Effect of Aqueous Extract of Fig (*Ficus carica*) Fruit on Some Hematological Parameters in Female Rabbits

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Abstract:

The effect of the aqueous extract of fig (*Ficus carica*) fruit was studied by using different concentrations of this fruit (15, 20 and 25 mg/kg of body weight) on some hematological parameters (hemoglobin concentration, packed cell volume, white blood cell count and platelets count) in female rabbits.

Twenty female rabbits were randomly divided into four groups (five animals in each group). Three groups were dosed with the concentrations mentioned above, while the last was administered with distilled water and considered as control group. These animals were orally dosed by aqueous extract using a micropipette.

The results showed that there was a significant ($P<0.05$) increase in hemoglobin concentration and packed cell volume means for all groups treated with aqueous extract of fig fruit compared with the control animals. In addition, there was a significant ($P<0.05$) increase in white blood cells count at levels of 20 and 25 mg/kg of body weight compared with the control group while there was no significant difference between 15 mg/kg group and control group. Concerning the platelets count, the results showed that there was a significant ($P<0.05$) increase at level of 25 mg/kg of body weight compared with the control group while there was no significant difference at both levels of 20 and 15 mg/kg of body weight as compared with control group.

It was concluded from this study that the effect of aqueous fig extract in hematopoiesis and improving blood parameters in female rabbits.

Key words: fig, *Ficus carica*, hemoglobin, packed cell volume, white blood cells, platelets

Introduction:

Fruit and vegetable consumption have been shown by wide epidemiological studies to reduce the risk of chronic diseases such as cancer, heart disease and stroke [1]. In addition, there is an inverse relationship between fruit and vegetable intake and blood pressure. A diet rich in fruits and vegetables has recently been found favorably to affect serum antioxidant capacity and protect against lipid peroxidation [2].

Among these fruits, the cultivated fig, *Ficus carica* L., which is distributed in the tropics and subtropics and clearly of greatest importance as a source of human food. Fig contains high amounts of carbohydrates and rich in many mineral elements such as calcium, phosphorus, iron, sodium and potassium. It also contains phenol antioxidants (flavonoids, monophenols and polyphenols) which are at least partly responsible for its beneficial

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effects on heart disease and cancer [2, 3].

In addition, fig has many medicinal uses, for example; it is used in inflammations and paralysis [2]. It is also claimed to be useful for liver and spleen disorders, to cure piles and in treatment of gout. Locally the leaves are being used in the treatment of jaundice and taken as a remedy for diabetes [4]. Figs are used in the treatment of obesity may be of help with weight reduction [4, 5].

Recently the extract of *Ficus carica* was tested for hepatoprotective in rats treated with rifampicin (hepatotoxic agent) and this appears to support traditional use of the medicinal plant in the treatment of liver diseases [5].

The present study was conducted to study the effect of aqueous extract of fig fruit on some of the hematological aspects (hemoglobin concentration, packed cell volume, total white blood cells count and platelet count) in female rabbits.

Materials and Methods:

Preparation of extract

Fig, *Ficus carica* fruits were washed, cut into small pieces, dried in oven at 40° C. (the fig pieces were dried at this temperature in order to conserve the active materials in fig) and ground. The powder was mixed with five fold of distilled water. Then, the mixture was put in the reflex at 100° C temperature. for three hours. After that, the extract was filtered and put in the rotary evaporator to concentrate the fluid. Then, the crude extract was further dried in oven at 45° C [6]. After drying, the crude extract was collected and stored at -20° C until used [7].

Experimental animals

Twenty female local rabbits with an average age of about 3-3.5 months and weight between 1150 – 1600 g were used. They were bred in special cages in Al- Nahrain University Research Center for Biotechnology, fed pellets (contain 20 % crude protein and 11% crude fibre, rich in protein and energy) and given tap water *ad libitum* during the experimental period which last from February to April. Concerning conditions of the laboratory, average temperature was about 21 - 24 °C and the light cycle was divided into 12 hours light: 12 hours dark [8].

Doses and design of the experiment

Female rabbits were orally dosed by aqueous extract by micropipette. The powder was mixed with distilled water to prepare the different doses of extract. The volume of administrated dose was 1ml/ day for 30 days. These doses were determined through the amount of effective dose for human. The effective dose was 700 mg/ kg of body weight for fig extract [9]. Therefore, it was selected the concentrations 15, 20 and 25 mg/ kg of body weight.

The animals were randomly divided into four groups (five animals in each group). The first, second and third groups were dosed with 15, 20 and 25 mg/kg of body weight, respectively, while the last group was considered as a control and daily administrated with 1 ml distilled water.

Collection of blood sample

After the period of dosing was elapsed (30 days), blood was collected by heart puncture. The volume of collected blood was approximately 2 ml and was collected in tubes containing K_2EDTA to estimate the values of hemoglobin concentration, packed cell volume, total white blood cells count and platelets count [10, 4]. After blood collection in K_2EDTA tubes, it was slowly expressed into the

vial to reduce the risk of hemolysis after removing of the needles from syringes [11].

Hematological parameters

Packed cell volume (PCV) or hematocrit

Immediately after collection the blood, PCV was determined by the microhematocrit method using hematocrit capillary tubes and microhematocrit centrifuge [10, 11].

Hemoglobin concentration (Hb)

Hemoglobin concentration was determined using kit (Crescent Diagnostics, Saudi Arabia) which depends on the cyanmethaemoglobin method [11].

Total white blood cells count

To do this, a hemocytometer and the white cell counting pipette that dilutes the cells 20 times were used. The blood was diluted with the white blood cells diluting fluid (Türk's solution). It was prepared by mixing 2 ml of glacial acetic acid and 98 ml of distilled water with adding 2 drops of methylene blue [11, 12]. The general formula is used:

$$\text{WBCs} / \mu L = \frac{\text{Cells counted in 4 squares X Dilution factor of the blood (200)}}{\text{Volume (0.4 } \mu L)}$$

Platelet count

The platelet count was performed using red cells counting pipette and hemocytometer. The blood was diluted with Rees-Eker solution. It was prepared by dissolving 32 gm of tri-sodium citrate in 1000 ml of distilled water, and then the solution was mixed with 10 ml of formalin [11]. The following formula is usually used:

$$\text{Plaletets count} = \frac{\text{Average number of platelets per mm}^2 \text{ X 200}}{0.1\ \mu L}$$

Statistical analysis

The results were analyzed statistically using analysis of variance (ANOVA) applicable to a completely randomized design. Then, the significance among means was tested depending on Duncan Multiple Range Test using SPSS program [13, 14].

Results and Discussion:

Table (1) illustrates the effect of aqueous extract of fig on means of hemoglobin concentration and packed cell volume means in female rabbits. The results show that there was a significant ($P<0.05$) increase in hemoglobin concentration for the three treated groups compared with control animals. The hemoglobin concentration mean was 13.584 g/dl in control group, while it was 15.354, 16.185 and 16.560 g/dl in groups treated with 15, 20 and 25 mg/kg of body weight, respectively. The results also show that there was a significant ($P<0.05$) increase in the packed cell volume in treated animals compared with control group. The means were 47.063, 49.555 and 50.681 %**,** while the PCV mean was 41.752 % for control group. No significant differences in both parameters among treated groups were observed.

Table (1): Effect of aqueous extract of fig on hemoglobin concentration and packed cell volume of female rabbits (Mean ± SE).

Treated groups	Hemoglobin concentration (g/ dl)	Packed cell volume (%)
15 mg/kg	b 15.354 ± 0.394	b 47.063 ± 0.181
20 mg/kg	b 16.185 ± 0.570	b 49.555 ± 1.711
25mg/kg	b 16.560 ± 0.443	b 50.681 ± 1.330
Control	a 13.584 ± 0.544	a 41.752 ± 1.633

* Similar letters indicated that there were no significant differences between treatment groups and different letters indicated that there were significant differences between treated groups at $p< 0.05$.

The significant increase in hemoglobin concentration and packed cell volume in the animals treated with aqueous extract of fig fruit compared with the control might be related to the small content of iron in fig fruit (about 3 mg per 100 gm of fig) [15]. Furthermore, it has been found that in each 100 gm of fig, there are 4.3 and 1.3 gm of protein in dried and fresh portion respectively [16]. The iron and protein are essential elements in hemoglobin synthesis [17]. It was estimated that only small amounts of iron are required in the daily diet to replace the small amounts lost in urine and feces [18]. The iron is recycled within the process of breakdown of hemoglobin by macrophages in the liver and spleen. The globin part is broken down into amino acids that are reused to produce other proteins. The iron is released from here and can be used to produce new hemoglobin molecules in the bone marrow [17, 18]. Therefore, this small amount of iron and protein in the fig extract might caused this significant increase in hemoglobin concentration and packed cell volume.

The results also show (Table 2) that there was a significant ($P<0.05$) increase in the white blood cells count at the level of 20 and 25 mg/kg of body weight (group 2 and 3) compared with control group. The white blood cell count mean was 9.640 and 9.760 $X10^3$cell/µL, respectively. There was no significant difference at the level of 15 mg/kg of body weight (group 1) as compared with control group. The white blood count mean was 9.240 $X10^3$cell/µL in 15 mg/kg of body weight while it was 8.640 $X10^3$cell/ µL in control group. The increase in the white blood cells count in animals treated with the aqueous extract of fig fruits might be related to the presence of phenolic compounds (such as, furanocomarins, Psoralens, angelicin and carotenoids) in fig which act as immunostimulatory agents (immunopotentiation or strengthening of the immune reactions) [19]. The immunostimulant activity boosts the body's macrophage response, which stimulates the lymphocytic system, and boosts production of white blood cells [19, 20]. The changes in the white blood cell count were within the normal range, however, the increase in the leukocyte counts outside the normal range may be an indication that the blood cell production increases in attempt to combat the toxin assault in the diets, since leukocytes are known to be among body defense mechanisms that fight against non-self or pathogenic organisms [21].

Concerning the platelets count, the results also show (Table 2) that there was a significant ($P<0.05$) increase in platelets count mean at the level of 25 mg/kg of body weight (group 3) but there was no significant difference at the level of 20 and 15 mg/kg of body weight (group 2 and 1) compared with control group. The platelets count means were 373.6, 392, 383.2 and 380.8 $X10^3$cell/µL for control, 25, 20 and 15 mg/kg of body weight, respectively.

In rabbit models, thrombocytes (platelets) and hematocrit (packed cell volume) have been shown to be

independent variables which influence haemostasis. Additionally metabolically active erythrocytes have been shown to enhance the platelet release reaction, eicosanoid synthesis and further platelet recruitment. Erythrocytes provide agonists for platelet action such as adenosine diphosphate (ADP) which facilitates platelet accretion to the endothelium and enhance platelet activity [22].

Recently, the fig fruit was found to be rich in fatty acids and vitamins. In addition, the non-essential fatty acids such as palmitic, stearic and oleic acids and essential fatty acids such as linoleic and linolenic acids were also identified in this fruit. Therefore, the fig fruit was confirmed to have important nutrients in term of human nutrition rather than just a fibrous fruit [16]. These elements are necessary for the process of blood cell formation (hematopoiesis) which occurs in red bone marrow and gives rise to the cell lines that produce the formed elements [17, 18].

From above, it could be concluded that the potency of aqueous fig extract in improving the blood parameters and affecting the hematopoiesis in female rabbits.

Table (2): Effect of aqueous extract of fig on white blood cell and platelets count of female rabbits (Mean ± SE).

Treated groups	WBC count ($X10^3$ cell/µL)	Platelets count ($X10^3$cell/µL)
15 mg/kg	a b 9.240 ± 0.248	a 380.8 ± 4.127
20 mg/kg	b 9.640 ± 0.172	a b 383.2 ± 2.416
25mg/kg	b 9.760 ± 0.172	b 392 ± 3.033
Control	a 8.640 ± 0.292	a 373.6 ± 4.166

* Similar letters indicated that there were no significant differences between treatment groups and different letters indicated that there were significant differences between treated groups at $p< 0.05$.

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تأثير المستخلص المائي لثمرة التين *Ficus carica* في بعض صفات الدم لإناث الأرانب

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الخلاصة:

تمت دراسة تأثير المستخلص المائي لثمرة التين و بتراكيز مختلفة (15و 20و25 ملغم/ كغم من وزن الجسم) في بعض صفات الدم (تركيز الهيموغلوبين, وحجم الخلايا المضغوطة, وعدد كريات الدم البيض, وعدد الصفيحات)لإناث الأرانب المحلية.

استخدمت في البحث عشرون انثى من الأرانب قسمت عشوائيا على أربع مجموعات طبقا للتراكيز المذكورة سابقا فضلا عن مجموعة السيطرة وبواقع خمسة حيوانات في المجموعة الواحدة. جرعت المجاميع الثلاث الأولى بالتراكيز المذكورة سابقا من المستخلص فيما جرعت المجموعة الرابعة بالماء المقطر بوصفها مجموعة سيطرة باستخدام الماصة الدقيقة ولمدة 30 يوما.

أظهرت النتائج أن هناك ارتفاعا معنويا ($P<0.05$) في معدل تركيز الهيموغلوبين وحجم الخلايا المضغوطة للمجموعات الثلاث الاولى مقارنة بالسيطرة.كما تبين ان هناك ارتفاعا معنويا ($P<0.05$) في عدد كريات الدم البيض في الحيوانات المعاملة بالتركيزين 20 و 25 ملغم/ كغم من وزن الجسم مقارنة بالسيطرة, فيما لم يظهر هناك أي فرق معنوي في المجموعة المعاملة بالتركيز 15 ملغم/ كغم. أما فيما يتعلق بعدد الصفيحات الدموية فقد وجد ان هناك ارتفاعا معنويا ($P<0.05$) في المجموعة المعاملة بالتركيز 25 ملغم/ كغم من وزن الجسم مقارنة بالسيطرة, كما لم يظهر أي فرق معنوي في المجموعتين المعاملتين بالتركيزين 20 و 15 ملغم/ كغم من وزن الجسم.

يمكن الاستنتاج من هذه الدراسة أن للمستخلص المائي لثمرة التين تأثيرا في الاتزان الدموي وتحسين بعض صفات الدم في اناث الأرانب.

الكلمات المفتاحية: ثمرة التين, الهيموغلوبين, حجم الخلايا المضغوطة, كريات الدم البيض, الصفيحات.