

The Effect of *Larinus maculatus* F. Cocoon Aqueous Extract in Some Immunological Aspects of Male Albino Mice

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Abstract

Traditional folk medicine is applied in the treatment of diversity chronic and acute conditions all over the worlds. This study was carried out to assess the capability of aqueous beetle cocoon extract (*Lorinus maculatus* F.) commonly known as (Tihan) in stimulating immune response. Adult mice (5 in each group) were divided into two groups: the first one orally administered with 150 mg/kg and the second group received normal saline as a control group for 14 days. Bone marrow and spleen were proceed for mitotic index and phagocytosis estimated. The results showed a significant increase in mitotic index and phagocytosis in treaded mice in comparing with the negative control. These results concluded that Tihan has a potential role in innate immune response.

Key words: *Larinus maculatus* F, phygocytotic index, mitotic index.

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Introduction

Over years, the expansion and mass syntheses of chemically drugs have developed health care all over the world. However, wide section in many countries still uses traditional physicians for their primary care. The most reasons for using folk remedy are that it is more affordable, economical and alleviates concerns about the adverse effects of chemical drug. Moreover, usage of folk medicine increases when conventional medicine is unsuccessful in the cure of illness, such as in cancer [1]. Traditional treatment provide valuable health care facility whether individuals have physical or economic admission to allopathic prescription, and it is a prosperous universal saleable enterprise [2].

Cocoon shell produced by salivary glands beetle larva of *Larinus maculatus* F from Curculionidae family. Known in Iraq as (Tihan) and in other countries (Iran and Syria) known as Trehala manna (Shekar tighal). People commonly use it in treatment of respiratory system diseases and viral or bacterial infection because of its potential ability in activation of immune system [3]. Additionally to its ability in purgative, antitussive, anti-asthmatic, febrifuge, anti-infective, and antioxidant properties which make it largely used in Iranian folk treatment [4]. Karam and Hamed [5] have reported the contains of water Trehala manna which is a different water soluble polysaccharides with variety structure and molecular weight. However, there is incomplete understanding regarding its analysis compound and its effects in immune system. For our knowledge, this is the first study in this aspect by using Iraqi Tihan and farther studies are needed to elucidate its different properties.

Materials and Methods:

Beetle cocoon extract preparation

Tihan cocoon shells were obtained from local shelf market (Iraq/Baghdad), identified by expert in Iraqi Natural History Museum (Figure 1). For hot aquatic extract [6]; cocoons were firstly emptied from inside insect, grounded using a laboratory electric grinder. 10 g from the powder dissolved in 100 ml of distal water using heating stirrer for two hour. The solution was centrifuged at 4000 rpm for 30 min and the supernatant then filtered and dried using incubator at temperature of 37° C for two days. A dose of 150 mg/kg were prepared and kept at 4°C.



Figure (1): Morphology of cocoon shell (Tihan) and *Larinus maculatus* F

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Experimental design:

Adult male albino mice (6-8 week age) were obtained from College of Education for Pure Sciences / Ibn Al-Haitham animal house for Department of Biology. All animals were housed for 7 days for adaptation before beginning the experiments. The animals were maintained at a temperature of 23 – 25°C with 12 hours light provided per day. They had free excess to food (standard pellets) and water. Animals mice were divided into two groups; the first group were administered 0.2 ml of normal saline (0.9%Nacl) as a control group, while the second group administered 150 mg/kg/bw for 14 days of Tihan aquatic extract.

Determination of mitotic Index:

Mitotic index was determined for cells obtained from bone marrow and spleen; according to Allen *et al.*, [7]. The cells of bone marrow and spleen collected using insulin syringe and gently suspended, centrifuged at 2000 rpm/min for 10 minutes). Cell pellet was then suspended in 5 ml of KCl; 0.075 M and incubated in a shaking water bath at 37°C for 30 min. after centrifuging at 3000 rpm/min for 10 min. pellet was fixed and 3-4 drops of cells were dropped on the slide and stained with Giemsa stain. Slides were observed under oil emersion lens (100X), and the percentage of mitotic index was calculated as the following:

$$M1 = \text{number of dividing cell} / \text{total number of cells} * 100$$

Determinations of phagocytotic index:

Macrophages were isolated from peritoneum as described by Lin *et al.*, [8]. Macrophages were collected from peritoneum by syringe and placed in tube, centrifuged at 2000 rpm/min for 5 minutes. The supernatant was discarded and pellet washed 3 times with Hanks buffer and Phagocytotic index was calculated as the following:

$$P1 = \text{number of phagocytic cells} / \text{total number} * 100$$

Statistical analysis

The statistical analysis was performed using Minitab 16 (Minitab Ltd, Coventry, UK). Differences between groups were determined using Students t-test. Data were expressed as mean and standard error and difference were accepted at $p \leq 0.05$.

Results

Determination of mitotic Index:

The mitotic index of spleen cells in control mice was significance increased ($58\% \pm 0.49$) compared with control group ($30\% \pm 0.63$) as shown in Figure 2.

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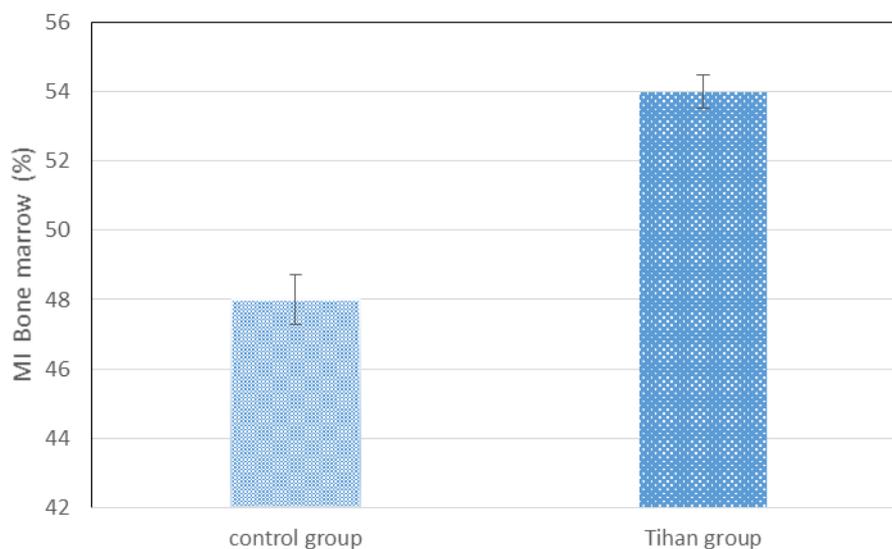


Figure (2): Mitotic index from bone marrow cells in albino male mice treated with aqueous extract of Tihan (*Larinus maculatus*) and control group (normal saline). Data express as mean \pm standard error.

In bone marrow cells no significance difference between treatment (48% \pm 0.37) and control group (54% \pm 0.44) was as shown in Figure 3

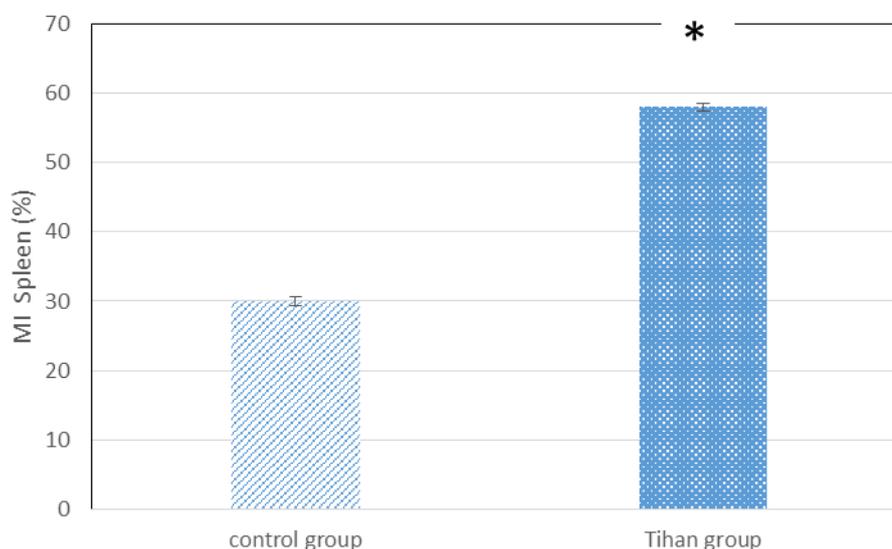


Figure (3): Mitotic index from Spleen cells in albino male mice treated with aqueous extract of Tihan (*Larinus maculatus*) and control group (normal saline). Data express as mean \pm standard error. * $P \leq 0.05$

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Determinations of phagocytotic index:

The results of phagocytotic index shown interesting highly significant increased $26.81\% \pm 0.92$ compared with control group 6.19 ± 1.05 as shown in Figure 4

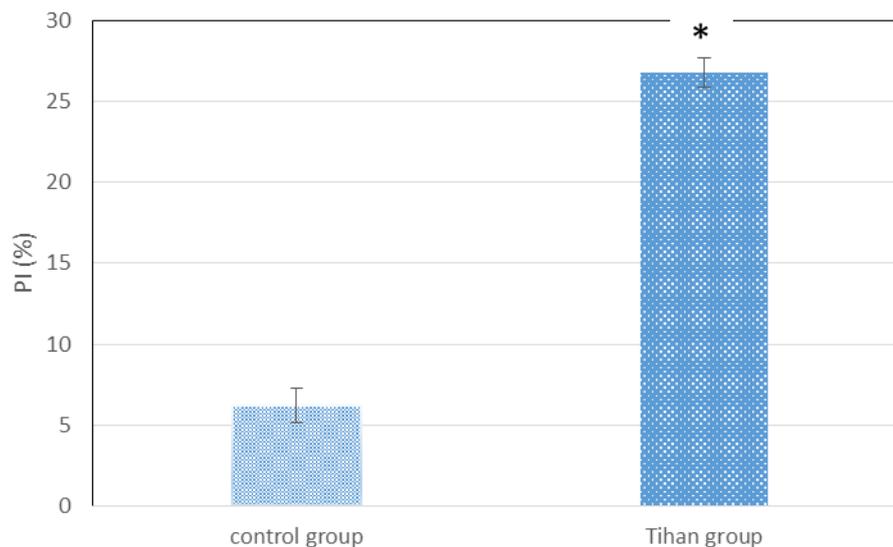


Figure (4): Phagocytotic index of peritoneum Macrophages in albino male mice treated with aqueous extract of Tihan (*Larinus maculates*) and control group (normal saline). Data express as mean \pm standard error. * $P \leq 0.01$

Discussion

The results of this study showed a significant increase in the phagocytosis index (PI) after treatment with the aqueous extract of COCOON *Larinus maculates* (Tihan) (150 mg / kg) for 14 days. The (PI) was (26.81%) compared with control group (6.19%). This increase can be attributed to the effect of the active component of the extract in stimulating the phagocytosis by stimulating the release of some attractive cellular mediators specifically TNF and IL-1 which stimulates macrophage cells, including neutrophils to leave the bloodstream to the peritoneal cavity [9]. The extract contains Polysaccharide acids (Data not show) which several studies have indicated the role of polysaccharides in increasing the phagocytotic index, whether these polysaccharides derived from plant extracts [10] or edible fungus [11]. These results agree with present study, that reflect the therapeutic application of aqueous extract of Tihan especially against infectious diseases. The results of this study showed that there was a significant increase in splenic cell division in the group of mice treated with aqueous extract of Tihan (58%) compared with 30% in control group. This indicates the role of polysaccharides in the extract of the cocoons in stimulating the mitotic activity of the spleen cells, as many studies have indicated the role of polysaccharides, including PHA in stimulating the divisional activity of T- lymphocytes

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as its role is similar to the role of IL-2, which stimulates the T-lymphocytes division and multiplication [12]. The effects of polysaccharides are also shown to stimulate the production of IL-2, which also plays a role in the activation of NK cells [13] as well as its role in T cell division. This indicates the role of polysaccharides in the development of immune function and its potential for therapeutic applications such as bacterial infections and cancer diseases [14; 15].

The results of this study showed a non significant decrease in bone marrow mitotic index compared with control (48% and 54%) respectively. This may be due to the concentration of the extracted dose used in this study, which significantly affected splenic cell division comparing with the division of bone marrow cells, and that such effects can be a kind of privacy to a particular organ without the other organs because of the difference in the location of immune cells in different lymphatic tissues [16].

Thus, we concluded that the high PI of phagocytes and the MI of splenic cell after treatment with the aqueous extract of Tihan can provide an indication of its role in modulating the immune response and it can be used in therapeutic applications against many pathogens.

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