The Effect of Furosemide on Some Physiological and Immunological Parameters In Male Albino Rats

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Abstract:
The present study was performed to examine the effect of furosemide (laxis) on certain hematological and immunological criteria in rats. The animals were divided into three groups (5 replicates for each): first and second treatment groups give orally suspended furosemide 0.1 and 0.2 mg/kg/day respectively, while the third group (control) gives orally normal saline (0.9%), and all groups primed for 5 days. The results showed a significant decreases (P<0.05) in RBCs count, platelets count, WBCs count, concentration of Hb and PCV% in treatment groups compare with control group; furthermore, results revealed a non significant differences (P<0.05) between treatment and control groups in concentration of total protein, while there was a significant increases (P<0.05) in concentration of the cytokine GM-CSF in first treatment group compared with second treatment and control groups.

key words: furosemide. blood parameters . GM-CSF.

Introduction:
Laxis is a brand name for the drug furosemide a very potent diuretic, which will cause the body to excrete water as well as potassium, sodium, and chloride (Richard and Mary, 2006). The use of drugs to enhance performance at olympic games started during the games in ancient Greece, when hallucinogens form fungi and possibly strychnine were used by competitors (Caltine et al., 2008), principally, drugs were prohibited because of their capacity to enhance performance.

In 2012, Word Anti-Doping Agency (WADA) assumed responsibility for the list from the International Olympic Games (IOG) – Medical Commission (MC). Currently WADA have three criteria for prohibiting a drug: if it enhances sport performance; if it would be harmful to health if with held; or if it is against the spirit of sport. However, the most important reason for prohibiting beta-blockers and diuretics is for weight-classified athletes, because diuretics have the capacity to enable
an athlete to compete in a lower weight(Ken,2012), meantime , Yajiro et al., (2013) reported that diuretics such as furosemide induced mild hypovolemia with hemocencentration. In present work, the changes in some hematological and immunological parameters in rats primed with furosemide are being examined.

**Materials and methods:**

1- **Laboratory Animals**

Fifteenth native rats *Rattus rattus* have been brought from animal house of College of Veterinary Medicine / University of Al-Qadisiya. These rats were kept throughout experimentation periods *ad libitum* for ration and housing (Schneider et al., 1990) in animal house. The animals have been subjected to laboratory conditions divided into 12 hours light and 12 hours of darkness and the temperature is set at 28±2 C°. The average weight of such animals ranged between 200-250gm and their ages ranged 4-4.5 months.

2- **Preparation of Drug Suspension:**

The furosemide (Laxis) was obtained from pharmacy and their equipment from company of Sanofiaventis (France) as a tablets in concentration of 40mg/kg. The tablets were macerated by blender and each tablet dissolved in 10 ml of physiological normal saline ,and the concentration for experiments were done according to the doses for human (Criado-Sanchez et al., 1999).

3- **Dosing Protocol:**

The rats were divided into three groups, (5 replicates for each) the first (T1) and second (T2) treated groups were orally received furosemide suspension at concentration 0.1 and 0.2mg/kg/day for five days respectively , while the third group (Control) was orally received normal Saline(0.9%) 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 tables containing anticoagulant (heparin) AFM-Dispo and placed in order to measure some hematological parameters , while the remainder of these samples were kept in sterile centrifuge tubes to separate the sera , and then measuring the concentrations of total protein and the cytokine GM-CSF.

1- **Hematological Assays:**

a- The RBCs count were done by using hemocytometer according to the Powers (1989).

b- The platelets count, total and differential WBCs count were done according to the Dacie and Lewis (2001).

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

2- **Immunological Assays:**

a- **Total 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).
6- Statistical Analysis

The results for experiments were analyzed using statistical programe (2007 version 3), mean , standard error as well as multiple comparisons in averages of animal groups by least significant difference (L.S.D.) and below the probability(0.05).

Results:

Results illustrated that the animals treated by furosemide showed a significant decreases (P<0.05) in RBCs count, Platelets count, WBCs count , also there was a significant difference between treated groups, concentration of Hb and percentage of PCV in comparison with control group as revealed in table (1).

Table (1): Hematological parameters in rats primed orally by furosemide for 5 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nature of treatment</th>
<th>BRCs count (cell/mm(^{3})x10(^{6})) mean ± S.E</th>
<th>Platelets count (cell/mm(^{3})x10(^{3})) mean ± S.E</th>
<th>WBCs count (cell/mm(^{3})x10(^{3})) mean ± S.E</th>
<th>Hb conc. (g/dl) mean ± S.E</th>
<th>PCV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>5.11 ± 1.23</td>
<td>497 ± 1.7</td>
<td>103 ± 2.34</td>
<td>11.8 ± 175</td>
<td>28.5±2.86</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>(T1)</td>
<td>1.41 ± 0.61*</td>
<td>40 ± 3.5*</td>
<td>1.6 ± 0.03*</td>
<td>3.3 ± 0.78*</td>
<td>9.1 ± 1.41*</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>(T2)</td>
<td>1.68 ± 0.54*</td>
<td>47 ± 3.5*</td>
<td>5.4 ± 1.25 **</td>
<td>3.7 ± 0.82 *</td>
<td>10.4 ± 2.5*</td>
</tr>
</tbody>
</table>

*(P<0.05): between treatments & control

***(P<0.05): between treatments

Table (2) showed that the concentrations of immunological parameters including ; total protein and the cytokine GM-CSF. Results revealed that their were no significant difference (P<0.05) in concentration of total protein between treated groups and control , while their was a significant differences (P<0.05) in the concentration of cytokine GM-CSF in second treatment group in comparison with first treatment and control groups .

Table (2) : The concentration of total protein and GM-CSF in rats primed orally by furosemide for 5 day

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nature of treatment</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>Control</td>
<td></td>
<td>6.37 ± 0.45</td>
<td>0.099</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td></td>
<td>7 ± 0.4</td>
<td>0.105*</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td></td>
<td>6.47 ± 0.41</td>
<td>0.097**</td>
</tr>
</tbody>
</table>

*(P<0.05): between treatments & control

**(P<0.05): between treatments

Discussion:

The results of the current study showed that a significant decreases in mean of RBCs count, WBCs count , platelets count, concentration of Hb and PVC%, and this can be attributed to the renal damage, which lead to a decline in erythropoietin hormone (Maravelias et al.,2005), therefore, reduce the bone marrow's ability to produce BRCs. Meantime , the decrease of platelets count may be related to inhibition of megakaryocytes, which the origin of the platelets in bone marrow. Steven et al., (2009) revealed that dehydration in males was induced with 40 mg furosemide and 8 hours water restriction, and this drug reduced plasma volume by 10±2% as well as blood volumes by 6±2% , also Richard and Mary (2006) reported that loop diuretics such as furosemide can cause a severe and rapid reduction in blood volume.
There was a significant increases (P<0.05) in the concentration of GM-CSF in first group compared with second treatment and control groups, this means that the first dose (0.1 mg/kg/day) may be stimulate growth and differentiation of myelomonocytic Lineage cells into macrophages, particularly dendritic cells (Kenneth et al., 2012). The results showed a non signification deference's (P<0.05) in the concentration of total protein in primed rats compared with control group, these agree with the study conducted by Moussa and Bashandy (2008) on rats, this explains the significant decline in the concentration of plasma protein may be to the lack of food intake as a results of furosemide poisoning (Banh, 2006).

References: