Histological Study of the Effect of Aqueous Extract of the Beetle Cocoon on Liver Tissue of Male Mice

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Abstract

The long history of traditional medicine in different cultures based on inheritance of experiences, principles, ability and observes, still used nowadays to prevent and/or treatment of diseases worldwide. Among which the extracts used in traditional medicine is aqueous beetle cocoon extract of Larinus maculates popularly known as Tihan in Iraq. To determine the histological effect of this extract on mice liver tissue, the animals were divided into four groups (n = 5) were treated with (150, 200, 400) mg/kg respectively of Cocoon aqueous extract of Larinus maculates for 14 days. Histology and immunohistochemistry test was performed to evaluate changes in liver tissue and TNF-α levels. The results showed dose depended changes, various pathological changes in the liver tissue observed including infiltrations, congestion and vaculation along with some dead cells, necrotic hepatocyte were observed in the liver of highly concentrated treatment group (400 mg kg⁻¹). Also, TNF-α level in the liver tissue increased by increasing the concentration of the extract. Immunohistochemistry result of positive reaction to TNF-α revealed high reaction in the liver tissue of mice treated with 200 mg kg⁻¹ and 400 mg kg⁻¹ compared with control group. The present study showed that changes in hepatocytes and the severity of pathological changes in the liver depends on the concentration of extract of Larinus maculates

Keywords: Larinus maculates, Histological Changes, Liver, Male Mice
1. Introduction

In many countries, traditional medicine, which has been used as alternative medicine for a long time to maintain health and treatment of diseases because of its effectiveness, economic and less side effects. Also, in failure of chemical drugs in the treatment of some diseases such as cancer (Cohen and Ernst 2010). People used plants, animals, insects and some marine organisms to treat diseases; Cocoon aqueous extract of Larinus maculates, known as Tahan, which is produced from the salivary glands (beetle larva of Larinus maculates), has been used in the treatment of many diseases such as respiratory infections, asthma and viral diseases (Evans et al. 2007; Hamedi et al., 2015). The main compounds of the cocoon extract have been characterized in previous chemical studies (Al-Shmgani et al. 2020, Karami 2012) and it has been found to increase the mitotic division of the spleen and bone marrow cells and increases the growth of phagocytic cells (Alasady et al., 2017). Effect of aqueous cocoon extract of L. Maculates on some physiological parameters has been reported and found to have some adverse effects on lipid profile in mice treated with high doses (Tawfeeq 2018). Im et al., (2018) showed ethanol extract of three common insect used as a food in Korea can prevent free fatty acid-induced lipid accumulation in an in vitro cellular nonalcoholic fatty liver disease (NAFLD) model. In addition, the fat fraction of the beetle Ulomoidesdermestoides Chevrolat, used in traditional medicine in many countries, enhanced the treatment of diabetes in mice. (Jasso-Villagomez et al., 2018).

As the liver is the critical organ in the body for metabolism of nutrient and drug, therefore it is the most affected organ by drugs and toxins (Anbarasu et al., 2012; Yewon Kang et al., 2020); and it is important to understand the underlying mechanisms of liver damage by any edible natural extract. The aim of this study was to demonstrate the effect of different concentrations of Larinus maculates on the producing of TNF-α and the histological structure of liver in albino mice.

2. Materials and Methods

2.1. Beetle cocoon extract preparation

The whole shell was collected from local markets (Baghdad / Iraq), the pupae were collected after removed the insect inside. The extract was prepared based on the method of DeFoliart (2002), 10g of powder was dissolved in 100 ml of distilled water and heated for two hours using magnetic stirrer. The solution was then centrifuged at 4000 rpm for 30 minutes, then
the supernatant was dried at 37°C. Concentrations at of 400, 200, 150 mg kg⁻¹ of beetle cocoon extract were prepared and store at 4°C.

2.2. Experimental Design

20 male Balb/c mice aged 6-8 weeks were obtained from the Animal House at the College of Education for Pure Sciences - Ibn Al-Haytham / Department Biology/ Baghdad University. The mice were housed at 22.5±5 °C and had freely accessed to water and food adlibitum. The mice were divided into four groups and treated for 14 days; the three groups were orally administration aquatic extract of 150, 200 and 400 mg kg⁻¹ concentrations respectively while the fourth group were fed 0.2 ml of 0.9% normal saline as a control group.

2.3. Histological and Immunohistochemistry Test

At the end of experiment, after necropsy of mice, the liver specimens removed surgically and fixed by formalin 10%. Liver histology section was prepared by Bancroft and Stevens method(1982). Sections of 5 µm were stain either with hematoxin-eosin to study histological changes, Also for measuring the amount of TNF-α in liver tissue by IHC technique according to the kit manufactured by SANTA CRUZ BIOTECHNOLOGY, INC.USA Taylor and Rudbeck (2013).

3. Results and Discussion

Comparative histological examination of liver tissue structure in the treatment groups (150, 200, 400 mg / kg) and the control group showed that the hepatic and radial cords did not change and no necrotic tissue was seen in the liver.(Figure 1). Examination of liver tissue in the control group showed intact hepatocytes with well-defined lobule central vein and sinusoid, on the other hand vaculation in cytoplasm of hepatocytes observed in the three treated groups (150,200,400) mg kg⁻¹ and the volume of vaculation was more at 400mg kg⁻¹ concentration (Figure 1D). This is probably due to the process of osteotosis caused by fat accumulation and active oxidation (β-oxidation) along with the production of free radicals and low levels of ATP due to mitochondrial degradation (Amacher and Chalasani, 2014; Hussain, 2015). Also results of present research showed moderate aggregation in central vein and moderate to sever infiltration (Figure 1B) of the white blood cells within the liver tissue at a concentration of 150 mg kg⁻¹ compared with control group, while there was sever aggregation and infiltration in the liver tissue of the mice treated with the (200,400) mg kg⁻¹
(Figure 1C,D) compared with control group. The appearance of severe aggregation and filtration can due to the necrotic hepatocytes resulting from treatment and accelerate the inflammatory response against pathogenic effect of tissue. Due to the dilation of blood vessels and the change in their permeability and the increase of adhesion molecules to transfer defense hepatocytes from the bloodstream to adjacent tissues to remove dead hepatocytes (Kumar et al., 2007) necrotic cells were observed in the liver of highly concentrated treatment group. (400 mg kg\(^{-1}\)). Several mechanism can explain hepatocytes damage, one of that increasing the concentration of calcium ions in the cell which stimulates a group of enzymes, including phospholipase, which degeneration lipids in the membranes, proteases, and endonucleases responsible for cleave DNA, and the analysis of the phospholipid in the plasma membrane of hepatocyte causing the necrosis of hepatocytes by increasing the permeability of the plasma membrane and releasing the contents of the cell (Kumar et al., 2013). The lipid oxidation enzymes also affect the mitochondrial membranes, changing their permeability and release various enzymes, including Cytochrome C, which stimulates the mechanisms of cellular death (Kumar et al., 2013; Semisch, et al., 2014). This result was consistent with the increase in sever congestion and infiltration observed in the liver sections of mice treated with a concentration of 400 mg kg\(^{-1}\).

![Image of liver sections](https://example.com/image.png)

Figure 1. Sections in the liver of Albino mice, a-control group, B. Mice treated with 150 mg/kg of aqueous extract of cocoon, C. Mice treated with 200 mg/kg of aqueous extract of cocoon, D. Mice treated with 400 mg/kg of aqueous extract of cocoon, (H&E, 40X), C=central vein, HC=Hepatic-cord, Cg=congestion, Vac= vaculation, Inf = Infiltration.

Immunohistochemistry result of positive reaction to TNF-\(\alpha\) revealed high reaction in the liver tissue of mice treated with 200 mg kg\(^{-1}\) and 400 mg kg\(^{-1}\) compared with control group.
(Figure 2A,B,C), the positive effect was more observed in the 400 mg kg$^{-1}$ comparing with 200 mg kg$^{-1}$. The occurrence of inflammation stimulated the release of TNF-$\alpha$ which worked to attract and infiltrated T-lymphocyte cells and neutrophil, express on the surface of endothelial cells to aggregation inflammatory cells in liver tissue and subsequent release of free radicals that contribute to the development of osteosis in the liver (Kumar et al., 2013). This confirms the cause of increased necrotic cells at 400 mg kg$^{-1}$ (Sawa, 2014).

The appearance of these histopathological changes is likely to result from the high dose (dose dependent) resulting increase concentration of active substances which stimulated endonucleases enzyme to cleave DNA and hepatocytes death.

The present study showed that changes in hepatocytes and the severity of pathological changes in the liver depends on the concentration of extract of *Larinus maculates*

**References**

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Evaluation of Drug-Induced Liver Injury Developed During Hospitalization Using Electronic Health Record (EHR)-Based Algorithm


