

## Effect of Cottonseed Extracts on Haematological Parameters of Albino Male Mice

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### الخلاصة:

تهدف الدراسة الحالية الى معرفة تأثير المستخلصات الزيتية والمائية لبذور القطن بمختلف التراكيز على معالم الدم. جرعت مجموعة من ذكور الفئران فمويًا بالمستخلص المائي [20 ملغم/كغم/يوم (مجموعة 2) و 40 ملغم/كغم/يوم (مجموعة 3)] ومجموعة اخرى جرعت بالمستخلص الزيتي [20 % (مجموعة 4) و 40% (مجموعة 5)] لمدة ستة اسابيع. أظهرت النتائج حدوث نقصان معنوي ( $P < 0.05$ ) في قيم الهيموكلوبين لفئران عوملت ب 20% و 40% مستخلص زيتي مقارنة مع السيطرة. كذلك لوحظ حصول نقصان معنوي ( $P < 0.05$ ) في النسبة المئوية لحجم كريات الدم المضغوطة في المجاميع المعاملة بكلا التركيزين من المستخلص المائي والزيتي مقارنة مع مجموعة السيطرة. بينما بينت نتائج تعداد كريات الدم الحمر والبيض عدم حدوث نقصان معنوي ( $P < 0.05$ ) في المجموعة الخامسة والسادسة مقارنة مع السيطرة. لوحظ حدوث تحسن معنوي ( $P < 0.05$ ) في تعداد الصفيحات الدموية في المجاميع م 2 وم 3 و م 5 و م 6 مقارنة مع مجموعة السيطرة. اما نتائج حساب العدد التفريقي لكريات الدم البيض فأوضحت حصول نقصان معنوي ( $P < 0.05$ ) في النسب المئوية للخلايا اللمفية وزيادة معنوية ( $P < 0.05$ ) في الخلايا العدلة في المجاميع م 2 و م 3 و م 6 مقارنة مع السيطرة. بينما اظهرت نتائج النسب المئوية للخلايا الوحيدة حدوث نقصان معنوي في المجاميع المعاملة بكلا التركيزين من المستخلص الزيتي. كذلك استخدام جرعة 40 ملغم /كغم/يوم من المستخلص المائي سببت تحسن معنوي ( $P < 0.05$ ) في النسب المئوية للخلايا الحمضة والقعدة مقارنة مع السيطرة.

### Abstract:

The objectives of the present study are to determine the effect of different concentrations of aqueous and oil extracts of cottonseed on haematological parameters. Groups of male mice were orally administered aqueous extract [20mg/kg/1 day (G2) and 40 mg/kg/day (G3)] and other groups were administered oil extract [20% (G5) and 40% (G6)] for six weeks.

The results showed that there was a significant ( $P<0.05$ ) reduction in Hb values for mice treated with 20% and 40% oil extract as compared to the control. Also, significant ( $P<0.05$ ) reduction in the percentage of PCV were noticed for both concentrations of extracts as compared to the control groups. While non significant ( $P>0.05$ ) differences were shown in RBC and WBC count among G5 and G6 as compared to G4. Significant ( $P<0.05$ ) increment in the platelet count was shown in G2, G3, G5 and G6 as compared to the control. Differential leukocyte analysis showed that a significant ( $P<0.05$ ) reduction was observed in the percentage of lymphocyte and significantly ( $P<0.05$ ) increase neutrophils in G2 and G3 as compared with G1 and in G6 as compared to G4. While, monocytes (%) were significantly ( $P<0.05$ ) decrease in G5 and G6 as compared to G4. However, 40mg/kg/day aqueous extract dose demonstrated a significant ( $P<0.05$ ) increment in the percentage of eosinophil and basophile cells as compared to G1.

### **Introduction:**

Cottonseed contains 7% moisture, 23% protein, 23% fat, 3% minerals, 24% fiber, and 43% carbohydrate <sup>[1]</sup>. Also, it contains quercetin derivatives which represent the main flavonoid species in cottonseed and rutin is one of the major quercetin glycosides, quercetin and rutin are shown to possess potent antioxidant activity <sup>[2]</sup>. Cottonseed oil has a mild, nut like taste. It is generally clear with a light golden color, but like most oils, the degree of color depends on the amount of refining <sup>[3]</sup>. The crude oil contains about 2% non glyceride materials, such as terpenoid phytoalexin, cyclopropenoid fatty acids, phospholipids, sterols, resins, carbohydrates and related pigments. The most notable terpenoid phytoalexin is gossypol <sup>[4]</sup>. Cottonseed oil is a pure source of fatty acids.

Cottonseed oil is useful for many applications starting with food application; it is used as a salad oil, used in mayonnaise, salad dressings, sauces, and marinades <sup>[5, 6]</sup>. Also, it is rich in tocopherols, these natural antioxidants have varying degrees of vitamin E activity, also contribute to its stability giving products that contain it a long shelf life <sup>[7]</sup>.

Gossypol is a pigment found in cottonseed oil, the levels of most of cottonseed pigments are reduced during the normal processing of oils to give them acceptable color, flavor and stability <sup>[8, 9]</sup>. Ingestion of gossypol or whole cottonseed extracts could bind to dietary iron in the small intestine and thus reduce its absorption and retention <sup>[10]</sup>. Moreover, haematocrit, expressed as packed cell volume, and haemoglobin, as an indicator of dietary Fe adequacy, show the physiological status of the growing animal. The aim of the present study was to determine the effects of orally administered oil and aqueous extracts of cottonseed on haemoglobin, haematocrit, RBC count, WBC count and platelet count in addition to the differential WBC (%).

**Materials and Methods:**

Healthy mature male Swiss albino mice were obtained from the animal house of the Institute of Embryo Research and Infertility Treatment, Al-Nahrain University. The average weight of mice was  $24 \pm 1$  g. They were kept in an air conditioned room (22–24°C) with an automatically controlled photoperiod of 14 hours (hrs) light and 10 hrs darkness. Mice were fed with the standard balanced pellet diet that prepared in the animal house of the above institute and given tap water ad libitum.

**Preparation of extracts:**

Cottonseeds (Ashour1, Lashata and Coker 310) were obtained from the General Company for Industrial Crops in Baghdad. Seeds were dried and powdered with a grinder. The oily extract of cottonseed was prepared according to Chernenko et.al. (1978)<sup>[11]</sup> by weight fifty grams of the dry powder of cottonseed and dissolved it in 700 mL of 95% of hexane alcohol by using soxhlet apparatus for 7-8 hrs. Then the solution was evaporated in a rotary evaporator under a reduced pressure at 60 °C. The oil was collected, put in glass containers and stored at 4 °C in refrigerator until used in the study. While the aqueous extract of cottonseed was prepared by weight fifty grams of the dry powder of cottonseed were mixed with 500 mL of cold distilled water in a closed vessel and left subjected to a continuous mixing for 24 hr at 40 °C in the magnetic stirrer, then the whole mixture was filtered by a piece of gauze, and followed by centrifuged at 3000 r.p.m for 15 min, then, filtrated through filter paper (Whatman No.1) and dried by placing it in the oven under 40-45 °C for 24 hr. The dried material was weighed and saved in dark place until use<sup>[12]</sup>.

**Experimental design:**

The experimental mice were divided randomly into 2 major groups (30 male/group) according to the type of extract and each major group was subdivided into 3 minor groups (5 male/group) according to the concentrations of extracts and handled as follows: G1: orally administered normal saline for 6 weeks. G2: orally administered 20mg/kg/day aqueous extract for 6weeks. G3: orally administered 40mg/kg/day aqueous extract for 6weeks. G4: orally administered olive oil for 6 weeks. G5: orally administered 20% oil extract of cottonseed for 6 weeks. G6: orally administered 40% oil extract of cottonseed for 6 weeks.

**Hematological parameters:**

Hemoglobin (Hb) was analyzed by the cyanmethemoglobin procedure using Drabkin's solution. While, Hematocrit was determined by the microhematocrit method<sup>[13]</sup>. RBC count was determined by haemocytometer slide<sup>[13]</sup>. Also, WBC and platelet count was determined by haemocytometer slide<sup>[14]</sup>. Differential WBC count was measured by made blood film for each group, count 100 W.B.C, then each type of WBC (Neutorophils, Lymphocyte, Monocyte, Eosinophils and Basophile) was calculated and estimated as a percent for each cell<sup>[14]</sup>.

**Statistical Analysis:**

Statistical analysis was performed with the Statistical Package for Social sciences software (SPSS; version 14.0) and also, Microsoft Excel Worksheet 2003. Differences between groups were examined by ANOVA test (analysis of variance of means) for figures with mean and standard error (S.E.).

### Results:

There was non significant ( $P>0.05$ ) decrease in Hb concentrations of mice treated with 40 mg/kg/day of aqueous extract of cottonseed in comparison to the control group (Table-1). Level of Hemoglobin (Hb) in the blood (g/dl) of mice treated with oil extract for 6 weeks showed that a significant ( $P<0.05$ ) reduction in Hb values were evident after six weeks of treatment with both concentrations of oil extract as compared with the control group (Table-2). Also, significant ( $P<0.05$ ) reduction were shown in the PCV values among G2 and G3 when compared to G1 (Table 1), and among G4 and G5 as compared with G4 (Table-2). Male mice received 20 and 40 mg/kg/day aqueous extract caused significant ( $P<0.05$ ) decrease in RBC count when compared with control group. While male mice received 20% and 40% oil extract of cottonseed caused non significant ( $P>0.05$ ) depression in the RBC and WBC count (cell/L) when compared with the control (Table-2). The results showed that there was significant ( $P<0.05$ ) increase in platelet count in all treated groups as compared with control (Table-1 and 2). Differential leukocyte analysis showed that a significant ( $P<0.05$ ) reduction was observed in the percentage of lymphocyte in G2 and G3 as compared with G1 and in G6 as compared to G4 (Tables 3 and 4). While the percentage of neutrophils were significantly ( $P<0.05$ ) increase in G2 and G3 as compared to G1 and in G6 as compared to G4. Also, the same tables show that the percentage of monocytes were significantly ( $P<0.05$ ) decrease in G5 and G6 as compared to G4. However, 40mg/kg/day aqueous extract demonstrated a significant ( $P<0.05$ ) increment in the percentage of eosinophil and basophil cells as compared to G1 (Tables 3 and 4).

Parameters	Groups G1 (Control)	Aqueous extract	
		G2 (20m /kg)	G3 (40mg/kg)
Hb g/dL	11.19 ± 0.60 <sup>a</sup>	10.73 ± 0.61 <sup>a</sup>	10.15 ± 0.70 <sup>a</sup>
PCV (%)	34.13 ± 1.90 <sup>a</sup>	32.13 ± 0.83 <sup>ab</sup>	31.50 ± 0.60 <sup>b</sup>
RBC count ×10 <sup>12</sup> (cell/L)	7.98 ± 0.19 <sup>a</sup>	7.39 ± 0.61 <sup>ab</sup>	6.28 ± 0.34 <sup>b</sup>
WBC count ×10 <sup>9</sup> (cell/L)	7.53 ± 0.29 <sup>a</sup>	7.78 ± 0.86 <sup>a</sup>	8.47 ± 0.46 <sup>a</sup>
Plateletcount×10 <sup>9</sup> (cell/L)	224.88 ± 9.38 <sup>a</sup>	234.13 ± 8.74 <sup>b</sup>	271.5 ± 12.55 <sup>c</sup>

**Table-1: Haematological parameters of male mice received two concentrations of aqueous extract of cottonseeds for six weeks**

Each value represents mean ± S.E. Means having different letters at the same row are significantly ( $P<0.05$ ) different. Means having same letters at the same row are non significant ( $P>0.05$ ) difference.

Parameters \ Groups	G4 (Control)	Oil extract	
		G5 (20% /♂)	G6 (40%/♂)
Hb g/dL	12.0 ± 0.28 <sup>a</sup>	11.04 ± 0.22 <sup>b</sup>	9.93 ± 0.55 <sup>c</sup>
PCV (%)	36.30 ± 0.84 <sup>a</sup>	34.20 ± 0.71 <sup>b</sup>	30.70 ± 1.78 <sup>c</sup>
RBC count ×10 <sup>12</sup> (cell/L)	10.08 ± 0.29 <sup>a</sup>	9.20 ± 0.28 <sup>a</sup>	9.10 ± 0.21 <sup>a</sup>
WBC count ×10 <sup>9</sup> (cell/L)	11.48 ± 0.38 <sup>a</sup>	11.69 ± 0.32 <sup>a</sup>	12.12 ± 0.24 <sup>a</sup>
Platelet count ×10 <sup>9</sup> (cell/L)	285.38 ± 19.53 <sup>a</sup>	296.0 ± 18.79 <sup>ab</sup>	305.25 ± 5.29 <sup>b</sup>

**Table-2: Haematological parameters of male mice received two concentrations of an oily extract of cottonseeds for six weeks period.**

Each value represents mean ± S.E. Means having different letters at the same row are significantly (P<0.05) difference.

Means having same letters at the same row are non significant (P>0.05) difference.

Parameters \ Groups	G1(Control)	Aqueous extract	
		G2 (20mg/kg)	G3 (40mg/kg)
Lymphocytes (%)	76.40 ± 1.91 <sup>a</sup>	74.90 ± 1.10 <sup>ab</sup>	72.10 ± 0.60 <sup>b</sup>
Neutrophils (%)	15.80 ± 1.50 <sup>a</sup>	17.60 ± 3.25 <sup>ab</sup>	19.20 ± 0.92 <sup>b</sup>
Monocytes (%)	6.60 ± 1.21 <sup>a</sup>	6.0 ± 0.32 <sup>a</sup>	5.80 ± 0.37 <sup>a</sup>
Eosinophils (%)	0.80 ± 0.20 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	2.10 ± 0.60 <sup>b</sup>
Basophils (%)	0.40 ± 0.24 <sup>a</sup>	0.40 ± 0.40 <sup>a</sup>	0.80 ± 0.2 <sup>b</sup>

**Table-3: Differential leukocyte analysis of male mice received two concentrations of aqueous extract of cottonseeds for six weeks.**

Each Value represents mean ± S.E. Means having different letters at the same row are significantly (P<0.05) different.

Parameters \ Groups	G4 (Control)	Oil extract	
		G5 (20% /♂)	G6 (40% /♂)
Lymphocytes (%)	75.10 ± 0.33 <sup>a</sup>	74.80 ± 0.2 <sup>a</sup>	70.60 ± 0.6 <sup>b</sup>
Neutrophils (%)	16.70 ± 0.77 <sup>a</sup>	17.40 ± 0.40 <sup>a</sup>	23.40 ± 0.51 <sup>b</sup>
Monocytes (%)	6.0 ± 0.63 <sup>a</sup>	5.80 ± 0.49 <sup>b</sup>	4.40 ± 0.51 <sup>c</sup>
Eosinophils (%)	1.40 ± 0.24 <sup>a</sup>	1.6 ± 0.77 <sup>a</sup>	1.7 ± 0.2 <sup>a</sup>
Basophils (%)	0.2 ± 0.12 <sup>a</sup>	0.4 ± 0.19 <sup>a</sup>	0.5 ± 0.16 <sup>a</sup>

**Table-4: Differential leukocyte analysis of male mice received two concentrations of oily extract of cottonseeds for six weeks.**

Each value represents mean ± S.E. Means having different letters at the same row are significantly (P<0.05) different.

**Discussion:**

According to the results obtained in this study, Hb concentrations was decreased in mice treated with cottonseed extracts which may be due to that gossypol can inhibit glucose-6-phosphate dehydrogenase, causing a decrease in NADPH production which is necessary to reduce glutathione, an important components of the cell antioxidant systems. Enzyme inhibition in this pathway or excess oxidants may cause reduced Hb and PCV [15]. Yue and Zhou [16] showed that when replacing of CSM with soybean meal in fish diets caused significant differences in Hb, PCV, RBCs and WBCs content. Adeyemo [17] showed that PCV and RBCs were significantly affected by the cottonseed cake in the first four weeks of feeding with 60% cottonseed cake to chickens, this reduction due to low protein intake or mild anemia. Gossypol administration may interfere with normal utilization of iron by chelating liver iron stock and therefore reduce the synthesis of Hb. Lindsey et al. [18] reported lower Hb concentration and haematocrit percentage in animals receiving gossypol in solvent extracted cottonseed meal. Khogali et al. [19] showed that the changes in the PCV and Hb values may be due to the malabsorption of nutrients and minerals or ions which are necessary for synthesis of Hb.

The present study also showed that there were non significant differences in RBCs count when male mice received oil extract of cottonseed as compared with the control group which may be due to high iodine value in cottonseed oil [20]. This results was disagree with results of de Peyster et al. [21] they found a reduction in RBC count which due to an increase in membrane permeability of RBCs because of gossypol treatment and suggested that degeneration of cell membranes at all levels of organization may exist with acute prolonged exposure to gossypol in vivo.

The results of this study showed that WBC count elevated in all treated groups, the increase in the absolute values of WBC count which was accounted by changes in the No. of neutrophil, in addition to the role of T in maintaining the values of RBC, Hb and PCV in the rat [22]. The elevated of WBC count may attributed to the direct effects of cottonseed on bone marrow to produce large numbers of WBC or indirect effects by necrosis of cells in different organs which stimulate bone marrow cell to mitosis and generation large numbers of WBC for defense to the body [23]. The present study also, revealed that orally given cottonseed extracts for 6 weeks caused the reduction in the percent of lymphocyte (lymphopenia) which was related to the decreased cell-mediated immune response. Since neutrophil and lymphocytes have a major role in fighting foreign organism, so the variation in WBCs count were essentially caused by variations in neutrophil and lymphocyte count [24].

Cottonseed contain phenols, alkaloids and saponins that have negative biological effects on microflora of the stomach in vertebrates and rumen in the cows which lead to reducing in their growth and reduction in the immunity [25]. Also, platelets count was elevated in mice received cottonseed extracts as compared with control group; this may be a manifestation of iron deficiency [26].

The other reason may be that the cells within bone marrow produce more platelet or the spleen was removed less platelet from the blood <sup>[27]</sup>.

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