Effect of Flavonoids Extracted from Hawthorn (*crataegus oxyacantha*) on some hematological parameters of female mice

A. I. O. Al-Abdaly

College of Veterinary Medicine / University of Baghdad

Abstract

This experiment was designed to study the effect of flavonoid extracted from Hawthorn (*crataegus oxyacantha*) on some hematological parameters in female mice. The experiment was performed on fifteen female mice which were divided randomly into three equal groups (5/group) and were treated daily as follows for four weeks: the first group was received distilled water and served as control, mice of the second group (T<sub>1</sub>) was administrated 9 mg /kg B.W. flavonoids and the third group (T<sub>2</sub>) was administrated 18 mg /kg B.W. flavonoids for four weeks. Blood samples were collected at the end of the experiment to study the following parameters:-RBCs count, PCV ratio, Hb concentration, differential leukocytes count, MCV, MCH and MCHC. The results showed that animals treated with flavonoids extracted from Hawthorn (T<sub>1</sub> and T<sub>2</sub>) have not significant differences (P>0.05) in RBC count, MCV and MCH, as compared to control. There was significant decrease in packed cell volume in both treated groups as compared to control but there was significant increment (P<0.05) in Hb concentration of third group ( T<sub>2</sub>) as compared to second (T<sub>1</sub>) and control groups. The results also showed marked increases of the MCHC percentage in both second and third treated group as compared to control. The effect of flavonoids extracted from hawthorn showed significant decrease of neutrophil percentage and significant increase of lymphocyte percentage in treated groups as compared to control, while there was no significant increase in the percentage of monocytes and eosinophils.

**أثر الفلافونويدات المستخلصة من ثمار الزعورر على بعض المعايير الدموية في إناث الفئران**

أناهذ إيبراهيم عبد العبدلي

كلية الطب البيطري/ جامعة بغداد

**الخليصة**

صممت هذه التجربة لدراسة تأثير الفلافونويدات المستخلصة من الزعورر على بعض المعايير الدموية في إناث الفئران. تم استخدام خمس عشرة فأرة قسمت بصورة عشوائية إلى ثلاث مجموعتين متساويتين (5/مجموعة) وعدت المجموعة الأولى كمجموعة سيطرة، وجرعت المجموعة الثانية (T<sub>1</sub>) بجرعة 9 ملغ/كم من وزن الجسم والمجموعة الثالثة (T<sub>2</sub>) بجرعة 18 ملغ/كم من وزن الجسم من الفلافونويدات المستخلصة من ثمار الزعورر. عن طريق الفم يوميا لمدة أربعة أسابيع. تم جمع نماذج الدم لدراسة المعايير التالية: العدد الكلي للكرائات الدم الحمراء، حجم الخلايا المرسومة، تركيز الهيموغلوبين، العد التفريقي للخلايا الدموية البيضاء، كمية خضاب كريات الدم، تركيز خضاب كريات الدم. لم تظهر النتائج أي فروقات معنوية (0.05>P) في معدلات أعداد كريات الدم الحمراء، حجم الكريات الدم الحمراء، كمية خضاب كريات الدم في المجموعتين المعاملتين مقارنة بمجموعة السيطرة. كما أشارت النتائج إلى وجود انخفاض معنوي (0.05>P) في النسبة المئوية لحجم الخلايا المرسومة في المجموعتين المعاملتين بفلافونويدات الزعورر مقارنة بالسيطرة. بينما لوحظ وجود زيادة معنوية (0.05>P) في
Introduction

Crataegus oxyacantha is the biological name for the plant commonly known as “Hawthorn”. It belongs to the Rosaceae family. Hawthorn (crataegus) is an odorless, thorny, deciduous tree that can grow up to 10 meters high (1), at altitudes of 180-300 meters (2). The leaves are lobed with white flowering tops that end in a red berry fruit (1). Active ingredients found in hawthorn include, flavonoids (such as vitexin, rutin, quercetin, and hyperoside), oligomericproanthocyanidins (OPCs, such as epicatechin, procyanidin, and particularly procyanidin B-2), flavone-C, triterpene acids (such ursolic acid, oleanolic acid, and crataegolic acid), and phenolic acids (such as caffeic acid, chlorogenic acid, and related phenolcarboxylic acids), Saponins and Tannins, Vitamin C, Cratetegin (most prevalent in flowers, leaves, berries), (3,4,5,6). The recommended daily dose of hawthorn is 160-900 mg of a native water-ethanol extract of the leaves or flowers (equivalent to 30-169 mg of epicatechin or 3.5-19.8 mg of flavonoids). (7, 8, 9, 10, 11, 12). Hawthorn has recently been shown antioxidant properties (13, 14, 15). Hawthorn also exhibits anti-inflammatory property by preventing synthesis and release of inflammatory promoters such as histamines, serine proteases, prostaglandins, leukotrienes etc., as well as, inhibiting enzymatic cleavage by enzyme secreted by leukocytes during inflammation (16), it has mild to moderate sedative effect has been demonstrated in humans and animal studies with hawthorn constituents and OPC’S are reported to be partially responsible for this effect (17,18). Today, hawthorn is used primarily for various cardiovascular conditions. The cardiovascular effects are believed to be the result of positive inotropic activity, also exert considerable collagen-stabilizing effects, enhancing integrity of blood vessels wall and improve coronary blood flow, and positive effects on oxygen utilization. Flavonoids are postulated to account for these effects. (3, 8, 12, 19, 20). The aim of this study is to examine the effect of flavonoids of crataegus oxyacantha on some hematological parameters.

Materials and Methods

The method of Harbone (1984) (21), was used for the extraction of flavonoids .to 100 gm of hawthorn berry after removing of the seed and mixing with mixture in one liter conical flask, 200 ml of 2N HCL was added and covered by aluminum foil then mixed and boiled in a water bath at 100ºC for 45 minutes (to complete hydrolysis) with a gentle mixing every 15 minutes intervals then the mixture was cold to 25-27 ºC and filtered under vacuum using whatman No.2 Filter paper. The filtrate was transferred into separatory funnel and the caratinoids, chlorophyll and waxes were separated from the filtrate by 100 ml petroleum ether using 25 ml at each interval. Then, flavonoid was extracted from the filtrate residues by 100 ml ethyl acetate in which 25 ml was used at each interval. Finally, flavonoids were dried under vacuum using rotary evaporator at 40±2 ºC; purified flavonoid was weighted and kept in a dark glass container at -20ºC till use. fifteen adult female mice were randomly divided into three groups (5 mice/ group) and treated as follows for four weeks: animals in group one had free access to food and water and served as control; group two (T1) animals in this group were subjected to oral
intubation of 9 mg/kg B. W. flavonoids extracted from hawthorn, while animals in group three received orally 18 mg/Kg B. W. flavonoids. Blood samples were collected by heart puncture technique for measuring the following parameters: Total red blood cells count (RBCs), packed cell volume (PCV%) according to (22,23,24), estimation of hemoglobin concentration (26), Mean corpuscular volume (MCV).

\[
Mcv = \frac{PCV\%}{\text{Total RBC count}} \times 10
\]

\[
(MCH = \frac{Hb\text{ concentration (g/dl)}}{\text{Total RBC count}} \times 10)
\]

Mean corpuscular hemoglobin (MCH)

\[
(MCHC\% = \frac{Hb\text{ concentration (g/dl)}}{PCV\%} \times 100)
\]

Mean corpuscular hemoglobin concentration (MCHC) count according to (25, 26). Differences between experimental groups were evaluated by using one way analysis of variance (ANOVA). Specific group differences were determined using least significant differences (LSD). For all analysis of P value 0.05 were considered to be significant (27).

Results

The yield of crude flavonoids extracted from crataegus oxyacantha samples revealed that out of each 100 gm Hawthorn berry, approximately 0.2 gm of crude flavonoids was obtained. The effect of flavonoids extracted from crataegus oxyacantha on blood picture of female mice was shown in tables (1, 2, 3). Data pertaining to red blood cells count, packed cell volume and hemoglobin concentration of control and flavonoids treated groups are depicted in Table (1). The results revealed that non significant differences (P>0.05) in total red blood cells count but there was significant decrease in packed cell volume in two treated groups as compared to control. On the other hand there was significant increase (P<0.05) in hemoglobin concentration in group treated with 18mg/kg B. W. of hawthorn flavonoids as compared to (T1) and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Red blood cells /mm3</th>
<th>Packed cell volume (%)</th>
<th>Hemoglobin concentration (gm / dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.78±0.65 A</td>
<td>45.4±1.20 B</td>
<td>14.21±0.34 A</td>
</tr>
<tr>
<td>T1 9mg flavonoids /kg B.W.</td>
<td>6.56±0.21 A</td>
<td>38.6±0.67 A</td>
<td>14.84±0.35 A</td>
</tr>
<tr>
<td>T2 18mg Flavonoids /kg B.W.</td>
<td>7.13±0.62 A</td>
<td>41.0±0.44 A</td>
<td>16.09±0.20 B</td>
</tr>
</tbody>
</table>

Values expressed as means ± SE. n= 5 / group.
Capital letters denote between groups differences, P<0.05 vs. control.

Table (1) Effect of flavonoids of crataegus oxyacantha on Red blood cells count, packed cell volume and hemoglobin concentration in female mice

The effect of oral intubation of flavonoids extracted from hawthorn on MCV, MCH, and MCHC in adult female mice was explained in table (2) showed significant increase (P<0.05) in the Mean corpuscular hemoglobin concentration (MCHC) in the groups T1 treated with 9 mg/kg B.W. and T2 treated with 18 mg/kg B.W. of hawthorn flavonoids respectively as compared with control. On the other hand there were non significant differences (P>0.05) in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) between the groups.
Table (2) effect of different doses of *crataegus oxycantha* flavonoids on MCV, MCH, MCHC, of female mice

<table>
<thead>
<tr>
<th>Group</th>
<th>MCV (vito liter)</th>
<th>MCH ( pico gram )</th>
<th>MCHC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.14± 8.45</td>
<td>A</td>
<td>18.95±2.04</td>
</tr>
<tr>
<td>T1 9 mg/kg B.W. of Flavonoids</td>
<td>59.07±2.48</td>
<td>A</td>
<td>22.71±0.98</td>
</tr>
<tr>
<td>T2 18 mg/ kg B.W. of Flavonoids</td>
<td>59.06±4.46</td>
<td>A</td>
<td>23.25±1.97</td>
</tr>
</tbody>
</table>

Values expressed as means ± SE. n= 5 / group. Capital letters denote differences between groups, P<0.05 vs. control.

The effect of two doses of flavonoids of *crataegus oxyacantha* on percentage of differential leukocytes count were observed in table (3). Significant suppression in the percentage of neutrophils in, two treated groups (T1, T2) As compared to control, meanwhile treatment of animals with flavonoids cause significant increase (P<0.05) of lymphocytes percentage of T1and T2 comparing to control. Finally there were non significant differences (P>0.05) in the percentage of monocytes andesinophils between treated groups and control.

Table (3) Effect of *crataegus oxycantha* flavonoids on differential leukocytes count in female mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neutrophil %</th>
<th>Lymphocyte%</th>
<th>Monocyt%</th>
<th>Eosinophil%</th>
<th>Basophil%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.57±2.31</td>
<td>B</td>
<td>55.87±2.40</td>
<td>A</td>
<td>22.81±3.33</td>
</tr>
<tr>
<td>T1 9 mg/kg B.W. Of Flavonoids</td>
<td>8.23±1.02</td>
<td>A</td>
<td>75.47±1.22</td>
<td>B</td>
<td>15.41±1.87</td>
</tr>
<tr>
<td>T2 18 mg/ kg B.W. of Flavonoids</td>
<td>5.88±0.87</td>
<td>A</td>
<td>78.21±3.59</td>
<td>B</td>
<td>15.44±3.12</td>
</tr>
</tbody>
</table>

Values expressed as means ± SE. n= 5 / group. Capital letters denote between groups differences, P<0.05 vs. control.

**Discussion**

*Crataegus oxyacantha* has significant activity because of the high content of flavonoid compounds, particularly the OPCs; it increases coronary blood flow, enhancing oxygen flow and utilization by the heart. Crataegus extracts also have a positive inotropic effect on the contraction amplitude of myocytes. Due its flavonoid content, hawthorn exerts considerable collagen stabilizing effects, enhancing integrity of the blood vessels (28, 29, 30). The results obtained in the present study clearly show that, the flavonoids extracted from *crataegus oxyacantha* were effectively improving some parameters of blood homeostasis in female mice. There were no significant effect in total erythrocytes count, MCV, and MCH, significant decrease in hematocrit ratio and significant increase in hemoglobin concentration and MCHC percentage. Hawthorn, as had been mentioned by many authors, can significantly reduce the amount of fibrinogen, decreased blood viscosity, plasma and serum viscosity, RBC aggregation index and lower RBC hematocrit, indicate that Hawthorn can improve blood stasis state (31, 32). The hawthorn fruit and methanolic extract of this herb showed significant increased of hemoglobin (33, 34). Consequently the MCHC will be increased (24, 35). This investigation also pointed to the role of flavonoids extracted from *crataegus oxyacantha* on differential leukocytes count especially neutrophils and lymphocytes, the results showed significant decreased in neutrophils percentage and significant increased in lymphocytes percentage. It has been found that howthorn exert as anti-inflammatory effect by preventing synthesis and release of inflammatory promoters such as histamines, serine proteases, prostaglandins, leukotrienes (16, 17, 36). Besides,
hawthorn inhibited enzymatic cleavage by Myeloperoxidase, which present in
neutrophils and has been successfully used to confirm inflammatory cell
activation during inflammation (37, 38, and 39). Lymphocytes participate in specific
immune responses (35). The increased lymphocytes due to the hawthorn, particularly its
flavonoids constituents with antioxidative activity, reduced the oxidative stress and
genotoxicity induced by toxic compounds, protection lymphocyte from genetic damage
(40) in addition to flavonoids, hawthorn is rich in minerals and contain a small amount
of active principle oligomeric procyanidine (1-epicatechol) which improved immunity
and increased lymphocytes (41). It is concluded from this study that flavonoids
extracted from hawthorn fruits maintained blood homeostasis, improved immunity and
possessed anti-inflammatory effect.

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