amygdalin</strong></td>
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<td><strong>Boldjan</strong></td>
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<td><strong>Boldjan+Amygdalin</strong></td>
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Data are expressed as mean ± SE of 10 observations. * Significant difference from the control at \( p < 0.05 \), **Significant difference from the Boldjan at \( p < 0.05 \).
Table 3: Changes in serum electrolytes level in different Gps.

<table>
<thead>
<tr>
<th></th>
<th>K⁺ (mmol/l)</th>
<th>Na⁺ (mmol/l)</th>
<th>Ca²⁺ (mmol/l)</th>
<th>Cl⁻ (mmol/l)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>3.95±0.19</td>
<td>137.0±10.4</td>
<td>1.21±0.08</td>
<td>100.5±7.25</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>3.91±0.22</td>
<td>136.2±10.1</td>
<td>1.23±0.09</td>
<td>100.3±7.07</td>
</tr>
<tr>
<td>Boldjan</td>
<td>4.41±0.48</td>
<td>125.5±9.8</td>
<td>1.27±0.12</td>
<td>114.0±6.50</td>
</tr>
<tr>
<td>Boldjan+Amygdalin</td>
<td>4.03±0.35</td>
<td>133.6±10.5</td>
<td>1.22±0.08</td>
<td>110.8±8.66</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE of 10 observations. * Significant difference from the control at p < 0.05, # Significant difference from the Boldjan at p < 0.05.

Kidney structure investigation:

Renal corpuscles and proximal and distal convoluted tubules were among the completely normal components of the renal cortex that were visible in kidney slices from the control and amygdalin groups (Figs. 1A&1B). The kidney sections in the Boldjan group showed significant degeneration of the renal tissues, hemorrhage, and considerable glomerular necrosis, and most of the glomeruli appeared to have lost their attachments (Fig. 1C&1D). However, after receiving amygdalin treatment, Boldjan showed a modest recovery with slight deteriorated renal tubules, mild glomeruli atrophy, and mild leucocytic infiltrations (Fig. 1E&1F).

Figure 2: Haematoxylin & Eosin-stained kidney sections. A&B: Kidney sections in control and Amygdalin Gps showing a normal structure of glomeruli (G) and renal tubules (RT). C&D: Kidney sections in Boldjan Gp exposed marked damage and most of the glomeruli (G) seemed to have lost their attachments, marked glomerular necrosis, degenerated to the renal tissues (arrows), hemorrhage and leucocytic infiltrations (arrowheads). E&F: Kidney sections in post-treated Boldjan with Amygdalin exposed mild glomeruli (G) atrophy, mild leucocytic infiltrations (arrowheads), and moderate degenerated renal tubules (arrows).
Discussion

Naturally occurring, anabolic hormones like testosterone promote protein synthesis within cells. The goal of the current study was to determine whether amygdalin extract could protect male rats against renal damage caused by Boldjan. According to the current findings, male rats given the anabolic steroid Boldjan intramuscularly showed a significant increase in their relative body weight rate and kidney weights when compared to the control group. However, these increases were decreased when the rats were given amygdalin along with Boldjen. In accordance with Hoseini et al. [29], mice's body and kidney weight increased in response to nandrolone decanoate. The present findings are consistent with the findings of Holt et al. [30], who found that the anabolic steroid methenolone enanthate increased the growing rat's rate of weight gain and kidney weight. Additionally, the current findings corroborate the findings of Hussain et al. [6], who found that injections of boldenone considerably raised the body weight and total protein concentrations in male rabbits.

The present findings, however, contradict the findings of Oda and El-Ashmawy [31], who found no evidence of a substantial effect of the anabolic steroid boldenone on body weight or weight increase. The present findings corroborate those of Alm-Elddeen and Tousson [2], who report that an injection of the growth promoter Boldenone resulted in a large increase in the amounts of creatine, urea, and total protein in male rabbits as well as a significant drop in the A/G ratio. These findings are consistent with those of Urhausen et al. [32] who found that intramuscular injections of boldenone undecylenate greatly improved the renal functions of weaned male lambs. Our findings also corroborated those of Tousson et al. [5], who discovered that injections of the growth promoter boldenone in rabbits caused damage to the kidneys and liver.

The current findings are consistent with the findings of Daher et al. [33] who showed that acute renal injury was caused by anabolic steroids, together with elevated serum urea, creatinine, and calcium ion levels. In contrast to the current findings, Gabr et al [34], observed that intramuscular boldenone injection
dramatically reduced urea levels. The noted reduction in Na+ and a significant increase in K+ levels following Boldjan injection could perhaps be attributed to inhibition of the Na+/K+ ATPase pump, a crucial mechanism for maintaining Na+ and K+ equilibrium in eukaryotic cells. Overdosing on steroids inhibits Na+/K+ATPase and its signaling pathways, which raises intracellular Ca2+ and Na+ levels. The changes in electrolyte balance that are currently observed are consistent with the findings of earlier studies by Elmasry et al. [10] and Tousson et al. [4]. Furthermore, it has been proposed that glomerular hyperfiltration and elevated body mass are the root causes of AAS-induced direct glomerular toxicity [35]. Both hypophosphatemia and elevated uric acid contribute to the increased urea level in serum [34]. Because AASs cause an increase in muscle mass, they also raise the body's creatinine level. These findings are consistent with those of Mutar et al. [17] who report that vitamin B17 significantly increased calcium and sodium ion levels while significantly decreasing urea, creatinine, K, and Cl levels. Additionally, our findings are consistent with Guo et al. [36] findings that amygdalin improves the biochemical parameters in chronic kidney disease and suppresses renal fibrosis.

The current findings showed that the injection of Boldjan caused substantial glomerular necrosis, atrophy, and degeneration of the renal tissues and that treating Boldjan with amygdalin resulted in a moderate improvement in these abnormalities in the structure of the kidneys. Our findings concur with those of Tousson [28], who noted that injections of the growth-promoting drug boldenone in rabbits can cause kidney damage. Our findings also corroborate the findings of Hoseini et al. [29] and Tousson et al [37], which showed that nandrolone decanoate caused renal impairment in mice. Treatment with herbal medicine improved kidney function and structure [38]. Also, Tsitsimpikou et al. [39] reported that; long-term nandrolone decanoate adminis induced renal toxicity and damage. Current results revealed that; treatments of Boldjan with amygdalin improved the damage in kidney structure after Boldjan treatments.

**Conclusion**

In conclusion, Boldjan injection in male rats induced renal toxicity and injury and the treatments with Amygdalin (Boldjan+Amygdalin) markedly restored the kidney damage generated by Boldjan. Amygdalin may be a useful prophylactic supplement to reduce kidney toxicity caused by Boldjan, perhaps through regulating oxidative stress reactions.

**Acknowledgments**

I would like to thank Prof. Dr. Ehab Tousson (Tanta University, Egypt) for his comments on an earlier version of this manuscript.

**Funding:** No fund.

**Authors’ contributions**

Sawsan Alsadee conceptualized the research idea, and data analysis, wrote and edited the manuscript, and approved the final manuscript.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article. If detailed data are required, they can contact the correspondence of the study, Sawsan Alsadee (Email address: sawsanalsadee@gmail.com).

**Declarations**

Ethics approval and consent to participate.
Consent for publication:
Not applicable

Competing interests
The author declares that has no competing interests.

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