Some pharmacological and toxicological envestigation of 
*Haloxylon salicornicum* in rabbits

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Abstract

This study aimed to investgate the effect of aqueous extract of *Haloxylon salicornicum* on some hematological parameters in rabbits and toxicity in rabbits and mice. Eighteen rabbits approximately 700-900gm body weight and 20 week old, were divided into 3 equal groups to study the effect of this plant on body weight and hematological parameter. The first group was regarded as a control, where as the other two groups were given (500-1000) mg/kg/day (safety dose) of aqueous extract of *Haloxylon salicornicum* orally by stomach tube for 8 successive weeks. The results showed significant increase of body weight, hemoglobin content, red blood cell count and packed cell volume. A decrease leukocytic in rabbits which was administrated (1000) mg/kg of extract. No significant effect was found in differential leukocytic count. The presence study investigated the toxicity of *Haloxylon salicornicum* in rabbits through examination of possible biochemical and histopathological changes. Animals were treated daily with a normal dose of 5g/kg, b.wt/day of *Haloxylon salicornicum* for 12 week. Changes in hepatic enzymes levels and histopathological changes in lung, liver, kidney, stomach, intestine and spleen, were observed. On the other hand, no mortality was noted for acute toxicity in mice, during 15 days period after aqueous toxic effect was noted.

Introduction

Plants have always plays a major role in treatment of human and animal diseases. Medical plants are a therapeutic resource much used in the traditional population of the world specifically for health care. *Haloxylon salicornicum* is a desert plant which belongs to family *chenopodiaceae*. The plant is typical of arid regions and represents xerophytic species adapted to extremes salinity. This plant contain, among other chemical constituent, sodium (28.48%), potassium (9.49%), carbonate (61.06%), alkaloid, saponin, phenol. This plant was used effectively in folk medicine as diuretic, anti ulcer, hypoglycemic and anti microbial. Valuable therapeutic uses of the tested plants as home remedy encouraged the author to study the effect of aqueous extract on hematological parameter and their toxicity to know the safety dose for administration.

Materials and Methods

Preparation of the aqueous extract: fifty grams from the plant were extracted with 500ml distilled water for 16 hrs. Then solution was concentrated by rotary evaporator (Puchi Rotavapor,RE) at 40°C, the final dryness was done by the evaporation of remnant solvent by leaving the residue in room temperature. The resultant was 10gm. This process was repeated 3 times.

Experimental

1- Effect on body weight and Blood parameters.

This experiment was performed on 3 groups, each of 6 rabbit, first group was regarded as the control group were as the other two groups were administrated (500 & 1000) mg/kg/day receptively of aqueous extract of *Haloxylon salicornicum* orally by a stomach tube for 8 successive weeks.
sample (1ml) was taken from each rabbits from heart in a clean dry test tube containing heparin as anticoagulant. Hemoglobin%, erythrocyte and leukocyte were counted immediately after blood sampling.

A- Determination of hemoglobin content:
Determination of hemoglobin content was carried out as explained by (10). Hemoglobin reacted with hydrochloride to form hematin.

B- Blood Cell count :
Erythocytic and leukocytic count was done using the double improved neubaur counting chamber as described by (11).

C- Packed Cell Volume (P.C.V.)
The method described by (9) was used for the determination of hematocrit values.

D- Differential Leukocytic Count:
Differential leukocytic count was done using blood smear,as in the method described by (12).

II- Acute toxicity:
A trial was carried out to determine the minimum lethal dose (MID) of aqueous extract on 15 adult mice of 25-30 gram body weight. The tested extract was administrated orally in graded increased doses (1-10g/kg b.wt). A control group was given the respective volume of solvent.

III- Effect of prolonged administration:
This experiment was preformed on two groups each of 5 mature rabbits of 1.5-2kg b.wt and 8-9 months old. The first group was taken as control where as the second groups was given aqueous extract of Haloxylon salicornicum (5g/kg b.wt./day) orally by stomach tube for 12 weeks. The rabbits were fed by concentrated diet and watered ad. Libitum. This experiment was done to study the effect of prolonged oral administration of the extract on chronic toxicity and changes in key hepatic enzymes level and histopathological changes in lung, liver, stomach, intestine, kidney and spleen. Chose this dose according to (5).

Statistical analysis :-
Statistical analysis of the obtained data was carried out using ANOVA test according to (1).

Results
Effect of eight weeks administration on:-
A- body weight:- The means difference increase in body weight of rabbits previously administrered aqueous extract (500&1000) mg/kg/day for eight weeks are recorded in table (1).

Table (1): Means values ± SD of the difference increase in body weight of rabbits given orally aqueous extract of plant for 8 successive weeks (n=6)

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Dose in mg/kg B.wt.</th>
<th>Body weight (gm) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>850±0.18</td>
</tr>
<tr>
<td>Haloxylon salicornicum</td>
<td>500</td>
<td>1005±0.42*</td>
</tr>
<tr>
<td>aqueous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haloxylon salicornicum</td>
<td>1000</td>
<td>1080±0.57**</td>
</tr>
<tr>
<td>aqueous</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P<0.05  ** P<0.01

The obtained date revealed that the extract of plant significantly increased the body weight of the treated groups as compared with that of the control group given the respective volume of the solvent.

B- Blood parameters:-
Changes in erythrocytic count, leukocytic count, hemoglobin content and packed cell volume following the oral administration of aqueous extract of the plant for 8 successive weeks are recorded in table (2). The obtained results showed a significant
increase in erythrocytic count in rabbits administered 1000mg /kg/day of extract after 4 weeks and highly after 8 weeks. A significant increase in hemoglobin content and P.C.V. was also recorded in both groups treated with aqueous extract after 4 weeks and 8 weeks in rabbits administration 500&1000 mg/kg of extract. The obtained results showed non significant change in leukocytic count during eighteen weeks of oral administration of aqueous extract in rabbits treated with (500mg/kg/day) of extract but in rabbits treated with (1000mg/kg/day) of extract the obtained result showed significant decrease in leukocytic count after 2 weeks and 8 weeks.

C- Differential leukocytic count:- Changes in differential leukocytic count following the oral administration of aqueous extract of plant for 8 weeks are recorded in table (3).The obtained results show a non significant change in differential leukocytic count .

Table(3):The effect of the 8 weeks oral administration of aqueous extract of studied Plant on differential leukocytic count (n=6).

<table>
<thead>
<tr>
<th>Period</th>
<th>Groups</th>
<th>Lymphocyte%</th>
<th>Monocyte %</th>
<th>Neutrophil %</th>
<th>Basophil %</th>
<th>Eosinophil %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>Control</td>
<td>41.5±0.35</td>
<td>20.17±0.46</td>
<td>53.86±0.51</td>
<td>3.915±0.40</td>
<td>2.41±0.99</td>
</tr>
<tr>
<td></td>
<td>500mg/kg of extract</td>
<td>30.7±91</td>
<td>26.18±0.65</td>
<td>56.19±0.43</td>
<td>5.00±0.41</td>
<td>4.5±0.69</td>
</tr>
<tr>
<td></td>
<td>1000mg/kg of extract</td>
<td>45.9±0.73</td>
<td>28.31±0.83</td>
<td>44.20±0.29</td>
<td>4.27±0.74</td>
<td>5.89±0.89</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Control</td>
<td>45.61±0.71</td>
<td>25.5±0.63</td>
<td>55.5±0.73</td>
<td>3.5±0.82</td>
<td>2.1±0.76</td>
</tr>
<tr>
<td></td>
<td>500mg/kg of extract</td>
<td>42.79±0.11</td>
<td>20.2±0.46</td>
<td>45.3±0.58</td>
<td>2.1±0.64</td>
<td>3±0.49</td>
</tr>
<tr>
<td></td>
<td>1000mg/kg of extract</td>
<td>44.80±0.26</td>
<td>21.1±0.37</td>
<td>44.1±0.24</td>
<td>1.62±0.13</td>
<td>1.9±0.52</td>
</tr>
<tr>
<td>6 weeks</td>
<td>Control</td>
<td>48.25±0.31</td>
<td>29.6±0.33</td>
<td>50.25±0.31</td>
<td>3.6±0.23</td>
<td>3.9±0.40</td>
</tr>
<tr>
<td></td>
<td>500mg/kg of extract</td>
<td>48.6±0.91</td>
<td>28.4±0.27</td>
<td>42.68±0.26</td>
<td>2.4±0.28</td>
<td>3.6±0.66</td>
</tr>
<tr>
<td></td>
<td>1000mg/kg of extract</td>
<td>50.1±0.75</td>
<td>28.7±0.13</td>
<td>50.87±0.17</td>
<td>2.03±0.57</td>
<td>2.9±0.52</td>
</tr>
<tr>
<td>8 weeks</td>
<td>Control</td>
<td>57.21±0.42</td>
<td>28±0.34</td>
<td>59.99±0.09</td>
<td>3.4±0.49</td>
<td>3.1±0.11</td>
</tr>
<tr>
<td></td>
<td>500mg/kg of extract</td>
<td>52.77±0.64</td>
<td>27±0.59</td>
<td>48.83±0.10</td>
<td>2.8±0.61</td>
<td>2.6±0.12</td>
</tr>
<tr>
<td></td>
<td>1000mg/kg of extract</td>
<td>56.92±0.85</td>
<td>28±0.35</td>
<td>52.21±0.34</td>
<td>1.9±0.99</td>
<td>1.6±0.85</td>
</tr>
</tbody>
</table>

D- Acute Toxicity :-Oral administration of aqueous extract in doses (1-10g/kg b.wt.) in adult mice respectively showed no toxic and mortality effects where as doses over these levels showed symptoms of toxicity characterized by colicy pain, shallow and rapid respiration and depression. The minimum lethal dose of aqueous 10g/kg/day .

E- Chronic Toxicity :-Chronic toxicity was studied in the rabbits treated with daily oral dose (5g/kg/day) for 12 weeks.

1- changes in key hepatic enzymes such as GOT and GPT:
Table (4) Effect of prolonged oral administration (12 weeks) of aqueous extract of Haloxylon salicornicum on some enzymatic activity of rabbits (N=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT unit /L</th>
<th>SGPT unit/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (3 ml of normal saline)</td>
<td>6.03 ± 1.32</td>
<td>6.85 ± 0.34</td>
</tr>
<tr>
<td>Aqueous extract of <em>Haloxylon Salicornium</em> 5g/kg/day</td>
<td>15.85 ± 1.05*</td>
<td>19.09 ± 2.18*</td>
</tr>
</tbody>
</table>

* P<0.01

2- changes in histopathological such as spleen, lung, liver, kidney, stomach and intestine.

**Spleen:** increase depletion and increase in haemorrhage.

**Lung:** fibrinoid pneumonia proliferation of the pneumocyte, hemocidrine, dilatation of some bronchioles, thickening of the wall of some arterioles.

**Liver:** hemocidrine into the hepatic cells, degeneration, and there are inflammatory cells, (mononuclear cells), dilatation of sinusoid.

**Kidney:** There are swelling of the epithelium cell of renal tubules & haemorrhage and inflammatory cells and lymphocyte.

**Stomach:** There are haemorrhage & sloughing of mucosal layer.

**Intestine:** infiltration of inflammatory cells in mucosa and submucosa, degenerated of epithelium cells.

Fig (1): Section of rabbit spleen treated with *Haloxylon salicornicum* (5 gm/kg/day) for 12 weeks orally, note depletion of follicular and haemorrhage. H&E 10 X

Fig (2): Section of rabbit lung treated with *Haloxylon salicornicum* (5gm/kg/day) for 12 weeks orally, note fibrinoid aggregation, thickening of alveolar wall, dilatation of some bronchioles. H&E 10X
The obtained results proved that acute toxicity and chronic toxicity respectively. Theses finding indicate that tested plant have very low toxicity was recorded by very large dose of tested extract which obtained from a larger amount of the studied plant. In this respect (5), reported that Haloxylon salicornicum showed no toxicity at tested dosage (2g/kg.b.wt) in mice and (7g/kg b.wt.) in alkaloid fraction Isolated from Haloxylon salicornicom devoid of toxic. The author reported that aqueous extract of the tested plant significantly increase serum glutamic -oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), the increment of these two enzymes in the serum related to the hepatic cells destruction as a result of the detoxification effect of the plant extract on these cells. The destruction of hepatic cells lead to exudation of these two enzymes to the blood circulation (15). The histopathological changes in tissues (liver, lung, stomach, spleen and intestine) related to of the plant content such as saponin, alkaloid and phenols after administration of the extract for 12 weeks.

**Discussion**

Fig (3) : Section of rabbit liver treated with *Haloxylon salicornicum* (5 gm/kg/day) for 12 weeks orally, note hemocidrosis and degeneration of hepatic cells H&E 10 X

Fig (4) : Section of rabbit kidney treated with *Haloxylon salicornicum* (5 gm/kg/day) for 12 weeks orally, note swelling of the epithelium cell of renal tubules and haemorrhage. H&E 10 X

Fig (5) : Section of rabbit stomach treated with *Haloxylon salicornicum* (5 gm/kg/day) for 12 weeks orally, note haemorrhage, sloughing of mucosa layer. H&E 10 X

Fig (6) : Section of rabbit intestine treated with *Haloxylon salicornicum* (5 gm/kg/day) for 12 weeks orally, note infiltration of inflammatory cells in mucosa and submucosa. H&E 10 X
On the other hand excessive irritation of saponin to kidney tissues lead to their inflammation. Saponin irritation effect also seen in other tissues of rabbits (16). The obtained results demonstrated that both of extract significantly increased body weight in rabbits administrated this extract for 8 successive weeks, this effect was found to be due to increase the adipose tissue in the bodies of animals. This result my be due to the excretion of thyroxin hormone and this hormone increased adipose tissue and increased from erythropoiesis which lead to the increase metabolism (17). In addition, the result shows that this extract significantly increased erythrocytic count, hemoglobin content and packed cell volume of treated groups compared with control. This effect may be due to the stimulation of cells of bone marrow for division or maybe due to stimulation of the excretion of thyroxin hormone increase the erythropoisis which lead to increasing the erythrocytic count and lead to increase the hemoglobin content and packed cell volume (17). The obtained result demonstrated that dose of 500mg/kg of extract non significantly change the leukocytic count and dose of 1000mg/kg of extract decreased significantly the leukocytic count and the change of differential leukocytic count non significant lead to stress because the plant contains saponin and alkaloid and this effect lead to migration leukocytic from blood stream to inflammation tissues. Erythrocyte count affect in the animals administrated 1000mg/kg/day may be not adapted to the salinity of the plant and effected on fragility of erythrocyte (18).

Conclusion

The aqueous extract of plant can be used effectively in the therapy of cases of anemia in animals in dose of (500mg/kg), such as food deficiency.

References


البحث عن التأثيرات الدوائية والسمية لمستخلص نبات الشنان في الأرانب

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الخليفة

استهدفت هذه الدراسة معرفة تأثير المستخلص المائي لنبات الشنان على المعايير الدموية في الأرانب وسميتها في الأرانب والفئران. استخدمت فيها 18 أرنب وفئراً يتراوح وزنهم 200-900 غرام وبعمر 20 أسبوع قسمتهم إلى ثلاث عشرة مجموع متساوي لمعرفة تأثير النبات على الوزن والمعايير الدموية. المجموعة الأولى اعتبرت مجتمعة سيطرة، أما المجموعتان الأخريتان فاعتبرتا 500-1000 ملم كغم يومياً، المستخلص المائي لنبات الشنان (كجرعة أمنية) عن طريق الفم بواسطة ابتعدي معدني ومدة ثمانية أسابيع متتالية. أظهرت النتائج أن المستخلص المائي لنبات الشنان للأرانب المعالجة أدى إلى زيادة معنوية في وزن الجسم وكمية الهيموغلوبيين وإعداد كريات الدم الحمر وحجم الخلايا المرصوصة. وقفة في إعداد كريات الدم البيضاء في الأرانب في المجموعة التي تم إعطاؤها 1000 ملم/كغم ممن المستخلص ؛ في حين لم يحصل أي تأثير معنوي على الخلايا التفريغية لكريات الدم البيضاء. تم التقصي عن سمية نبات الشنان من خلال دراسة بعض المعايير الكيميائية مثل إنزيمات الكبد والدهليزات السنية للأعضاء الحيوانية مثل (الرئة، الكبد، الكلية، المعده، الأمعاء، الطحال) بالاستدمار بجرعة 1000 ملم/كغم عن طريق الفم لمدة 12 أسبوع كذلك لم تلاحظ أي وفيات عند دراسة السمية الحادة للفئران خلال 15 يوم.