

**EFFECTS OF DRENCHING AQUOUS EXTRACTS OF LICORICE ROOT (*Glycyrrhiza galabra*) AND *Oreganum vulgari* ON HEMATOLOGICAL CHARACTERISTICS OF ADULT MALE RABBITS (*Lepus cuniculus*).**

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(Received 28 February 2018 ,Accepted 17 May 2018)

**Keyword:** licorice, oregano, hematology.

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**ABSTRACT**

This study was done on thirty mature male rabbits of 1500 gm mean body weight, those rabbits were randomly divided into three equal experimental groups of ten animals each .The first group was regarded as control group and drenched 3 ml normal saline daily for one month. The second group animals were drenched 3ml of licorice root water extract daily for one month also. While the third group animals were drenched 3ml of water extract of oregano daily for one month also. At the end of the drenching period blood samples were drawn from all animals by heart puncture and blood was analyzed for total RBC count; Hb %; pcv%; total leukocytic count and blood indices of MCH; MCV, and MCHC were calculated in addition to doing blood smears from each animal to estimate percentages of differential leukocytic count. Results revealed that drenching licorice water extract had a significant increase ( $p<0.05$ ) on total RBC count; total WBC count and neutrophils also, significant decrease in MCHC and eosinophil, whereas results of drenching water extract of *Oreganum vulgari* caused significant increase on total leukocytic count , MCV and neutrophil while RBC had no significant effect so, PCV, Hb, had no effect in the two experimental.

**INTRODUCTION**

Licorice which is the root of the Leguminous *Glycyrrhiza* plants species, has been consumed for over 4000 years since the area of ancient Egypt and is among the

botanical most frequently employed in foods and tradition medicine in both eastern and western countries (1).

Although licorice has such a long history of consumption, safety information on the hydrophobic fraction of Licorice (*G. glabra*) is limited. In order to confirm the safety of Licorice extract, a series of nonclinical studies were conducted, As a result of these studies, it was concluded that preclinically Licorice extract is safe and non carcinogenic (2) and (3) stated that Licorice is a plant of ancient origin and steeped in history. Its extract and principle components glycyrrizin have been used in food and in both traditional and herbal medicine. As a result there is a high level of use of Licorice ( glycyeehizin) in the world with an estimated consumption of 300 mg licorice per Kg/day (3).

Biochemical studies indicated that glycyrrihin inhibits  $11\beta$  hydroxysteroid dehydrogenase enzyme which is responsible for inactivating cortisol. As a result the continuous highlevel exposure to glycyrrhizin compounds can produce hypermineralocorticoid-like effect in both animals and human. These in vivo and clinical studies have reported beneficial effects of licorice ,including antiulcer, antiviral and hepatoprotective responses. Various genotoxic studies have indicated that Licorice extract is neither teratogenic nor mutagenic and may possess antigenotoxic properties under certain conditions (4).

Also the other herb *Oreganum vulgari* is a hardly perennial plant which grows wild in its nature areas in Mediterranean countries().Oregano are widely used in pharmaceutical and cosmetic industries as flavoring substances of food products. Alcoholic beverages and perfumery for their spicy fragrance (6) and (7).It has also been used as a traditional remedy to treat various ailment such as spasmodic; antimicrobial; expectorant; carminative aromatic for whooping and convulsive coughs; digestive disorders and menstrual problems (7, 8,9 10). It contains phenolic terpenoids (thymol; carvacrol); flavonoids; luteolin; apigenin; Tannis; hydroquinone; Oleanolic acid; showing strong antioxidant activity (11).Extract of *Oreganum vulgari* decreases responses to histamine; serotonin and nicotin (12).In folk medicine oregano is used for cramps depression, dizziness; gastrointestinal dis orders; migraine; nervous headache and paroxysmal cough and as diuretic(13).So according to the information available about licorice and oregano plants and very little published information about

their effects on hematology, we hypothesized that treatment by them may cause some hematological changes in male rabbits, as blood parameters are considered the most physiological indicator of whole body and therefore are important in diagnosing the structural and functional status of rabbits(14).

In addition, hematological studies provide quite frequently and routinely accepted procedures in diagnosis of mammal research diseases (15)So according to information available about the two plants we hypothesized that treatment by them may cause some hematological changes in rabbits.

## **MATERIALS AND METHODS**

This work was done on 30 mature local male rabbits purchased from Basrah market and kept in the animal house of College of veterinary medicine/university of Basrah, they were left there for one week to acclimatize before treatment begun. Rabbits were of 1500gm body weight mean and between 6-7 months. They were feed alfa alfa adlibrtum and bread and provided with clean drinking water adlibitum also. The animals exposed to 12 hours light to dark cycle. The animals were randomly allocated to three equal experimental groups of 10 animals each. The first group is regarded as control group and drenched 3 ml normal saline daily for one month .The second group animals were drenched 3 ml of licorice root extract for one month also and the animals of the third group were drenched 3ml of oregano extract for one month also.

**Plant Materials:-** Licorice root and *Oreganum vulgari* plants were purchased from market and taxonomic identification and authentication was done in the herbarium of College of Science .

**Preparation of water extract:** After cleaning of each of licorice root and oregano plant and ground them to fine powder, 6 gm of each of the powdered plant added to (200)ml of distilled water and boiled in the reflex instrument found in the research lab unit until the volume was reduced to 100 ml. The extract was then stirred at room temperature for 24 hours. Water soluble extracts were obtained following centrifugation at (10000)rpm for 14 minutes(16).

**Obtaining of blood sample:** 5ml of blood samples were collected from each animal of all groups by cardiac puncture using sterile disposable syringes, the blood was

immediately was transferred to test tubes containing disodium salt of ethylene diamine tetra acetic acid(EDTA) as anticoagulant, and the blood was used for analysis of hematological characteristics.

#### **Hematological parameters studies:**

The value of total erythrocytic counts (RBCs);packed cell volume (PCV); hemoglobin(Hb%); total leukocytic count(WBCs)and blood films were done from each blood sample to estimate percentages of differential leukocytic counts. Also blood indices mean corpuscular volume (mcv); mean corpuscular hemoglobin(MCH);and mean corpuscular hemoglobin concentration (MCHC) were calculated. All those hematological parameters were determined according to methods described by ( 17).

#### **Statistical analysis:**

Statistical analysis was performed using student (T) test after applying one way analysis of variance(ANOVA)between the groups according to (18).Means and their standered deviations(SD)were calculated using spss software according to (19).A value of ( $p<0.05$ ) was considered statistically significant.

## **RESULTS AND DISCUSSIONS**

Table (1) shows the values of the hematological parameters resulted after drenching the rabbits aquaus extract of Licorice root and *Oreganum vulgari* at a dose of 20mg/kg body weight daily for one month. It is clear from the table that there was no significant difference in Hb%;PCV; and MCH values of both drenched groups compared with the control group. These results are in agreement with those found by (2) when they found no significant adverse effects during two weeks ingestion period of Licorice and also resembles those found by (16) in rats but it is completely opposite to those found in fish recorded by (20)and these differences may be due to different responses between mammals (rats and rabbits)and aquatics( fish). Same trends were found due to hematological indices(MCH) and (MCHC) in animals of this study (rabbits).While there is significant increase in (MCV) And RBCs and total WBCs for *Oreganum vulgari* treated group. But there is significant increase in total RBCs and significant decrease in total WBCs in licorice treated group. ( 2) stated

that those results indicates that Licorice is a safe ingredient for functional food for human and animals even for a long term or excessive ingestion.

Same trends was also found by (21). Also (22) states that glycyrrhizin which is the major effective components of Licorice extract was found to protect erythrocytes against hemolysis induced by other saponin compounds in a short period treatment even at a high concentration. Also (4) found results resembles our result when they found all the hematological parameters remain within the normal range related to safety assessment at predose and four weeks after the initiation of treatment in human. Also in broiler chickens (23) found same results when he found that addition of 450mg of Licorice extract to drinking water of broiler chicken caused significant increase ( $p<0.05$ ) in total number of RBCs. (24) noted that LD<sub>50</sub> of glycyrrhithia extract containing 53% glycyrrhizin aid in mice and rats depends upon various routs of administration and it is high ranging from 7500-8000 mg/kg body weight orally so our used dose is very safe (20mg/kg) body weight. He also found that long term treatment of rats with high concentration of Licorice extract containing 2500 mg/kg /day for 90 days caused significant decrease in total RBCs count accompanied by significant decrease also in PCV%. The dose used in this study 20 mg/kg/day for 30 day is believed safe and caused no significant changes in Hb%; PCV% but caused significant ameliorative increase in RBCs and WBCs it means that drenching water extract of licorice root did not cause any type of anemia even when drenched for a long period. (25) did not find any significant differences in all hematological parameters and blood indices in rats drenched *Origanum syriacum* oil; so they declared that drenching such oil did not cause any possible secondary effects after administration because (26) considered blood parameters are the most physiological indicators of whole the body so there for they are important in diagnosing the structural and functional status of rabbits. The significant increase in total leukocytic count due to drenching of oregano in this study (table 1) which is due to increase in neutrophils (table 2) is demented by similar results found by (27) when found higher value of phagocytic activity in rabbits fed oreganum and they found prolonged immune stimulatory effects which is regarded as beneficial effect of originum plant extract oral administration; so the dose of *Origanum vulgari*

Extract used in this study so as the Licorice extract also are regarded beneficial like those results mentioned by other authors but if the doses used are high it may

exert adverse effect as that found by (28) when they found that high dose of *Origanum vulgari* oil had adverse effect on metabolism of mice on the contrary to the lower concentration which have apposite effects on oxidant status.

**Table (1) Effect of drenching aqueous extract of Licorice root and *Oreganum vulgari* on hematological characteristics of mature male rabbits(means± SE)**

Treatment groups	Blood parameters						
	Hb%	PCV%	RBCx10 <sup>4</sup>	WBCs	MCV cmm	MCH pg	MCHC gm/dl
Control group	14.4±1.5 A	30±3.4 A	630±20.3 A	5200±50.4 A	40±1.54 A	14±1.7 A	35±1.5 A
Licorice group	12.4±1.72A	29±3.4 A	998±20.3 B	6300±50.4B	39±1.75 A	12.4±1.6 A	31.8±2.14 A
Oregano group	13.5±0.95A	36±2.3 A	594±25.4 A	26117±96 C	66±3.4 B	14±3.2 A	24±5.4 B

means bearing different letters vertically differs significantly at 5% level (P<0.05)

**Table(2) effect of drenching aqueous extract of licorice root and oregano on differential leukocyte count of mature male rabbits (±SE)**

Treatment group	Differential leukocyte count percent				
	Lymph.%	neutro.%	Eosin%	Mono.%	Baso.%
Control group	40±4.25 A	45±3.26 A	10.0±1.6 A	4.0±2.41 A	1.0±0.0 A
Licorice group	41±1.84 A	53±2.54 B	4±1.7 B	2.0±1.3 A	0.0±0.0 A
Oregano group	30±5.4 A	60±6.5 B	8±3.6 A	2±1.1 A	0.0±0.0 A

Means bearing different letter vertically differs significantly at 5%level

## تأثير تجريع المستخلص المائي لعرق السوس والمرد قوش على الصفات الدموية لذكور الارانب المحلية البالغة

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### الخلاصة

اجريت هذه الدراسة على ثلاثين ذكر ارنب بالغ وبمعدل وزن الف وخمسمائة غرام، قسمت الارانب بشكل عشوائي الى ثلاثة مجاميع كل مجموعة عشرة حيوانات المجموعة الاولى اعتبرت كمجموعة سيطرة وجرعة 3 مل محلول فسلجي يوميا لمدة شهر واحد. حيوانات المجموعة الثانية جرعت 3 مل من المستخلص المائي لعرق السوس يوميا لشهر واحد ايضا بينما حيوانات المجموعة الثالثة جرعت 3 مل م المستخلص لمائي للمرد قوش يوميا ولمدة شهر واحد ايضا. في نهاية فترة التجريع جمعت عينات الدم من كل الحيوانات بوخز القلب و تم تحليل عدد كريات الدم الحمراء، ونسبة الهيموغلوبين، ونسبة المئوية لحجم كريات الدم المرصوص، وحساب عدد كريات الدم البيضاء، كما تم حساب كل من معدل هيموغلوبين الكرية الحمراء الواحدة ومعدل حجم الكرية الحمراء الواحدة وكذلك معدل تركيز هيموغلوبين الكرية الحمراء الواحدة. بالإضافة لعمل مسحة دموية لكل العينات لتقييم النسبة المئوية للعد التفرقي لخلايا الدم البيضاء. النتائج: ادى تجريع الحيوانات بالمستخلص المائي لعرق السوس الى احداث زيادة معنوية في العدد الكلي لكريات الدم الحمراء وفي العدد الكلي لخلايا الدم البيضاء والخلايا البيضاء العدلة كذلك انخفض معدل تركيز هيموغلوبين الكرية والخلايا الحمضة في حين نتاج تجريع المستخلص المائي للمرد قوش سبب ارتفاع معنوي في العدد الكلي لخلايا الدم البيضاء وحجم هيموغلوبين الكرية والخلايا العدلة، بينما العدد الكلي لكريات الدم الحمراء لم تتأثر. كذلك حجم الخلايا لمرصوص، و الهيموغلوبين لم يتأثران في كلا التجريبتين

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