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Study the effect of hot aqueous extract of beetle cocoon *Larinus maculatus* F. on some physiological parameters in male albino mice

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Abstract: *The study aimed to investigate the effect of oral administration of hot aqueous extract of beetle cocoon Larinus maculatus Faldermann, in a two doses 50 and100mg/Kg/B.wt for 3 and 6 weeks respectively on the levels of serum glucose, weight of body, and lipid profile in male mice Mus musculus. The results revealed that there was a significant ($p<0.05$) decrease in serum glucose level was dose and period dependent. Weight of body also reduced significantly ($p<0.05$) with doses and period dependent. The lipid profile level significantly ($p<0.05$) decreased in dose and period's manner in each of Total cholesterol (TC), Triglyceride (TG), High Density Lipoprotein- (HDL), Low Density Lipoprotein (LDL), and Very Low Density Lipoprotein (VLDL). Results showed that the hot aqueous extract of beetle cocoon has side effect at two administered doses in present study, since the raw extract of this cocoon is used in people medicine to treat for respiratory inflammations in Iraq from decades. The study revealed that the extract have some undesirable effects on lipid profile, so must advise the people not to rush in using the extract of cocoon in therapy of chronic respiratory problems because it has other side effects in the body.*

Key words *cocoon, extract, glucose, lipid profile, mice*

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I. Introduction

From many centuries, Arabic medicine was the first form of original medicine whose plenty of their drugs derived from natural substances especially plants or parts of plant from the Mediterranean area, and Iraqi people from hundreds of years was a race and distinctive in this field (1). Nowadays evaluations revealed that a higher of eighty percent of the world's population depends on classic medicine for providing healthcare (2, 3).

The medicine of herbs is commonly used in Iraq, and hot watery extract of cocoon *Larinus maculatus* L. was one of such medicinal substances described to treat cough and problems of respiratory system (4). That cocoon was mostly used as food in Iraq especially in north areas and in countries around the White Sea (5, 6). Trehala manna is a name of cocoon of *Larinus maculatus* beetle has the low sweet tasting (7). Trehala manna is not the same manna, which has been mentioned in Sahih Muslim that the Muslims' prophet Muhammad told us that our God sent it down upon the people of Israel, and the water of it is use as a medicine for the eyes (8). Previous studies were carried out for knowing if the extract is accepted to use as food but their results revealed that there were unwanted effects in blood picture and many changes occurred in most of tissues and internal organs in male mice (9, 10). Because of using the cocoon was without consulting a specialist doctor, so we proposed to complete the search to ensure if there was another effects on the body weight, serum level of glucose, and lipid profile on male mice.

II. Materials and methods

2.1 Collection and preparation of the extract from cocoon

Cocoon of beetle of *Larinus maculatus* was purchased from a local market in Baghdad, Iraq .The cocoon was identified by Iraqi Natural History Museum. The cocoon was cleaned from the insects, milled using a laboratory electric mill (Arthur H. Thomas/ USA). The watery extract was obtained according to (11) by stirring 5 gm of milled powder with 50 ml of hot distilled water at room temperature for one hour. The suspension was centrifuged (by B&T/ England) at 3500 rpm for 60 min, and the supernatant was filtered through Whatman filter paper No.1. The extract was concentrated within 48 hours by using incubator at an optimum temperature of 34-37° C. The doses 50 and 100 mg/Kg/B.wt were prepared by weighing suitable quantities of dry extract of cocoon and dissolved each of them with distilled water.

2.2 Experimental mice, preparation and acclimatization

Sixty healthy mature albino male mice (*Mus musculus* L.), with age 45- 60 days and weighing 25± 0.5 gm, was purchased from the animal house unit, College of Medicine, University of Baghdad, Iraq. The mice

were housed in polypropylene cages, under well ventilated animal house conditions at temperature 22-25 °C, photoperiod with 12 hours natural light and 12 hours' natural dark. The mice were fed with balanced pellets with tap water *ad libitum*.

2.3 Experimental design

Sixty (60) mature male mice were randomly divided into 3 main groups 1, 2, and 3 (20 animals for each group). Then each group was subdivided into A, and B which represents the periods 3 and 6 weeks of administration respectively as follows: The group1 was given 0.25ml saline and served as control. Group 2 and 3 were given the extract at dose 50 and 100mg/ kg of body weight/day. The treatment was given orally for 3, and 6 weeks once daily between 8:00-10:00AM, then after 24h from the last treatment for each group, blood samples were collected by cardiac puncture, from control and extract treated mice. Blood samples with cool centrifuged (LKB/ Finland), collected by Pasteur pipette and put it in refrigerator at -20 °C for analysis within 24 hours. Glucose, Total cholesterol (TC), and Triglyceride (TG) were determined by enzymatic colorimetric method of Trinder (12). High density lipoprotein (HDL), Low density lipoprotein (LDL) were assayed using Randox diagnostic kits determined following the principle described by Trinder(12), then from the results very low density lipoprotein (VLDL) were calculated as mentioned in (13). Data were analyzed using SPSS for Windows, version 16.0 SPSS Inc. Chicago, United States) and the results are expressed as mean ± SE.

III. Results

3.1 changes in serum glucose levels

Table (1) represents the level of serum glucose and there was a significant ($p<0.05$) decrease in treated groups (G2) and (G3) as compared to control groups (G1), the reducing in the level of serum glucose was with low and high dose of the extract administration and dependent with long lasting exposure when compared to control group.

Table (1): Effect of 50, and 100 mg/ kg/day of cocoon extract after 3 and 6 weeks treatment on the level of serum glucose (mg/dl) in mature male mice

Groups	Glucose level after 3 weeks (A)	Glucose level after 6 weeks (B)
Control (G1)	96.19 ± 1.71 a	99.00 ± 1.51 a
treated (G2)	88.40 ± 2.26 b	83.90 ±1.45 b
treated (G3)	87.73 ± 2.47 b	80.13 ± 1.20 b

Each group n=10 male mice, each value represents mean± SE, Different letters refer to significant differences ($p<0.05$) in compared with control group (in same column).

3.2 Changes in the body weights

The results in Table (2) showed a significant ($P<0.05$) increase in the body weight of control groups (G1A) and (G1B) after 3 and 6 weeks of treatment as compared with initial weight of mice. Also results showed a significant ($P<0.05$) decrease in the body weight in treated groups (G2A) and (G3A) after 3 weeks, and there was another significant ($P<0.05$) decrement in the body weight in treated groups (G2B) and (G3B) after 6 weeks of treatment as compared to control groups in same periods. A significant ($P<0.05$) decrease in body weight was showed between treated groups in same period of treatment. The decreasing in the body weight of treated mice was dependent on dose and period of treatment with cocoon extract.

Table (2): Effect of 50 and 100 mg/ kg/day of cocoon extract on the body weight (gm) in male mice after 3 and 6 weeks treatment

period	Control (G1)	Treated (G2)	Treated (G3)
Initial weight	25.1 8± 0.24 a	25.26 ± 0.17 a	25.42 ±0.28 a
Treated for 3 weeks (A)	30.4 ± 0.90 b	22.52 ± 0.10 b*	21.01±0.17 b*
Treated for 6 weeks (B)	39.1 ± 0.45 c	18.73 ± 0.13 c*	17.51±0.16 c*

Each group n =10 male mice, each value represents mean ± SE, the different letters mean significant ($P<0.05$) difference between treated groups in the same column, the * mean significant ($P<0.05$) difference between treated groups in same period (same row)

3.3 Changes in lipid profile levels

Results in table (3) showed serum lipid profile level after 3 weeks of treatment with hot watery extract of cocoon, a significant ($P<0.05$) decrease in levels of T.C, T.G, HDL, LDL, and VLDL in serum of treated groups

G2A and G3A as compared to G1A. The reducing in the levels of all of them was dependent on dose and long lasting of exposure on treated mice after 3 weeks of oral administration of the extract.

Table (3): Effect of 50 and 100 mg/ kg/day of cocoon extract on the level of serum lipid profile(mmol/l) in male mice after 3 weeks treatment

Group	T.C	T.G	HDL	LDL	VLDL
Control G1 A	0.90±0.01 a	2.77±0.09 a	0.70±0.06 a	0.34±0.12 a	0.554±0.018 a
Treated G2 A	0.80.±0.10 b	2.60±0.10 b	0.65 ±0.11 b	0.30 ±0.10 b	0.52 ±0.02 b
Treated G3 A	0.75±0.03 c	2.30 ± 0.07 c	0.58 ±0.03 c	0.26±0.01 c	00.46±0.014 c

In each group n =10 male mice, each value represents mean± SE, the different letters mean significant ($P<0.05$) difference between treated groups in the same column.

Table (4) represents the results in relation to oral administration of extract after 6 weeks of treatment on serum lipid profile, showed a significant ($P<0.05$) decrease in the levels of T.C, T.G, HDL, LDL, and VLDL in serum of treated mice (G2B) and (G3B) when compared with control group (G1B). The decrement of the level in serum lipid profile was period and dose dependent.

Table (4): Effect of two doses of hot aqueous extracts (50 and 100 mg/ kg/day) after 6 weeks on the levels of serum lipid profile(mmol/l) in male mice

Group	T.C.	T.G	HDL	LDL	VLDL
Control G1 B	0.92±0.02 a	2.77±0.002 a	0.70±0.01 a	0.33±0.01 a	0.554±0.00 a
Treated G2 B	0.74±0.03 b	2.40±0.00 b	0.60 ±0.02 b	0.20 ±0.01 b	0.48 ±0.00 b
Treated G3 B	0.70±0.00 c	2.15 ± 0.00 c	0.50 ±0.03 c	0.13±0.03 c	0.43±0.00 c

Each group n =10 male mice, each value represents mean± SE, the different letters mean significant ($P<0.05$) difference between treated groups in the same column

IV. Discussion

Human beings from ancient times tried to made from the nature sources around him to extract medicines from local plants known to heal diseases with a hope to produce a special syrup for staying live forever. Trehala manna, the name of cocoon is known in Iraq as "Tehan" was described before decades for treat problems of respiratory system (11). Any substance in order to be described as a drug must have in some of their components beneficial chemicals and other of their component are not, this mean may be there was side effect in that substance (14). Trehala manna in our study is from northern of Iraq, a cocoon has been analysis by Leibowitz (15), and Sabry and Atallah (16) whom isolated sucrose, melezitose, and trehalose which is the important component in it (trehalose represents of 7% only from raw material of the cocoon). Trehalose is a sugar the most beneficial for human and other organisms (17). Our study showed that the hot watery of the extract of cocoon had decreased the levels of glucose, and also reducing the body weight in treated mice. The results may be expected because trehalose today's is used to decrease the level of glucose in patients with diabetes, and it is also used for loss the body weight in human (18), but we must not forget that trehalose which we talk about it is in a pure form and in present study the raw extract of cocoon is a mixture from it with other components. Results also revealed that there were decreases in all of levels of serum lipid profile, that changes are not expected, because trehalose alone has the ability to preserve and prevent any changes of lipids in the serum (18), and in previous studies we were used the same doses of the raw extract and it induced many changes in hematological parameters, adipose tissue, kidney, liver and other tissues in mice (9, 10), and in present study it induced changes on the levels of glucose, the body weight, and on the level of lipid profile. So we are in doubt about the extract of cocoon. There are may be another causes or may be the cause of raw extract components. So a new attempt to analyze the real components of the same trehala manna, and the primary results showed that cocoon consists of tannins, flavonoids, resins, carbohydrates, proteins, heavy metals such as cadmium, lead, zinc, iron, and maybe there are other materials because the study is not complete yet (Abdul Aziz, A., private communication). We should not be surprised about the presence of large quantities of heavy metals in the cocoon we obtained from northern Iraq because of the large number of wars that occurred and the pollution causing the whole area. Our results about decrease the levels of serum T.C, T.G, HDL, LDL, and VLDL can be accepted if we considered the cause is that the presence much of quantity of heavy metals in the extract of cocoon and its affect together much more than the effect of trehalose and other nutrient components that mentioned above on male mice, in this case the results about reducing serum levels of lipid profile in

present study is agreed with study about effect of heavy metals such as cadmium, lead, zinc, and iron on the levels of serum lipid profile in white mice (19).

V. Conclusion

In conclusion the present study revealed that there was a caution when hot watery of the extract of cocoon had been taken orally despite the presence of trehalose in its component, we recommend for another studies about cocoon components, and another studies about its effect and how it is affect. Also we must advising the people not to rush about using the raw extract of cocoon in treat chronic respiratory diseases because it has other side effects in the body.

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