Effect of Androgenic Anabolic Steroids Dianabol In Some Haematological and Immunological Criteria For Male Albino Rats

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Abstract
The present study was performed to examine effect of androgenic anabolic steroid dianabol in some haematological and immunological criteria for male Albino rat. The animals were divided in to four groups (6 animals for each group) first , second and third treatment groups gives orally suspended dianabol 10,20 and 40 mg/kg/day respectively ,while the fourth group (control) gives physiological normal saline(0.85%) , and all groups primed for 8 weeks . The results showed that significant increase(P<0.05) in RBC count , platelets count , Hb , p.c.v and percentage of lymphocytes in treatments groups compared with control , while the WBCs count show significant decrease (p<0.05) in percentages of granulocytes and monocytes, furthermore , the results refer that significant decrease (p<0.05) in concentrations of GM-CSF and total protein in treated groups compared with control group.

Keywords: Activated Sports for Albany Dinapol muscles, platelets, hemoglobin concentration.

Introduction
In recent times the phenomenon of the use of androgenic anabolic steroids by some sports athletes were spreaded , particularly in games that require large muscle force area built faster than usual(Calfee & Fadale, 2006) as noted in public life that a lot of workers who to need muscle strength to get their work done eating such real state without aprescription.

Doping defined as each substances or drug enters the body and unusual quantities and lead to increase efficiency facility for acomplete athlete and illegally that cause damage when health continue eating it badly(Khalil ,2006)because of the widespread use of these drugs and their side effect resulting from it(Yeasalis et al1989)appeared to study these complications,which is long-term negative effects.

Androgenic anabolic steroid (AAS) the most prevalent types of steroids in general and especially among young people as it works to increase the bodies metabolism , especially proteins , which increase physical strength significantly , it has been found that 63.9% of the samples examined for convicts using AAS...
Marcia and Anderson, 2000), where dianabol is one of the most important drugs that are used to express body building and raise heavies a strong androgenic effect because they are raising rats of male hormone which leads to stimulate muscle cells to maintain a high concentration of nitrogen which would create the cell to retain a larger amount of protein that works to build muscle mass and large this steroid hormone is one of the derivatives industry of testosterone, which is exerted in to the body by the testes and adrenal gland has been altered so that structural qualities overcome male (Llewellyns, 2007). The aim of this research is to evaluation the side effects of dianabol in rats through the study of Blood parameters including, RBCs, platelets, WBCs count, and differentiation, Hb and PCV and immunological assays including the concentrations of GM-CSF and total protein.

**Materials and methods**

1. **Laboratory animals:**
   Twenty four native rats *Rattus rattus* have been brought from animal house of college of veterinary medicine /university of AL-Qadissiya. These rats were kept throughout experimentation periods *Ad libitum* for ration and housing (Schnider et al., 1990) in animal house of college of science /university of babylon. The average weight of such animal ranged between 250-300 gm and their ages ranged from 4-5 months. The animals have been subjected to laboratory conditions divided in to 12 hours light and 12 hours dark and the temperature is set at 28±2 c°.

2. **Preparation of drug suspension:**
   The methandrostenolone (dianabol) was obtained from the pharmacy and their equipment from company of british dispensy as a tablets in concentration 5 mg/kg. The tablets were macerated by blender and each tablet dissolved in 10 ml of physiological normal saline, and the concentration of experiments were done according to the doses for human (Criado-Sanchez et al., 1999).

3. **Dosing protocol:**
   The rats were divide in to four groups, 6 replicates for each. The first (t1), second (t2) and third (t3) treated groups orally received dianabol suspension at concentrations 10, 20 and 40 mg/kg/day for 8 weeks respectively, while the last group control was orally received normal saline for such period.

4. **Blood samples**
   The blood samples were collected directly from rats by heart puncture, one day after the last dose. The small amount of blood samples were kept in sterile tubes containing anticoagulant (heparin) AFM-DISPO and placed in refrigerator at 4 c in order to the measure some haematological parameters, while the remainder of these samples were kept in sterile centrifuge tubes to separate the serum, and then measuring the concentrations the total protein and GM-CSF.

5. **Haematological assays**
   A-The platelet count, WBCs count and differentiation were done according to the Dacie and Lemis (2001).

   B-The percentage of PCV was measured according to the Hilman and Ault (2002), while the concentration of Hb was estimated according to the Rodak (2002).

6. **Immunological assays**
   A-Totai protein:
   The concentration of total protein was estimated according to the manual procedure in kits of Randox company (UK).
B - GM-CSF :

The concentration of the cytokine Granulocyte – Macrophage colony stimulating factor (GM-CSF) was determined by ELISA kit according to the manual procedure of komabiotech company (south korea).

7-Statistical analysis :

The result for experiments were analyzed by using statistical programe SPSS version 17 ,using one way Anova , mean and standard errors as well as multiple comparisons in average of animal groups by using least significant difference (L.S.D) and below the probability (0.05) (Jawda ,2008).

Results:

Result showed that the rats treated by dianabol has resulted a significant increase (p<0.05) in RBCs count , platelets count , concentration of Hb and percentage of P.C.V in all treated groups in comparison with control group , while there was significant decrease (p<0.05) in total WBCs count in third group (t3) than that in control rats (table 1).

Table (1): changes in some blood parameters in rats primed orally by dianabol for 8 weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBCs count (cell/mm³×10) Mean±S.E.</th>
<th>Platelets count (cell/mm³×10) Mean±S.E.</th>
<th>WBCs count (cell/mm³×10) Mean±S.E.</th>
<th>Hb concen. gm/100ml Mean±S.E.</th>
<th>p.c.v % Mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ± 0.12</td>
<td>494 ± 8.6</td>
<td>11.2 ± 0.6</td>
<td>14.1 ± 0.2</td>
<td>30.1 ± 1.3</td>
</tr>
<tr>
<td>10 mg/kg (t1)</td>
<td>6.3 ± 0.14*</td>
<td>588 ± 6*</td>
<td>10.8 ± 1</td>
<td>14.9 ± 0.1*</td>
<td>37.8 ± 0.4*</td>
</tr>
<tr>
<td>20 mg/kg (t2)</td>
<td>6.2 ± 0.21*</td>
<td>785 ± 7.9*</td>
<td>9.7 ± 0.4</td>
<td>15.2 ± 0.3*</td>
<td>40.5 ± 0.6*</td>
</tr>
<tr>
<td>40 mg/kg (t3)</td>
<td>7.6 4 ± 0.25*</td>
<td>746 ± 52*</td>
<td>7.9 ± 0.4*</td>
<td>15.5 ± 0.1*</td>
<td>41.6 ± 0.3*</td>
</tr>
</tbody>
</table>

* :P<0.05

The results of differential WBCs count showed a significant increase (p<0.05) in lymphocytes percentage in treated groups compared with control group (figure 1).

Figure (1) : the percentage of lymphocytes in rats treated with dianabol for 8 weeks.

while there is a significant decrease (p<0.05) in percentages of granulocytes and monocytes in all treatment groups when compared with control group , where the concentration 40 mg/kg is the most influential as illustrated in figures 2 and 3 respectively.
The results revealed that the presence of a significant decrease below the level (p<0.05) in the concentration of total protein of three treated groups (t1, t2 and t3) which reached 5.4±0.25, 4.4±0.55 and 5.1±0.40 gm/dl respectively compared with control rats (6.6±0.33), the results also showed a significant decrease (p<0.05) in concentration of granulocyte-macrophage colony stimulating factor (GM-CSF) in all treatment groups which reached 3.2±0.3, 3.6±0.45 and 2.6±0.5 pg/ml consecutively, compared with control group which was 4.4±0.36 pg/ml as illustrated in table (2).
Table (2) : the concentrations of total protein and GM-CSF in rats primed orally dianabol for 8 weeks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration of total protein (gm/dl) Mean±S.E.</th>
<th>Concentration of GM-CSF (pg/ml Mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.6 ± 0.26</td>
<td>4.4 ± 0.20</td>
</tr>
<tr>
<td>10 mg/kg (11)</td>
<td>5.4 ± 0.19*</td>
<td>3.2 ± 0.17*</td>
</tr>
<tr>
<td>20 mg/kg (12)</td>
<td>4.4 ± 0.22*</td>
<td>3.4 ± 0.26*</td>
</tr>
<tr>
<td>40 mg/kg (13)</td>
<td>5.1 ± 0.20*</td>
<td>2.6 ± 0.29*</td>
</tr>
</tbody>
</table>

* P<0.05

Discussion:

The results of the current study revealed a significant increase in the mean of concentration of hemoglobin (Hb) and RBCs count, and this can be attributed to the effect of androgenic anabolic steroids (AAS) which increased the concentration of erythropoietin hormone which stimulate bone marrow to increase production of RBCs (Gallicchio et al., 1984; Alex et al., 2011), also that the increase in number of red blood cells will increase the percentage of packed cell volume (P.C.V) (Chung et al., 2007).

The studies indicated increase in the average of platelets count when sport doping by athletes (McCrohon et al., 2000). The results illustrated there was a significant increases (p<0.05) in percentage of lymphocytes, the possible explanation of this results, the dianabol well known to induce inflammatory responses, which lead to increases in lymphocytes percentages (Garg et al., 1997).

There was a significant decrease (p<0.05) in the concentration of the cytokine GM-CSF, which plays an important role in the stimulate growth and differentiation of myelomonocytic lineage cells into granulocytes and macrophages, particularly dendritic cells (Kenneth et al., 2012), this means that the role of the drug may be suppressive to body immunity, and could be due to the renal damage, which lead to a decline in erythropoietin hormone (Maravelias et al., 2005; Marshal-Gradisnik et al., 2009), but this study differed with the (AL-Muhaissen et al., 2011) study proved that the use of AAS increase the capacity of the production of immune cells, especially those that play important role in humeral immunity, which lead to increase production of immunoglobulins and complement components. in study of Maktof (2009) on women suffering from the high proportion of the prolactin hormone which trigger the synthesis of B-cells, immunoglobulin and complement components, meantime (AL-Muhaissen et al., 2011) reported that the increase production of prolactin when athletes who use AAS.

The results showed a significant decrease (p<0.05) in concentration of total protein in reated rats compared with the control group and these agree with the study conducted by Moussa and Bashandy (2008) on rats, this explain the significant decline in the concentration of plasma protein to the lack of food intake as a result of dianabol poisoning (Banh, 2006), furthermore, there are some cases of diseases,
especially hepatic and renal diseases lead to a rapid loss in the plasma protein (AL-Hilaly, 1997).

**Conclusion**

The results of the current study revealed a significant increase in the mean of concentration of hemoglobin (Hb), packed cell volume (P.C.V), RBCs count, platelets count and lymphocytes percentage.

The current study revealed a significant decrease (p<0.05) in the percentage of granulocytes, monocytes, concentration of the cytokine GM-CSF and total protein in serum. These means AAS use lead to changes in blood parameters and immunosuppressive, and these changes depend on dose and using period.

**References**


Maravelias, C.; Dona, A.; Stefanidou, M. and Spiliopoulou, C. 2005


