

## Toxicopathological study of aqueous extract of beetle cocoon *Larinus maculatus* F. on some internal organs in male albino mice

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### Summary

The present study was carried out to investigate the effect of oral administration of hot aqueous extract of beetle cocoon *Larinus maculatus* Faldermann, in a two doses 50 and 100mg/Kg/Bw for 25 and 45 days respectively on some organs such as liver, lung, kidney, intestine, heart, spleen, and brain in male mice *Mus musculus*. The results indicated that there were toxicopathological changes in many tissues of experimental animals. Histo-pathological changes was dose and period dependent. It was found that the aqueous extract of beetle cocoon has undesirable effect at the administered doses, since the raw extract of this cocoon is currently being used in Folk medicine as treatment for cough, bronchitis in Iraq. This study revealed that the low doses of this extract have some deleterious effects on certain organs, so it should not be used for long durations in control of respiratory disease.

**Key words:** Aqueous crude, extract, beetle cocoon, histopathological effect.

دراسة مرضية سمية حول تأثير المستخلص المائي الخام لشرنقة خنفساء *Larinus maculatus* F. على بعض الأعضاء الداخلية لذكور الفئران البيض

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### الخلاصة

هدفت الدراسة لمعرفة تأثير التجريع الفموي للمستخلص المائي الحار لشرنقة خنفساء *Larinus maculatus* F. وبجرعتين (50 و100 ملغم/كغم من وزن الجسم) وللفترات (25 و45) يوماً على التوالي على أنسجة بعض الأعضاء الداخلية كالكلب، الرئة، الكلى، الأمعاء، القلب، والدماغ في ذكور الفئران البيضاء. أشارت النتائج إلى حدوث تغيرات سمية مرضية في أنسجة هذه الأعضاء، حيث اختلفت شدة التغيرات المرضية النسبية بزيادة كمية الجرعة وطول مدة التجريع. لقد تبين إن للمستخلص تأثير ضار وبالجرع المستخدمة في هذه الدراسة. بما إن المستخلص الخام للشرنقة يستخدم في الطب الشعبي العراقي لمعالجة السعال والتهاب القصبات فقد أوضحت نتائج الدراسة إن الجرع الواطئة من هذا المستخلص لها تأثيرات ضارة على بعض الأعضاء الداخلية، ولا يمكن استخدامه لفترة طويلة لعلاج الأمراض الخاصة بالجهاز التنفسي.

### Introduction

Medicinal plants have been a major source of therapeutic agents since ancient times. Natural products are the cornerstone of health care delivery especially in resource poor settings. Present estimates indicate that about eighty percent of the world's population relies on traditional medicine for health care delivery (1, 2).

The use of traditional and herbal medicine is widely practiced in Iraq, and the aqueous extract of cocoon *Larinus maculatus* L. (Family: Curculionidae) is one of such medicinal substances commonly employed to control bronchitis and inflammation of respiratory system. That cocoon is widely distributed in: Iraq especially in North Iraqi District, Syria, Egypt, Sinai, Turkey, and Iran (3).

Trehala manna, this is the name of the sweet-tasting cocoon of the *Larinus maculatus* beetle which is the food miraculously supplied to the Israelites during their wanderings journey in the wilderness(4), and is used as food in Syria (5). The name man-es-simma or manna wa salwa, has been preserved in Arabic by the Arab's Sinai who harvest it. It was said, manna which was "white like coriander seed and tasted like a wafer made with honey" (3), 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" (6). The manna of northern Iraq

(called gazzo) is used for sweetening pastry , also used as an aperients and expectorant (7); cures teeth-gum ; its seeds , fruit and leaves are anti-rheumatic and fever reducer. Ash manna is cures skin ulcers (6); and used as a laxative, Since Avicenna described in "Pharmacopoeia Persia" in 1681, is given for coughs and to relive respiratory organs (8).Despite of popular use of this cocoon , no information exists about its safety. Since the diseases of respiratory is a chronic disorder, this study conducted to evaluated the sub-acute and sub-chronic toxicity of the aqueous extract of the cocoon beetle *Larinus maculatus* Faldermann in male mice, so its histopathological effect on some organs need to be investigated for understanding such food with a view to determining its acceptability.

## **Materials and Methods**

### **\* Collection of cocoon and preparation of the aqueous extract**

Cocoon like shell larval beetle of *Larinus maculatus* was purchased from a local market in Baghdad and Karbala, Iraq .The cocoon like shell was identified by Iraqi Natural History Museum. The cocoon was cleaned from the insects ,milled separately using a laboratory electric mill (Philips/Holland). The watery extract was obtained according to (9) by stirring 10 gm of milled powder with (100ml) of hot distilled water at room temperature for one hour. The suspension was centrifuged(by centrifuge Jouan/France) at 4000 rpm 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° C for two days.

The higher doses of extract for sub acute(2,4,8,12, and 20gm/Kg/Bw)were prepared by using following weights: 2gm/Kg/Bw=50mg of dry extract/0.25ml distilled water/1 mouse 25 gm Bw , 4 gm/Kg/Bw =100mg of dry extract/0.25ml distilled water/1 mouse 25 gm Bw , 8 gm/Kg/Bw=200 mg of dry extract/0.25ml distilled water/1 mouse 25 gm Bw , 12 gm/Kg/Bw= 300mg of dry extract/0.25ml distilled water/1 mouse 25 gm Bw , and 20 gm/Kg/Bw= 500mg/Kg/Bw of dry extract/0.25ml distilled water/1 mouse 25 gm Bw. While the doses for sub chronic(50 and 100 mg/Kg/Bw) were prepared as following weights:50mg/Kg/ Bw = 1.25 mg of dry extract/0.25ml distilled water/1 mouse 25 gm Bw, and 100mg/Kg/Bw =2.5 mg of dry extract/0.25ml distilled water/1 mouse 25 gm Bw.

Adult male albino mice (*Mus musculus*) were about 6-8 weeks age, with average weight 25 ± 3 grams, were purchased from the animal house for the Department of Drug Quality and Control /Al-Andalous square in Baghdad. The animals were housed 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 prepared diet and water - ad libitum were provided.

Sub acute and sub chronic toxicity study of the aqueous extract of beetle cocoon orally administered to mice was done according to the method (10); the mice were observed for obvious toxic symptoms, or mortality determined twenty four hours after administration. Sixty (60) males mice were randomly assigned into 10 groups(6 animals for each group). Two groups for Control were administered 0.25 ml of normal saline (0.9%NaCl), while 4 groups of mice were administered (50mg/Kg and 100mg/Kg /Bw) of extract respectively. The extract were administered for (25 , and 45 days) for each group to the animals using oral rout by means of polythene cannula. The last 4 groups were divided into 4-5 mice for administration the high doses (2, 4,8,12, and 20 gm/Kg/BW) for sub acute study (once for 1 day). At the end of every period for each group; the animals were killed by cervical dislocation, scarified directly and specimens from kidney, heart, lung, liver, spleen, intestine and brain were taken for histopathological study and prepared routinely and stained with hematoxylin and eosin (11). Tissues were examined for lesions by light microscope under magnification power. Photographs were taken by Sony digital camera.

## **Results**

Mice treated with the crude aqueous extract of the cocoon beetle for *Larinus maculatus* showed at the higher doses (2 , 4, 8 ,12 and 20 g/ Kg) , anorexia (loss of appetite),

diarrhea, weakness or slow movement were observed , and no mortality observed after twenty four hours of oral administration. The mice of control shows no histopathological changes .

The histopathological effects of the administered doses (50, and 100 mg /Kg/Bw) of aqueous extract of beetle cocoon *L. maculatus* for (25 and 45 days) on different tissues are present as follows : the oral administration of the dose 50 mg/Kg/Bw for 25 and 45 days on liver, kidney, intestine, and heart in male mice are shown in Figs.(1-4). The results show that there were various degrees of histological alterations observed in the organs examined. Liver sections in this dose after 25 days illustrated in (Fig.1) showed congestion and dilation of central vein with vacuolar degeneration of hepatocytes, whereas in sections of kidney showed atrophy to glomerular tuft (Fig. 2).

Also; the results showed fatty degeneration in the heart , and increasing in numbers of goblet cells in the intestine of mice (Fig.3) and (Fig.4) respectively.



Fig.1: Liver of mouse administered orally aqueous extract of cocoon (50mg /Kg / Bw) for 25 days showing dilation & congestion of central vein with vacuolar degeneration of hepatocytes (H&E x400)

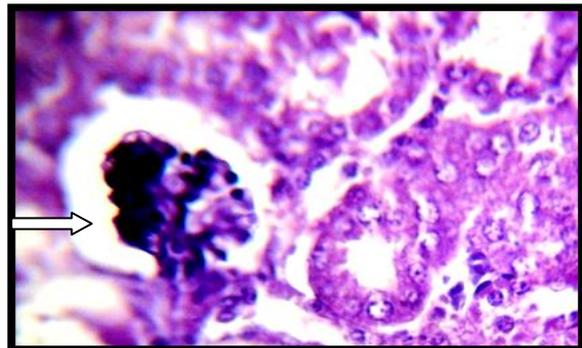


Fig . 2 : Kidney of mouse administered orally aqueous extract of cocoon ( 50 mg /Kg / Bw)for 25 days showing atrophy of glomerular tuft (H & E x400)

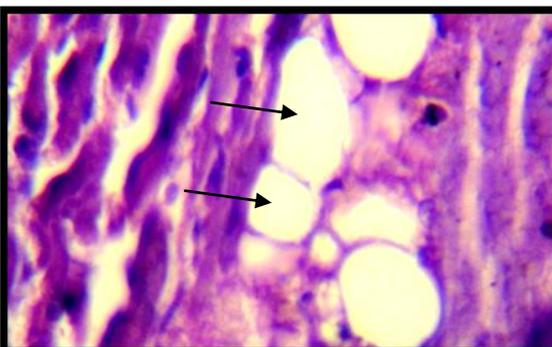


Fig. 3 : Heart of mouse administered orally aqueous extract of cocoon (50mg /Kg/Bw)for 25 days showing fatty degeneration (H&E x400)



Fig.4: Intestine of mouse administered aqueous extract of cocoon (50mg/Kg /Bw) for 25 days showing increase in numbers of goblet cells (H&E x400)

At the same period (25 days) but with dose of 100mg/Kg/Bw , the results showed that there were severely changes : in sections of kidney and lung which showed perivascular cuffing (Fig.5) and (Fig.6). Sections of intestine showed mononuclear cells infiltration in the lamina propria of mucosa (Fig.7), and in (Fig.8) showed severe fatty degeneration in heart's muscles.

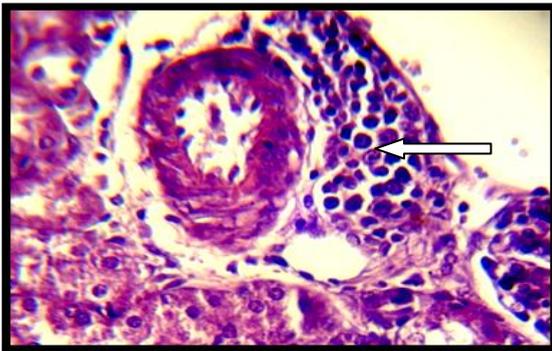


Fig.5:Kidneyof mouse administered orally aqueous extract of beetle cocoon (100mg / Kg /Bw) for25 days showing perivascular cuffing (H&Ex400) →

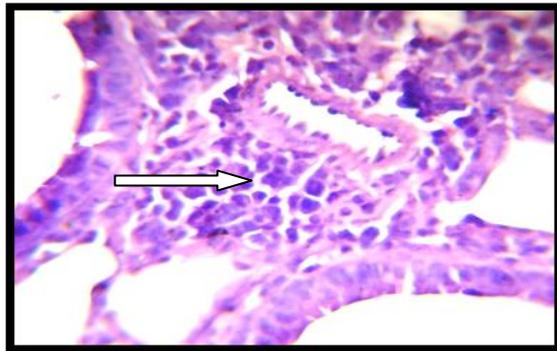


Fig.6: Lung of mouse administered orally aqueous extract of beetle cocoon (100mg /Kg/Bw) for 25 days showing perivascular cuffing → (H&Ex400)

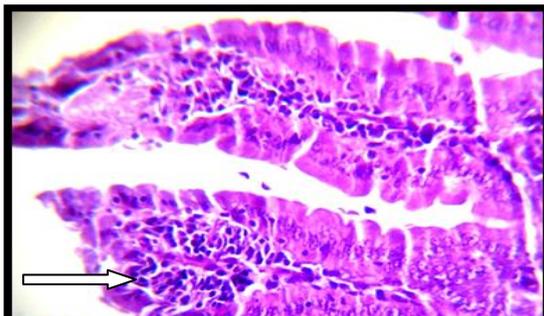


Fig.7: Intestine of mouse administered orally aqueous extract of beetle cocoon(100mg /Kg/Bw)for25days showing mononuclear cells infiltration in the lamina propria of mucosa (H&Ex400) →

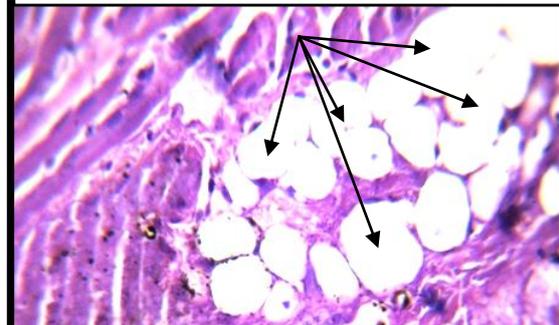


Fig 8: Heart of mouse administered orally aqueous extract of beetle cocoon (100mg /Kg/ Bw)for 25 days showing severe fatty degeneration → (H&Ex400)

**After administrations** for 45 days with dose 50mg/Kg/Bw, the histopathological changes are shown the similar lesions in intestine and heart sections as in the previous period. In sections of brain ,there were perivascular and perineural edema (Fig.9, and Fig.10), the kidney showed focal interstitial and periglomerular mononuclear cells infiltration. Thickening of interalveolar septa due to congestion and mononuclear cells infiltration in lung sections(Fig.11); whereas spleen sections showed depletion of lymphoid white pulp with increase in numbers of megakaryocytes and deposition of hemosidrin pigment(Fig.12) .

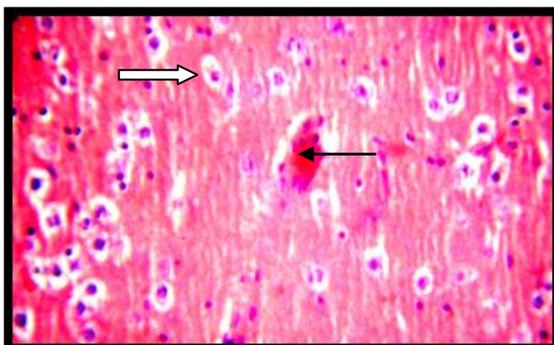


Fig.9: Brain of mouse administered orally aqueous extract of beetle cocoon (50mg/Kg /Bw)→for 45 days showing perivascular and perineural edema → (H&Ex400)

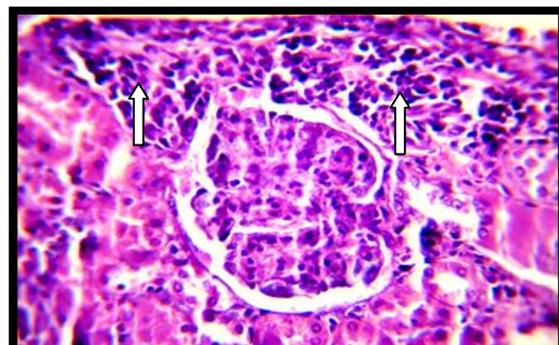


Fig.10: Kidney of mouse administered orally aqueous extract of beetle cocoon (50mg/Kg/Bw) for 45 days showing focal interstitial and periglomerular mononuclear cells infiltration → (H&Ex400)

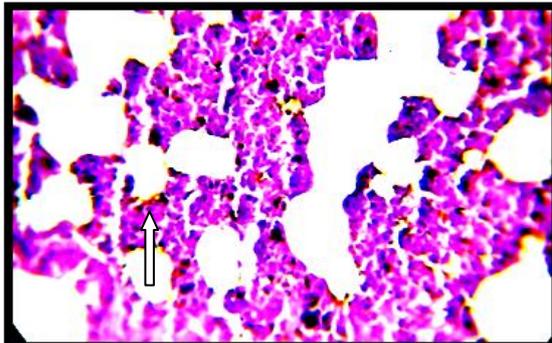


Fig. 11: Lung of mouse administered orally aqueous extract of beetle cocoon (50mg /Kg/Bw)for 45 days showing thickening of interalveolar septa , congestion and mononuclear cells infiltration  $\Rightarrow$  (H&Ex400)

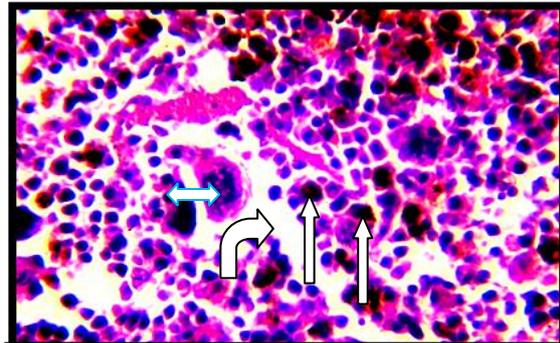


Fig12:Spleen of mouse administered orally aqueous extract of beetle cocoon (100mg /Kg/Bw)for 45 days showing depletion of lymphoid white pulp  $\curvearrowright$  with increase in numbers of megakaryocytes  $\Leftarrow$  and deposition of hemosiderin pigment  $\Rightarrow$  (H&Ex400).

The results with dose 100mg/Kg/Bw after 45 days of administration. Liver sections showed moderate prebiliary fibrosis with hyperplasia of epithelial lining forming papillary projections (Fig.13), whereas brain sections showed degeneration of pukinji cells with complete dissolution of the others (Fig.14). Kidney's sections showed periglomerular mononuclear cells infiltration with fibrous thickening Bowman's capsule fibrosis (Fig.15). Lung sections showed pneumonic area with adjacent emphysema (Fig.16). The histopathological changes in intestine: an increasing of goblet cells and fatty degeneration in the heart muscle are the similar with lesions of the previous periods (50mg /Kg /Bw for 25 and 45 days, and 100mg/Kg/Bw for 25 days)

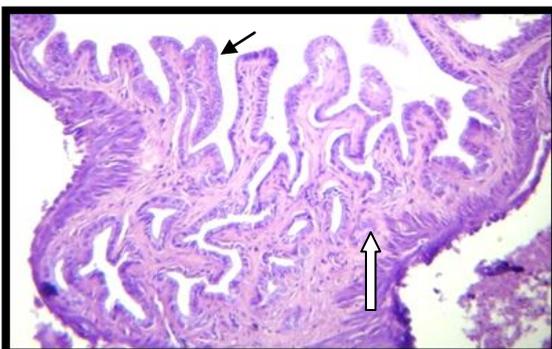


Fig.13: Liver of mouse administered orally aqueous extract of beetle cocoon (100mg / Kg/ Bw) for45 days showing moderate prebiliary fibrosis  $\Rightarrow$  with hyperplasia of epithelial lining forming papillary projections (H&Ex100)  $\rightarrow$

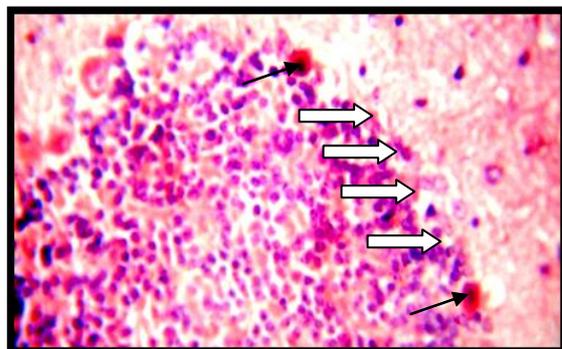


Fig.14 :Cerebellum of mouse administered orally aqueous extract of beetle cocoon (100mg/Kg/Bw) for 45 days showing degeneration of pukinji cells  $\rightarrow$  with complete dissolution of the others  $\curvearrowright$  ( H&EX400).

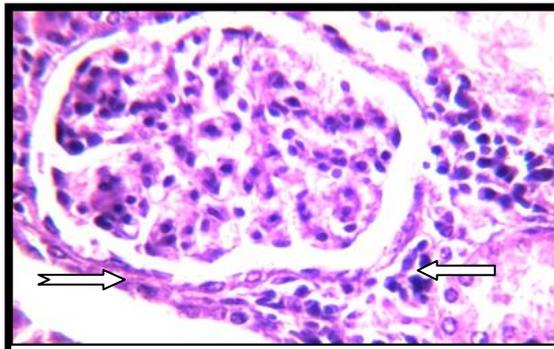


Fig.15:Kidney of mouse administered orally aqueous extract of beetle cocoon (100 mg / Kg /Bw) for 45 days showing periglomerular mononuclear cells infiltration with fibrous thickening Bowman capsule (H&Ex400)

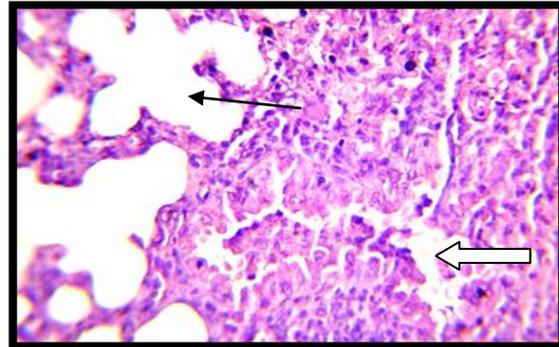


Fig.16:Lung of mouse administered orally aqueous extract of beetle cocoon (100mg/Kg/Bw) for 45 days showing pneumonic area with adjacent emphysema (H&Ex400)

### Discussion

Over the past decades, several reports in both developed and developing countries have indicated adverse effects allegedly arising from the use of medicinal plants (12). Some of these effects include abortion, dizziness, vomiting, diarrhea, abdominal pain, tachycardia, death, ulcer and loss of appetite (13). These effects could be attributed to the presence of phytotoxic compounds in the plant extracts and lack of actual dosage necessary for the treatment of diseases. The acute toxicity test (LD<sub>50</sub>) is 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 (14). The acute toxicity of the cocoon extract in mice showed that the crude aqueous extract of beetle cocoon of *Larinus maculatus* was not toxic even in the raw form at least at the administered doses (2, 4,8,12 and 20 gm/Kg/Bw) to mice; since there were no mortality and no histopathological changes observed after twenty four hours.

Kidney, liver, lung, heart, brain, spleen and intestine were chosen because they are the most sensitive organ of toxicity in both humans and animals. Liver has tremendous capacity to detoxify toxic principle and synthesize useful principles (15) (16), since the chronic toxicity of the extract were performed to it. It was obtained from the results; there were certain effects on all the tissues subjected in current study. The histopathological changes in the organs are clearly apparent; we supposed before starting this study that no side effects will happened when we will use the aqueous extract of beetle cocoon in administration of mice because of presence of trehalose; but we are surprised when the animals suffered from the loss of appetite, loss of activity. We supposed that the side effects are due to the other components in this cocoon, so perfect phytochemical analyses for its components are needed. Although the describing of the components had analyzed for the beetle cocoon have been done before 127 years ago (5); we hypothesized that the numerous chemical pollutions may resulted in certain biologically changes to these cocoons. It can be concluded that the aqueous extract of beetle cocoon *Larinus maculatus* F. caused toxicopathological changes in vital organs when used in low doses for long period and could not be used in beneficial treatment to contact diseases of respiratory system with long durations.

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