Effect of Garlic Powder (*Allium sativum*) on Blood Constituents in Male Rabbits

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Abstract

The effect of garlic powder on blood constituents in male rabbits was studied. These constituents were hemoglobin concentration, packed cell volume, red blood cell count, total and differential white blood cell count, platelets count and red cell indices. Fifteen male rabbits were randomly divided into three groups (five animals in each group). Two groups were supplied with pellets containing 1 and 5% garlic powder. The last group was considered as control and supplied with pellets and water. The period of experiment was lasted for 21 days. The results showed that there was a significant ($P \leq 0.05$) increase in the means of hemoglobin concentration, packed cell volume, platelets count, total white blood cells count, lymphocytes count, neutrophils count and mean corpuscular hemoglobin concentration in the group treated with 5% garlic powder compared with control group, while, there was no significant differences in these mentioned parameters at the treated group with 1% garlic powder compared with control group. In addition, there was a significant ($P \leq 0.05$) increased in red blood cells count at 1% and 5% garlic powder compared with control group. On the other hand, there were no significant differences in the means of monocytes count, eosinophils count, basophils count, mean corpuscular hemoglobin and mean cell volume in the treated groups with 1% and 5% garlic powder compared with control group.

Keywords**:** garlic, hemoglobin, packed cell volume, blood cells, platelets.

Introduction

Spices such as curry, garlic, cinnamon, cumin and ginger, have been used by man for the treatment of various diseases [1]. Garlic is one of the oldest cultivated plants [2]. The virtues of garlic (*Allium sativum*), as a medicinal plant, are known to most cultures of the world belongs to the family liliaceace [3, 4, 2]. It has been shown to have several effects in the body. This includes inhibition of platelet aggregation, reduction of arterial blood pressure and prevention of fat infilteration of the liver [3, 5]. Furthermore, it has been used for the treatment of digestive disorders, infestation with worms and renal disorders, as well as to help mother during difficult childbirth [6, 1].

Garlic is reported to contain volatile oil, allin (S-allyl-L-cysteine sulfoxide), S-methyl-L-cysteine sulfoxide and allinase [6]. Other major compounds present are diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide and allyl methyl disulphide [٦]. Allicin, diallyl disulfide-oxide, an active ingredient released from garlic is a systemic vasodilator [6, 4, 5].

Due to scanty information on the effects of garlic on hematological parameters, this study is undertaken to investigate the influence of garlic on certain hematological parameters in normal rabbits at varying concentration.

Materials and Methods

Experimental animals

Fifteen male local rabbits with an average age of about 2.5-3 months and weighting between 1200–1400 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. Concerning conditions of the laboratory, average temperature was about 22-25°C. and the light cycle was divided into 12 hours light: 12 hours dark [7, 8].

Design of the experiment

The animals were randomly divided into three groups (five animals in each group). The first group was considered as control and supplied with pellets and water. The second group was supplied with food (pellets) containing 1% garlic powder. The third group was supplied with food (pellets) containing 5% garlic powder. The pellets were supplied once

daily for each group and the period of experiment was lasted for 21 days.

Blood sample collection

After the period of dosing was elapsed (21 days), blood was collected by heart puncture. The volume of collected blood was approximately 2 ml and was collected in tubes containing K2EDTA anticoagulant to determine the values of hemoglobin concentration, packed cell volume, red blood cells count, total white blood cells count, differential white blood cell count, platelets count and red cell indices [7, 3]. The blood was slowly expressed into K2EDTA tubes to reduce the risk of hemolysis after removing of the needles from syringes [8]

Hematological parameters

Packed cell volume (PCV)

Immediately after collecting the blood, PCV was determined by the microhematocrit method using hematocrit capillary tubes, microhematocrit centrifuge and hematocrit reader [7, 8].

Hemoglobin concentration (Hb)

Hemoglobin concentration was determined using kit (Crescent Diagnostics, Saudi Arabia) which depends on the cyanmethaemoglobin method. Hemoglobin is converted into cyanmethaemoglobin under the influence of potassium ferricyanide and potassium cyanide. [8].

Red blood cells count

The hemocytometer and the red cell counting pipette that dilutes the cells 200 times were used. The Hayem's solution was used to dilute the blood sample [8]. The following formula was used to calculate RBC count:

$$\text{RBC (cell/}\mu\text{L)} = \frac{\text{N}}{80} \times 400 \times 200 \times 10$$

N= the total RBCs counted.

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 [8]. The following formula was used to calculate WBC count:

$$\text{WBC (cell/}\mu\text{L)} = \frac{\text{N}}{4} \times 20 \times 10$$

N= the total WBCs counted in the four corner squares of hemocytometer.

Differential white blood cell count

Differential WBC count was determined by counting 100 cells on blood smears stained with Leishman's stain. The absolute number of each type of leukocyte was calculated from the data on the total and differential WBC counts [10, 11].

Platelet count

The platelet count was performed using red cells counting pipette and hemocytometer. The blood was diluted with Rees-Eker solution [8, 11].

Mean corpuscular hemoglobin concentration (MCHC)

The mean corpuscular hemoglobin concentration was calculated using the formula according to [10]:

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/100 ml)}}{\text{PCV (\%)}} \times 100$$

Mean corpuscular hemoglobin (MCH)

The mean corpuscular hemoglobin was calculated using the formula according to [10]:

$$\text{MCH (pg/cell)} = \frac{\text{Hb (g/100 ml)}}{\text{RBC count (X10}^6\text{cell/}\mu\text{l)}} \times 10$$

Mean corpuscular volume (MCV)

The mean corpuscular volume was calculated using the formula according to [10]:

$$\text{MCV (fL)} = \frac{\text{PCV (\%)}}{\text{RBC count (X10}^6\text{cell/}\mu\text{l)}} \times 10$$

Experimental design and statistical analysis

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 [12].

Results and Discussion

Table (1) illustrates the effect of garlic powder on means of hemoglobin concentration, packed cell volume, red blood cell count and platelets count in male rabbits. The results demonstrated that there were a significant ($P \leq 0.05$) increased in hemoglobin

concentration, packed cell volume and platelets count in the treated animals with 5% of garlic powder, and no significant differences in these mentioned parameters in the treated animals with 1% of garlic powder compared with control group. The hemoglobin concentration mean was 12.90 mg/dl in control group, while it was 13.34 and 13.66 mg/dl in treated groups with 1 and 5% garlic powder, respectively. The packed cell volume mean was 39.70 in control group, while they were 41.02 and 41.98% in treated groups with 1 and 5% garlic powder, respectively. Concerning the means of platelets count, they were 370.60, 374.60 and 377.60 $X10^3$cell/µl in the control, 1% and 5% garlic powder groups, respectively.

The results also showed that there was a significant ($P\leq0.05$) increased in red blood cell count in the treated groups with 1% and 5% garlic powder compared with control group. The RBC count mean was 5.44 $X10^6$cell/µl in control group, while, they were 5.76 and 5.94 $X10^6$cell/µl in treated groups with 1% and 5% garlic powder, respectively.

Table (1)
Effect of garlic powder on hemoglobin concentration, packed cell volume, red blood cell and platelets counts in male rabbits.

Treatments	*Hemoglobin concentration (mg/dl)*	*Packed cell volume (%)*	*RBC count ($X10^6$cell/µl)*	*Platelets count ($X10^3$cell/µl)*
Control	a 12.90 ± 0.33	a 39.70 ± 0.99	a 5.44 ± 0.16	a 370.60 ± 2.06
1% Garlic powder	ab 13.54 ± 0.21	ab 41.62 ± 0.64	b 6.04 ± 0.15	ab 374.60 ± 1.43
5% Garlic powder	b 14.06 ± 0.23	b 43.18 ± 0.68	b 6.32 ± 0.20	b 377.60 ± 1.25

Values are means ± SE.
Similar letters indicate no significant differences and different letters indicated significant differences at P ≤ 0.05.

On the other hand, Table (2) revealed that no significant differences in MCH and MCV in the treated groups with 1% and 5% garlic powder compared with control group. The MCH were 23.75, 23.20 and 23.72 pg/cell, while, the MCV were 73.09, 71.31 and 70.81 fL. Furthermore, there was a significant ($P\leq0.05$) increased in MCHC at the treated group with 5% garlic powder and no significant difference at the treated group with 1% garlic powder compared with control group. The MCHC were 32.48, 32.52 and 32.56 g/dl in control, 1% and 5% garlic powder, respectively.

Table (2)
Effect of garlic powder on red blood cell indices in male rabbits.

Treatments	*MCHC (g/dl)*	*MCV(fL)*	*MCH (pg/cell)*
Control	a 32.48 ± 0.02	a 73.09 ± 1.71	a 23.75 ± 0.56
1% Garlic powder	ab 32.52 ± 0.01	a 71.31 ± 0.73	a 23.20 ± 0.23
5% Garlic powder	b 32.56 ± 0.01	a 70.81 ± 1.74	a 23.72 ± 0.26

Values are means ± SE.
Similar letters indicate no significant differences and different letters indicate significant differences at P ≤ 0.05.

The results in Table (3) demonstrated that there was a significant (P≤0.05) increase in the total white blood cells, lymphocytes and neutrophils counts at 5% garlic powder group compared with control group. The means of these parameters at 5% garlic powder was 8.90, 5.88 and 0.34 X10³cell/µl, respectively. While, they were 8.36, 5.42 and 0.28 X10³cell/µl, respectively, in control group.

In addition, there were no significant differences in the means of monocytes, eosinophils and basophils counts in the treated group with 1% garlic powder compared with control group. The means of these parameters at 1% garlic powder were 0.31, 0.14 and 0.16 X10³cell/µl, respectively, while they were 0.29, 0.15 and 0.16 X10³cell/µl, respectively in control group.

Table (3)
Effect of garlic powder on total and differential white blood cells count in male rabbits.

Treatments	*WBC count (X10³cell/µl)*	*Lymphocyte count (X10³cell/µl)*	*Monocyte count (X10³cell/µl)*	*Neutrophils count (X10³cell/µl)*	*Eosinophils count (X10³cell/µl)*	*Basophil count (X10³cell/µl)*
Control	a 8.36 ± 0.14	a 5.42 ± 0.13	a 0.29 ± 0.01	a 0.28 ± 0.01	a 0.15 ± 0.02	a 0.16 ± 0.02
1% Garlic powder	ab 8.66 ± 0.14	ab 5.76 ± 0.09	a 0.31 ± 0.01	a 0.31 ± 0.01	a 0.14 ± 0.01	a 0.16 ± 0.01
5% Garlic powder	b 8.90 ± 0.13	b 5.88 ± 0.11	a 0.30 ± 0.02	b 0.34 ± 0.01	a 0.14 ± 0.02	a 0.15 ± 0.02

Values are means ± SE.
Similar letters indicate no significant differences and different letters indicate significant differences at P≤ 0.05.

The increase in hemoglobin concentration, packed cell volume and platelets count at 5% garlic powder group as well the increased in the red blood cell count at 1 and 5% garlic powder group compared with control group may be possible related to the end product of garlic metabolism in the body that stimulates the kidney directly to cause formation and secretion of erythropoetin (a potent stimulator of the bone marrow) [3].

Moreover, it has been found that garlic has some constituents that may play a role in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow [13, 14].

In addition, the increased in these parameters may be explained as garlic plant is considered an active oxygen scavenger. It is thus possible that garlic components compete with hemoglobin in the red blood cell for oxygen resulting in hypoxia which then stimulates hemoglobin synthesis and red blood cell production [3].

Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anemia in most animals [13]. The increase in MCHC at 5% garlic powder group compared with control group blood may be attributed to a defense reaction against *A. sativum*, which occurs by stimulation of erythropoiesis [13, 14].

On the other hand, the increased in the total white blood cells count at 5% garlic powder group compared with control group came into agreement with the earlier reports in that supplementation of garlic had significantly improved leukocyte count, indicating the immunostimulant properties of garlic [4, 2, 14].

Immunostimulants attached to specific receptors on the cell surface of the phagocytes and lymphocytes activating this cell to produce some enzymes that can destroy pathogens [14]. Many defense mechanisms activated by garlic counteract the challenge infection including the production of superoxide anions against infections [2, 14].

Moreover, the increased in lymphocytes and neutrophils counts at 5% garlic powder group may be related to an increase in the production of some chemical cytokines (interferon, interleukins and complement proteins). These cytokines stimulate other arms of the immune system and increase the activity of natural killer cells as well as T- and B- lymphocytes [4, 3, 14]. Furthermore, it has been found that garlic contains a therapeutic factor (Germanium) which enhances natural killer cell and macrophage activity in experimental animals that stimulate the immune function [3, 14].

It concluded from the present study that garlic plant has a wide spectrum of actions; not only antiviral, antibacterial, antiprotozoal and antifungal but also has beneficial effects on the immune and cardiovascular systems. Thus, the consumption of garlic at culinary levels as is the practice today is beneficial.

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الخلاصة

درس تأثير مسحوق الثوم على مكونات الدم في ذكور الأرانب، وقد شملت هذه المكونات تركيز الهيموغلوبين وحجم الخلايا المضغوط وتعداد كريات الدم الحمر والتعداد الكلي والتفاضلي لكريات الدم البيض وتعداد الصفيحات الدموية وثوابت الكرية الحمراء. استخدم في البحث خمسة عشر ذكرا من الأرانب قسمت عشوائيا إلى ثلاث مجموعات وبواقع خمس حيوانات في المجموعة الواحدة. زودت مجموعتين منها بالعليقة المحتوية على ١% و٥% من مسحوق الثوم، فيما اعتبرت المجموعة الأخيرة كمجموعة سيطرة وزودت بالماء والعليقة واستغرقت مدة التجربة ٢١ يوما. أظهرت النتائج بأن هناك ارتفاعا معنويا ($P\leq0.05$) في معدل تركيز الهيموغلوبين وحجم الخلايا المضغوطة وتعداد الصفيحات والعدد الكلي لكريات الدم البيض وتعداد الخلايا اللمفية والخلايا العدلة ومعدل تركيز هيموغلوبين الكرية في المجموعة المعاملة بـ ٥% مسحوق الثوم مقارنة بمجموعة السيطرة، فيما لم يظهر هناك اختلافا معنويا في المتغيرات المذكورة للمجموعة المعاملة بـ ١% مسحوق الثوم مقارنة بمجموعة السيطرة. بالإضافة إلى ذلك فأن هناك ارتفاعا معنويا ($P\leq0.05$) في تعداد الكريات الحمر في المجموعتين المعاملتين بـ ١% و ٥% مسحوق الثوم مقارنة بمجموعة السيطرة. وفي الجانب الاخر فانه لم يظهر هناك فروق معنوية في معدلات تعداد الخلايا الوحيدة والحمضة والقعدة ومعدل هيموغلوبين الكرية ومعدل حجم الكرية في المجموعتين المعاملتين بـ ١% و ٥% مسحوق الثوم مقارنة بمجموعة السيطرة.