The effect of crude aqueous extract of cocoon beetle *Larinus maculatus* F. on some hematological parameters in male white mice *Mus musculus*

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Abstract :

The aim of this study to investigate the effect of crude hot aqueous extract of cocoon beetle *Larinus maculatus* Faldermann(Family: Curculionidae) in a different doses(50,100,200,300)mg/Kg/ day respectively with periods (15,25,35,45) days respectively on some hematological parameters such as hemoglobin(Hb),red blood cells count(RBCs),Packed cell volume(PCV),mean corpuscular volume(MCV),mean corpuscular hemoglobin(MCH),mean corpuscular hemoglobin concentration (MCHC)and white blood cells count(WBCs)were determined in mice *Mus musculus* administered orally the extract .The results showed that there was a significant decrease ($P<0.05$) in Hb ,PCV, RBCs values, and MCV,MCH and MCHC also affected by the extract for all of the treatments and in all the periods . In WBCs values there was a significant increase ($P<0.05$) with all of treatments and with different periods .The results therefore indicate there was anemia with experimental animals and the presence of undesirable effect in the use of aqueous extract of beetle cocoon in the raw form at least at the administered concentrations and for the duration of feeding. The findings are unhealthy since the raw extract of this cocoon is currently being used as drug as treatment for cough, bronchitis in many families in Iraq ,Syria, Turkey and Iran.

Introduction

Trehala, this is a name for a crude drug consisting of the larval chambers formed by insects belonging to the genus ***Larinus*** that live on several plants belongs to the ***Echinops*** species of the middle-east. This beetle cocoon is one of very few naturally occurring molecules that taste sweet, although it is half as sweet as table sugar. Trehala manna, the sweet-tasting cocoon of the *Larinus maculates* beetle which a lot of people believe that manna can simply drip spontaneously from a tree and, it is the food miraculously supplied to the Israelites during their wanderings journey in the wilderness(1).The name man-es-simma or manna wa salwa , has been preserved in Arabic by the Araƀ s Sinai

who harvest it. It was said ,manna which was "white like coriander seed and tasted like a wafer made with honey" (2), It is narrated in the hadith Sahih Muslim the Muslims' prophet Muhammad said " Truffles are a kind of ' manna ' which Allah the Glorious and Exalted, sent down upon the people of Israel ,and its juice is a medicine for the eyes" (3).

It was not improved that the cocoon of the parasitic beetles that was manna for Moses, or manna has been with gum resin produced by several kinds of trees ,especially the tamarisk tree , with dried sweet secretions of various insects that eat plant sap and with a species of lichen(4) . Anywhere this subject was historical origin (5) (6) (7) .

Hanbury (8) described the cocoons as:˝The cocoons *Larinus maculatus* Faldermann, a species occurring widely around the Mediterranean and as far east as Iran are described as ovoid or globular and about ¾ of an inch long. Their inner surface is composed of a smooth , hard ,dusky layer ,external to which is a thick, rough , tuberculated coating of a grayish-white color and earthy appearance .They are found on the stems of *Echinops* and sometimes contain spiny portions of the leaves. the cocoons were abundant in the shops of the Jewish drug-dealers in Constantinople(Istanbul),were Arab and Turkish physicians considered them of value in treating respiratory diseases˝ (9).

Trehala which is used as food in Syria, the substance has been examined by Apping (10),his chemical analysis yielded moisture 10.78 ash, 2.79 fat, albuminoids soluble in water 8.09 and insoluble 2.31,true starch 6.72; cellulose like substance derived from starch 24.90; mucilage soluble in water 7.60 , and mucilage insoluble in water 10.93,chlorophyll 0.16 ,tannin and citric acid traces and Trehalose 23.84 % , the cocoon is a product of the larva, but the material for this structure , although of vegetable origin, cannot have been derived from the plant , upon which it was built, since he found the pith and other portions of the tissue of the stems to be entirely free from starch and from trehalose(10), While Leibowitz(11)has isolated trehalose from manna of North Iraqian Dessert, which is about 60% of total carbohydrate and represents 7% of the raw material. Trehalose is the major component in trehala manna; it is white crystalline carbohydrate made of two glucose molecules, trehalose : that name was given by French chemist Berthelot in 1858, who isolated this sugar from a cocoon which *Larinus nidificans* as the main insect species responsible for its formation(12).Trehalose (α-D-glucopyranosyle - α-D-gluco- pyranose) is a non reducing sugar abundant in Nature. It is found in the prokaryotes(13),yeasts(14)(15) , brewing yeasts (16) ,young mushrooms(*17*),the trehala manna plant(11),invertebrates (18); in many insects ,trehalose constitutes the major haemolymph (blood) sugar ,whereas glucose is often present at much lower concentration (19) (20). In mammals trehalose absent in their bodies in spite of having trehalase ;a very special enzyme in the intestine which converts

trehalose into two units of glucose (21). Many functions of trehalose had been described for trehalose, in prokaryotes (22) (23).In mycobacteria, trehalose can be incorporated into glycolipids and therefore acts as a structural component(24), it is believed to be responsible for the low permeability of the membranes conferring appreciable drug resistance to the organisms (25)(26). Trehalose may plays fundamental role in fungal spore germination and serves as a source of carbon for synthesis of glucose or energy, and act as a regulator of metabolism and may affect the activity of key enzymes(27)(28)or its role as an antioxidant(29);while in some fungi the trehalose pathway has been as a selective fungicidal target for use in antifungal development(30).

In insects trehalose is the most abundant sugar in their haemolymph(31) in high concentration (80-90%)and in thorax muscles , were it is consumed during flight(32).In addition, trehalose may protect and regulate the different enzymes in the stages of insects' life against thermal denaturation(33)(34).

Trehalose may play a central role in carbohydrate utilization and storage in plants of prevailing carbon availability ;sugar signaling-pathways and regulate plant growth, adaptation and development (35)(*36*) (37). In low plants, , In *Arabidopsis thaliana* it has demonstrated that trehalose has a fundamental role in embryo development, and in abscisic acid and sugar signaling(38) .

Trehalose is a non-toxic, non reducing disaccharide of glucose that possesses an exceptional ability to stabilize and preserve cells and protect cell membrane structures under various environmental stresses such as drying, freezing, oxidation (39)and high temperature(40). For these purposes, trehalose has attracted for modern medicine requires preservation of tissues and/or blood cells for transplantations or transfusions (41). the utility of trehalose has been attributed to its protective interactions with lipid membranes stabilization of labile proteins against damage and the ability to form stable glasses during cooling of cells for cryopreservation(42)(43). Trehalose also useful in the cryopreservation of sperm and stem cells and in the development of a highly reliable organ preservation solutions (44) (45) , microinjection technique to introduce trehalose in the women in vitro fertilized oocyte followed by cryopreservation (42)and preservation of red blood cells (41) , hematopoietic stem cells (46), embryonic stem cells (47) ,adipose cell (48). Recent studies have shown that trehalose can also prevent damage to mammalian eyes, dry eye syndrome (49) (50) (51). The ability of trehalose to stabilize aggregation-prone protein molecules is under investigation as possible approach in the treatment of polyglutamine disease, such as Huntington's chorea(52)and Alzheimer's disease (53). Trehalose has proved quite useful in a number of industries including: dried , frozen and processed foods ,in the pharmaceutical industry; it is used as a moisturizer in many basic toiletries such as bath oils and hair growth

tonics, deodorization , cosmetics ,monoclonal antibody formulations (54),plant activation, antibacterial sheets, and nutrients for larvae (55).

We must clarify that all of the functions of trehalose we spoke it , happened as a pure trehalose either in nature or it has been manufactured by [Hayashibara biochemical laboratories and it include: the purity of not less than 98% of this sugar(trehalose),not more than 0.05% total ash,<1 ppm lead, < 300 viable colony counts(aerobic) per g of trehalose(CFU/g),<100 CFU/g of yeast or molds, and negative CFU/g for *Escherichia coli]* (56).

In general, any natural substance used as food contains various compositions of nutrients and antinutrients and could have important or deleterious effects in the body when consumed . The composition of the nutrients and antinutrients , usually leads to side effects in most substances which may lead to toxicity , Hyperlipidaemia ,excessive weight gain, hyperglycemia, carotenemia, constipation, kidney stones, body odour, bad breath, allergies, diarrhea, frequent urination, and acne(57). In most of these side effects, the biochemical and hematological parameters are usually altered. For a cocoon to be considered safe for human and animal health , its effect on these parameters need to be investigated to understand the nutritional potentials and safety of such foods with a view to determining their acceptability, and that's our aim for this study.

Materials and methods

*Collection and preparation the aqueous extract

Cocoon like shell larval beetle for *Larinus maculatus* was purchased from a local market in Baghdad & Karbala, Iraq .The cocoon like shell was identified by natural history museum in Bab al-Muʿaddam. The cocoon was cleaned from the insects ,milled separately using a laboratory electric mill (Philips/Holland) . The watery extract was obtained (the method from 9) by stirring the milled powder(10g were weighed by Sartorius sensitive electric balance) with (100ml.)of hot distilled water at room temperature for one hour. The suspension was centrifuged(by centrifuge Jouan/France) at 4000rpm for two hours and the supernatant was filtered through whatman filter paper No.1 . The extract was concentrated using incubator at an optimum temperature of 34-37^{O} c for two days. The weight of the dry extract was determined. The different concentrations (50,100,200 and 300 mg/Kg)of the extract were prepared.

* Experimental animals

Adult male albino mice(*Mus musculus*) were about 6-8 weeks age, with average weight ranged between 25±3grams,was purchased from the Department of Drug -the animal house /al-Andalous square in Baghdad. The animals were quarantined and acclimatized in the laboratory for 7 days before the beginning of the study. Environmentally controlled room at 10 changes of air per hour, 22±3°c , relative humidity of 30-70% with 12 hours of artificial (fluorescent)

light provided per day . the animals were housed in standard cages with closed diet and water were provided.

* Experimental design

An acute toxicity study of the aqueous extract of beetle cocoon were orally administered to mice was done by the method in(58); the mice were observed for obvious toxic symptoms, or mortality was determined twenty four hours after administration. Seventy (70)males mice were randomly assigned into different groups (of 3 animals each) . Control animals were administered 0.25 ml of normal saline (0.9%Nacl) while other groups of mice were administered (50mg/Kg ,100mg/Kg , 200 mg / Kg, 300mg/Kg)/day of extract respectively. The extract were administered for (15 ,25 ,35 and 45 days) for each group to the animals using oral rout by means of polythene cannula. At the end of every period for each group ,mice were killed with heart puncture for collecting blood samples for analysis of hematological parameters like, hemoglobin concentration was estimated using the cyanomethemoglobin photometric method, the packed cell volume was estimated using the micro-haematocrit centrifuge , red blood cells and white blood cells were estimated using the improved Neubar heamocytometer , then the mean corpuscular hemoglobin , mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated . The Statistical analysis of results are represents as means ± S.D. Significance between control and extract treated groups were determined using Duncan′s test . A P value ≤ 0.05 was considered significant .

* Hematological studies :

The packed cell volume was estimated using the micro-haematocrit centrifuge . The hemoglobin concentration was estimated from packed cell volume values. The red blood corpuscles count and total white blood cells count was estimated using improved Neubar haemocytometer (59)

Results

The acute toxicity of the cocoon like shell beetle for *Larinus maculatus* extract in mice showed at the higher doses(2000 , 4000, 8000 ,12000 and 20000mg/Kg), anorexia and diarrhea were observed , no mortality observed after twenty four hours of oral administration.

The results of the effect of administration of various doses (50,100,200 and 300 mg /Kg) of *L. maculatus* cocoon aqueous extract for (15,25,35 and 45 days) on haematological parameters such as red blood corpuscles count , haemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration and white blood cells count for every dose with different periods are presented in tables 1, 2, 3 ,4 , 5 ,6 & 7.

Table 1: Effects of the extract on Red blood cell (RBC)counts in mice

Mean Red blood corpuscles count($x10^6/\mu L$)				
Treatment	15^{th} day	25^{th} day	35^{th} day	45^{th} day
Control(10ml/Kg)	6.30 ± 0.2	6.26±0.24	6.35±0.22	6.33 ±0.17
50mg/Kg	4.93±0.37 *	6.35±0.13	6.66±0.28	6.55±0.15
100mg/Kg	4.9 ± 0.1 *	3.53 ± 0.25 *	2.33 ± 0.15 *	1.83 ± 0.20 *
200mg/Kg	4.09 ±0.49 *	5.44 ± 0.46 *	5.66 ± 0.15 *	4.96 ± 0.20 *
300mg/Kg	5.43 ± 0.15 *	5.4 ± 0.1 *	3.34 ± 0.13 *	1.73 ± 0.12 *

Dose in mg/Kg orally. n/g = 3 . *P<0.05 compared with control value

In table 1 represents the results of administration various doses of cocoon extract effects on red blood cell counts(RBC). the data showing that there was a significant decrease ($p\leq 0.05$)in number of red blood corpuscles (RBC) after treatments with 50mg/Kg, 100mg/Kg and 200mg/Kg ; and 300mg/Kg of extract in the first 15 days of administration, the result revealed that there was effect of the extract in a dose dependent in the same period , and A significant decrease ($p\leq 0.05$)with the second, third and fourth period of administration after treatments with100mg/Kg, 200 mg / Kg and 300mg/Kg; and in lower effect with 50mg/Kg in compare with control. the data showed a significant dramatically decrease in number of RBCs ($p\leq 0.05$) with increasing the time of administration and the dose of extract(50mg/Kg)was the lowest effect.

The results in table 2 showing a significant decrease ($p\leq 0.05$) in Hemoglobin levels(Hb) in the first 15 days of oral administration for all the doses using in our search ; and a significant decrease ($p\leq 0.05$) was noted in Hb levels dramatically with increasing the time (15,25,35,and 45 days) of oral administration, and with doses (50,200,and 300mg/Kg) in compare with control. The dose of cocoon extract (50mg/Kg) was noted the lowest effect in compare with other using doses .

Table 2: Effects of the extract on Hemoglobin (Hb) concentration in mice

Mean Hemoglobin level (Hb in gm/dl)				
Treatment	15^{th} day	25^{th} day	35^{th} day	45^{th} day
Control(10ml/Kg)	12.14 ± 0.16	12.15 ± 0.13	12.13 ±0.15	12.14 ± 0.12
50mg/Kg	9.61 ± 1.13 *	11 ± 1	11 ± 1	11.46 ± 0.45
100mg/Kg	9 ± 0.5 *	9.166 ± 0.57 *	9.33 ± 0.57 *	7.16 ± 0.76 *
200mg/Kg	10.77 ±0.38 *	10.44 ± 0.50 *	10.44 ± 0.38 *	10.44 ± 0.38 *

300mg/Kg	10.30 ± 0.5 *	10.23 ± 1.15 *	9.33 ± 1.15 *	9.00 ± 0.57 *

Dose in mg/Kg orally. n/g = 3 . *P<0.05 compared with control value.

Table 3: Effects of the extract on Packed cell volume (PCV) in mice

Mean Packed cell volume (PCV in %)				
Treatment	15th day	25th day	35th day	45th day
Control(10ml/Kg)	37.5 ± 0.5	37.5 ± 0.5	37.5 ± 0.55	37.5 ± 0.45
50mg/Kg	25 ± 1.73 *	32.66 ± 0.57 *	34 ± 1 *	35.66 ± 1.15
100mg/Kg	24.66 ± 0.57 *	28 ± 1 *	30.66 ± 3.05 *	27 ± 2 *
200mg/Kg	29 ± 1.5 *	28.33 ± 1.15 *	26 ± 3.46 *	31.33 ± 2.88 *
300mg/Kg	29 ± 1.0 *	34.36 ± 2.30 *	34.40 ± 0.57 *	34 ± 0.5 *

Dose in mg/Kg orally. n/g = 3 . *P≤ 0.05 compared with control value.

In table 3 the results showing a higher significant decrease (p≤ 0.05) was reported in packed cell volume (PCV)after 15 days from administration with all doses using in the research , and significantly decreased (p≤ 0.05) with increasing the days of administration for doses 100, 200, and 300mg/Kg in compare with control. In the dose 50mg/Kg; the data showing that in spite of the effect of the extract in decreasing PCV , that significant effect is lowered with increasing the time of oral administration (25,35, and 45 days) .

The mean corpuscular hemoglobin(MCH) is the average mass of hemoglobin per red blood corpuscles in a sample of blood. since the values of (MCH) are showed in table 4 , the data showing there was a decrease in MCH levels with no significant (P≤ 0.05) in the concentration 50mg/Kg with increasing of the time of oral administration; while in the second dose(100mg/Kg)there was a significant (P≤0.05)increased in MCH levels with increasing the days of oral administration, and in the dose 200mg/Kg there was a significant (P≤ 0.05)increased in MCH level after 15 days of oral administration; while in the other periods (25,35,and 45 days), the levels of MCH decreased with no significant (P≤ 0.05). in the dose 300mg/Kg there was a variance effect of extract, the data showed the level of MCH decreased with no significant level(P≤ 0.05)in the 15 & 25 days after administration ;while a significant increase in MCH levels(P≤ 0.05)in the days 35 &45 after oral administration.

Table 4 : Effects of the extract on mean corpuscular hemoglobin (MCH) in mice

Mean of mean corpuscular hemoglobin(MCH in Pg/cell)				
Treatment	15th day	25th day	35th day	45th day
Control(10ml/Kg)	19.27 ± 0.48	19.25± 0.5	19.10 ±0.49	19.25 ± 0.48
50mg/Kg	19.43 ± 0.85	17.30 ± 1.23	16.51 ± 1.69	16.54 ± 0.81
100mg/Kg	18.35 ± 0.64	25.96 ± 1.08 *	40.02 ± 3.79 *	39.45 ± 6.07 *
200mg/ Kg	26.22 ± 0.67 *	19.18 ± 0.43	18.42 ± 0.29	21.04.98 ±0.40

300mg/ Kg	18.96 ± 1.34	18.96 ± 1.83	27.98 ± 4.70 *	60.81 ± 7.85 *

Dose in mg/Kg orally. n/g = 3 . *P≤ 0.05 compared with control value.

The mean corpuscular volume (MCV) is a measure of the average size of the red blood corpuscles , the table(5) is showing the levels of MCV were already affected by the extract and a significant decrease (P≤ 0.05)was noted in the concentration 50mg/Kg after 15,25,35 ,and 45 days from oral administration. In the dose 100mg/Kg ,the levels of MCV were decreased significantly (P≤ 0.05) after 15 days of oral administration ; while a significant increase(P≤ 0.05) was noted after 25,35,and 45 days of administration. In contrast with the dose 200mg/Kg, the level of MCV was increased after 15 days and MCV levels decreased after 25,35,and 45 days of administration with a significant effect (P≤0.05) in compare with MCV level in control. the levels of MCV with dose 300mg /Kg of extract were a significant increased (P≤ 0.05) after 35 and 45 days of administration in comparing with control value.

Table 5 : Effects of extract on mean corpuscular volume (MCV) in mice

Mean of mean corpuscular volume(MCV in fL)				
Treatment	15th day	25th day	35th day	45th day
Control(10ml/Kg)	59.55± 1.66	59.53 ±1.68	59.53 ±1.70	59.55 ±1.69
50mg/ Kg	50.69 ± 0.60 *	51.44 ± 0.83 *	51.05 ± 2.64 *	52.46± 2.16 *
100mg/ Kg	50.34 ± 0.58 *	79.37 ± 2.80 *	132.06 ± 18.29*	148.73 ±22.04 *
200mg/ Kg	74.96 ±1.42 *	48.5 ± 1.80 *	45.83 ± 5.06 *	44.97 ± 4.04 *
300mg/ Kg	53.40 ±1.85	66.01± 3.30	108.27 ± 6.15 *	198.11± 32.39 *

Dose in mg/Kg orally. n/g = 3 . *P≤ 0.05 compared with control value.

In table 6 the results showed the mean of mean corpuscular hemoglobin concentration (MCHC), which is a measure of the concentration of hemoglobin in a given volume of packed red blood corpuscles. reported as part of a standard complete blood count. the range for it is between 32-36 mg/dl. The data in the table approximately with the ranges in general , in spite of there is a significant increase I the values in comparing with the values in the controls.

Table 6 : Effects of the extract on mean corpuscular hemoglobin concentration (MCHC) on mice

Mean of mean corpuscular hemoglobin concentration (MCHC in gm/dl)				
Treatment	15th day	25th day	35th day	45th day
Control(10ml/Kg)	32.37 ± 0.1	32.55±0.2	32.34 ±0.3	32.37 ± 0.3
50mg/ Kg	36.45 ± 2.08 *	33.64 ± 2.56	32.31 ± 1.98	32.14 ± 0.24
100mg/ Kg	35.47 ± 1.35 *	32.71 ± 1.21	30.51± 1.43	26.5 ± 0.93 *

200mg/ Kg	36.36 ±0.67 *	31.32 ±0.98	36.53 ± 4.54	33.55± 4.03
300mg/ Kg	33.81 ± 2.00	31.72 ±1.22	30.05 ± 2.62	34.4 ± 1.70

Dose in mg/Kg orally. n/g = 3 . *P≤ 0.05 compared with control value

The data in table 7 are showing the results of the mean of white blood cells count(WBC), A significant increase(P≤ 0.05) in numbers of WBCs with all of the using doses ,and with the periods. A higher significance(P≤ 0.05) was noted with the first dose (50mg/Kg)with the largest number in WBCs after oral administration of the extract in compare with control . in addition that in spite of increasing in number of WBCs with a high significant , there was a decrease in the number of WBCs with increasing the days of oral administration in the mice.

Table 7 : Effects of the extract on White blood cell (WBC)counts in mice

Mean of White blood cell count($x10^3$cell/µL)				
Treatment	15^{th} day	25^{th} day	35^{th} day	45^{th} day
Control(10ml/Kg)	3.483± 0.07	3.478±0.99	3.494 ±0.77	3.473 ± 0.97
50mg/ Kg l	19.166±0.30 *	18.766 ± 0.20 *	18.800 ± 0.70 *	5.833 ±0.30 *
100mg/ Kg	10.350± 0.44 *	4.200±0.36 *	5.400 ± 0.20 *	1.750 ±0.15 *
200mg/ Kg	9.150±0.40 *	12.383 ± 0.46 *	9.016 ± 0.68 *	8.066±0.58 *
300mg/ Kg	11.533± 0.20 *	9.233± 0.30b *	5.516 ±0.36 *	5.566 ±0.61 *

Dose in mg/Kg orally. n/g = 3 . *P≤ 0.05 compared with control value

Discussion

The acute toxicity test are generally the first test conducted in any toxicity study. They provided data on the relative toxicity likely to arise from a single or brief exposure to any substance . Different plants extracts have been known to possess different levels of toxicity which majorly depends on the levels of antinutrients inherent in the plants (60). Preliminary investigations on the acute toxicity of the cocoon extract in mice showed that the aqueous of cocoon like shell beetle for *Larinus maculatus* extract has safety profile when given orally , an indication of relative non- acute toxicity of the raw extract, since no mortality observed in animals after 24 hours of administration. The Blood in mammals helps distribute the nutrients, oxygen, and hormones the body needs. It also carries toxins and waste materials to the liver and kidneys to be removed from the body, for these reasons the haematological system has been proposed as being an important target organ for inducing toxicity(61) . The aim of this study is to ascertain if the raw cocoon extract could have beneficial effect or risk cautions with using it with the animals on hematological parameters in the mice. The red blood corpuscles (RBCs)count provides an indirect estimate of

the blood's haemoglobin content , A low Packed cell volume(PCV) and/ or haemoglobin concentration(Hb) indicates such conditions as anemia or over hydration in the mice (62). The effect of the extract on these parameters indicated likelihood of the extract to induce anemia even after long use (63).In order to know the types of anaemias, which is not a single disease but a group of disorders in which haemoglobin concentration of blood is below the normal ranges in the experimental animals (61), we calculate: the mean corpuscular hemoglobin (MCH) , which is the average mass of hemoglobin per red blood corpuscles in a sample of blood and the mean corpuscular volume(MCV),which is a measure of the average size of the red blood corpuscles . When we checking the values in MCH with the values in MCV in the tables 4 and 5 , as we expected actually that the MCH values is the mirror of MCV values . So we considered the first and third doses (50& 200mg/Kg)are causes microcytic anaemia (due to diminished the size of RBCs)in mice; While the second and fourth doses (100 & 300 mg/Kg) are causes macrocytic anemia (due to increase the sizes of RBCs) (64). The mean of mean corpuscular hemoglobin concentration (MCHC),which is a measure of the concentration of hemoglobin in a given volume of packed red blood corpuscles; It is reported as parts of a standard complete blood count. the range for it is between 32-36 mg/dl. The data in the table(6) approximately with the ranges in general ,and that means there was anemia with experimental animals after oral administration the extracts of cocoon with different doses and with different periods. when we examining the values in table 6, and comparing with tables 4 & 5 , we can consider that the doses 50 and 200mg/Kg are causes normochromic/microcytic anaemias (that's mean the low concentration of hemoglobin with small volume of corpuscles) and that case is results may be from a deficiency of the hormone erythropoietin from kidney failure (64). While the dose 100mg/Kg is causing hypochromic /microcytic anaemias (that's mean a little amount of Hb inside small size cell or failure to produce Hb results in smaller cell) that case is results may be from iron deficiency. While the dose 300mg/Kg is causing normochromic/macrocytic anaemia (that's mean a little amount of Hb inside large size cell) that case is results may be from Vitamin B12 deficiency or from folate deficiency (62).The white blood cells or leukocytes, are a part of the immune system and help the body fight the infection. They circulate in the blood so that they can transported to an area where an infection has developed when we comparing with the values in the control. the increasing of the number of leukocytes ,which is called leukocytosis; it may be normal to happened due to anemia in the mice' s bodies, since the increase in WBCs may be due to anaemia or severe emotional or physical stressor tissue damage (61), or it may probably due to the normal responses to foreign bodies or stress associated with the chronic administrations study. Hackbath *et al*.(65)had earlier recorded a strong influence of food components on haematological traits , packed cell

volume and haemoglobin concentration being very strong indicators of nutritional status of animals, and we expect that is happen in our study. The results obtained for all treatment groups indicate malabsorption , since the anaemias results from iron or folate deficiency; which the extract of cocoon like shell beetle for *Larinus maculatus* F. effects to the gut in the animals and suppress the absorption of the important iron and vitamins for synthesis the red blood cells. and may be affects on other organs or tissues in these animals ,since we used the raw extract which certainly contain the antinutrients components and may produced many undesirable effects . therefore other studies must be done in the future to detect the components, the ratios of the antinutrients in this cocoon and how much it affects on the different animal tissues .

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تأثير مستخلص مائي خام لشرنقة خنفساء السوس.F *Larinus maculatus* في بعض المعايير الفسلجية للدم في ذكور الفئران البيض *Mus musculus*

*إقبال ناجي توفيق

الخلاصة :

هدفت الدراسة إلى بحث تأثير مستخلص مائي ساخن خام لشرنقة الخنفساء *Larinus maculatus* Faldermann التابعة لعائلة السوس (Family: Curculionidae) ، بتراكيز مختلفة (200،100،50 و300ملغم/كغم/يوم) ومع فترات زمنية مختلفة (35،25،15 و45) يوما في بعض المعايير الدموية كتركيز الهيموكلوبين (Hb)،عدد كريات الدم الحمراء (RBCs count)، حجم الكريات المرصوصة (PCV)،متوسط حجم الكريه (MCV)، متوسط هيموكلوبين الكريه (MCH)، متوسط تركيز هيموكلوبين الكريه (MCHC) وعدد خلايا الدم البيضاء (WBCs count) في الفئران البيضاء التي تم تجريعها فمويا. أشارت النتائج إلى حدوث نقص وبشكل معنوي ($P\leq 0.05$) في قيم Hb،PCV و RBCs ، وان كلا من المتوسطات MCV، MCH وMCHC بالتالي تأثرت مع نقص معنوي فيها ($P\leq 0.05$) في كل تراكيز الجرعات المستخدمة ولجميع الفترات الزمنية . وبالنسبة لعدد خلايا الم البيضاء (WBcs) فقد تأثرت هي الأخرى وظهرت زيادة معنوية مصاحبة لجميع التراكيز ولكل الفترات الزمنية . تشير النتائج إلى تأثر حيوانات التجربة بحالة فقر الدم Anemia ناتج من عدة أسباب ، في مقدمتها قلة عدد كريات الدم الحمراء من جراء نقص الحديد أو من نقص فيتامين B_{12}. وان وجود مثل هذا التأثير غير المرغوب فيه يدلل على عدم صحية مستخلص الخام للشرنقة ، والمستخدم حاليا في الطب الشعبي كعقار للسعال ،والتهاب القصبات لدى كثير من العوائل في العراق ، سوريا ، تركيا وإيران .