Investigation of Effect of Black Seed (Nigella) and Honey Astragalus Extract on Changes of Liver Tissue in Male Mice following Injection of Thioacetamide

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ABSTRACT

It's found that the use of herbs has numerous advantages for the treatment of liver problems. The aim of this study was to evaluate the effects of ethanolic extract of black seed and honey astragalus on changes of liver tissue in mice following injection of thioacetamide. The study consists of seven treatment groups, a control group and an injection control group. Control and injection control were kept under natural conditions typical food and water for 40 days before injection of thioacetamide. Six treatment groups were treated by the concentrations of 125, 250 mg / Kg 500 Nigella seeds and honey for 40 days and 1 treatment group were treated by the concentration of 125 mg/Kg of combined Nigella seeds and honey at the same time as. After this time, liver tissue samples were taken from half of the rats in each group. After 41th day, the treated and injection control groups were intraperitoneally treated by thioacetamide (50mg/kg) for three consecutive days. After this time, similar factors were investigated in their liver tissues. The results showed that the changes in liver tissue was more after intravenous injection of thioacetamide than before injection, but the severity of injury in the treated group surprisingly decreased in comparison with injection control group injected. This decrease was statistically more significant in groups that treat by combination of Nigella seeds and honey. Due to the fact that the combined treatment with 125 mg/Kg Nigella seeds and honey effect on liver tissue changes, a combination of Nigella seeds and honey can be used for the prevention and treatment of Liver diseases after the injection of thioacetamide. Design of therapeutic agents in combination with the use of these two materials with properties effective properties against liver disease could be a new innovation in drug design.

Keywords: Black seed, Drug design, Honey astragals, Liver disease, Liver tissue, Thioacetamide

INTRODUCTION

Liver is one of the important organs in metabolism of carbohydrates, proteins and fats. Liver also plays an important role in secretion, excretion of toxins and it is a source for parasite, viral and microbial infection [1-3]. Since the liver is a complex organ, it is susceptible to a wide range of serious effects caused by high levels of alcohol or drugs, viral infections such as hepatocytes, cancer and other metabolic diseases. Some agents such as thioacetamide lead to necrosis and cirrhosis. Thioacetamid is a liver toxin and damages liver cells and tissues through free radical [4,5]. So, investigation of new therapies for the prevention and treatment of liver diseases is very important in scientific researches. In between, due to side effects of usual medicine, there is an increasing interest to herbal medicine in order to treatment of liver diseases [6]. Different parts of plants have many different biological activities such as anti-cancer, antioxidant, anti-inflammation and immunomodulation [7-10]. Recently, using of traditional compound with known properties is a goal

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to design of effective treatment of diseases like Honey Astragals and Black seed [11-13]. Honey consists 81 % carbohydrate and 21 % water which is considered as a plant with anti-microbial and anti-inflammation properties [14-16]. Black seed could be used for wound healing, anti-poising and anti-depressants. It could regulate the activity of liver that decreased gluconeogenesis [17,18]. Due to this, the use of these plants could be effective in detoxification and natural food like Black seed and Honey Astragals were used in treatment of liver diseases [19-21]. Considering to anti-oxidant property of Black seed in combination with Honey Astragals, in this study, we assessed the effect of Black seed and Honey Astragals on changes of liver tissue in mice following injection of thioacetamide. Due to similar physiologic human and mice, it can be used these compounds in treatment of liver disease, if there is an effective relationship.

MATERIALS AND METHODS

Preparation of Black seed and Honey Astraggalus

The powder of Black seed was poured into the flask with ethanol 96% as a solvent (200 gr powder, 600 ml ethanol). This compound was placed on shaker for 24 hours then was passed through filter. The filtrate solution was preserved in a cool place and the rest of them was placed on shaker with ethanol for 24 hours and then was passed through filter. Using by rotator, two filtrate solutions was combine and ethanol out and brown solution was prepared. Brown solution was poured in to the decanter with the same volume of chloroform in order to consist of two phases. The lower phase is discarded and top phase that is felanoides was poured to the plate and oven 40°C. The obtained solution is Black seed. Honey Astraggalus of this study was purchased from Koohrang hive of Chaharmahal and Bakhtiari. In order to preparation and serial dilution of Honey Astraggalus and Black seed, the weight of mice was measured then based on weight, the material was prepared. The volume of gavage was 0.5 ml to each mice. 4.8 gr Black seed extraction was combined to 10 ml of distilled water as a storage solution. Following that, three concentrations were prepared 125, 250 and 500 mg/kg. This preparation was done for Honey Astraggalus.

Injection of material to different groups of mice

72 mice (Balb/c) were analyzed with the weight of 28- 30 gr that is prepared from University of Isfahan. That was divided to 9 groups by chance and there were 8 mice in each group. Two groups were considered as a control and injection control group that were treated by normal condition, water, regular food. Seven groups were treated with Honey Astraggalus and Black seed separately (125, 250 and 500 mg/kg) or in combination of each other (125 mg/kg) for 40 days. The sample was drawn before and after injection of Thioacetamide. Following treatment of eight mice in each group during 40 days, the half of mice was selected in order to liver change detection. On second phase of study, 0.5 ml of Thioacetamide was injected to the rest of mice of all groups except to the control group for three days and the sample was drawn 24 hours after the last injection.

Detection of liver changes

For investigation of liver changes, the liver tissue of mice was carefully isolated and placed in 10% formalin solution and then, liver samples were studies for histological sections. For this purposes, three stages of dehydration, clearing, and impregnation was performed by a tissue processer. After this, stained samples were then put into a container, paraffin and wax is poured on it and put into refrigerator for a few minutes. At this stage, the slides were prepared by microtome. The paraffin was removed by xylene and then, slides were washed by 100% alcohol and distilled water. The

samples were immediately placed in hematoxylin and were then washed with water. After cleaning the slides, it was floating in acid-alcohol. Then, for staining of tissue background, the samples were placed in calcium carbonate. Sample was inside eosin for a few minutes. The solution was washed with distilled water. In the next stage, samples were placed in the three container of alcohol 70%, 90% and 100% for 3-5 seconds.

RESULTS AND DISCUSSION

The result showed that there are no changes in liver tissue before injection of Thioacetamide in control groups. Liver lobules, hepatocytes, and central vein are normal in this stage. This group has not received any thioacetamide. So, this group has normal tissue after normal saline injection (Figure 1A). In control injection group, the severe destruction of hepatocytes, very severe hyperemia in tissue, and disorderliness in the structure of liver lobule was observed after injection of Thioacetamide (Figure 1B). In Figure 2, the result of the effect of different concentration of Black seed and Honey on liver tissue was summarized. As shown in this figure, at 125mg/kg of Black seed, before the injection thioacetamide, liver lobules have lost part of their order. Central venous volume has increased, but hyperemia in tissue was not observed. After injection of thioacetamide, severe damage of liver tissue was happened in 125 mg/kg concentration of Black seed. Complete destruction of liver lobules, clear acidophilus of hepatocytes, nuclear accumulation of basophils, and central venous congestion was observed in this concentration (Figure 2A). The increase of concentration of Black seed (250 and 500 mg/kg) lead to increase of its inhibitory activity on severe side effects of injection of thioacetamide (Figure 2B,C). The similar status was observed in different concentration of Honey (Figure 3A-C). The result of combination group was shown in figure. In this group, before injection thioacetamide, the treatment with combination of Black seed and Honey lead to tissue destruction, but this change was less than single mode. This result likely shows a protective effect of combination of Black seed and Honey. The changes in liver tissue after injection of thioacetamide in presence of Black seed and Honey were also more less than single mode.

All changes including destruction of hepatocytes and accumulation of basophil cells around blood vessels and ports triad, acidophilus mass in some hepatocytes and congestion hepatic portal vein and artery was more less in comparison other groups (Figure 4). In this study, the changes of liver tissue was analyzes in treated condition of different concentration of Black seed, Honey Astraggalus separately, at one concentration of combination of Black seed and Honey Astraggalus in before and after Thioacetamide injection on mice. By analyzing the obtained results it can be concluded that nigella and honey have an important role in reducing the toxicity of thioacetamide due to their antioxidant properties and flavonoids compounds. Each of these two compounds can reduce the severity of this poison individually; however, this efficacy increases when they are used altogether by exhibiting better antioxidant properties through synergistic effects. The treatment with combination of Honey and black seed leads to changes in liver tissue. After comparing all the slides before intravenous injection of thioacetamide with controls and injection control groups, it was found that the combination of Honey and black seed can cause damage to the liver, including more acidophil, the destruction of hepatocytes, cells and basophils assembly (Figure 4). But the purpose of this study is assessment of their changes on liver tissue after injection of thioacetamide. According to obtained data, the combination of Honey and black seed reduce the severity of the liver injury induced by thioacetamide. It has been proven that one of the industrial toxins that has adverse effects on liver diseases like cirrhosis and cancer is thioacetamide [22]. After entering the body, Thioacetamide was changed to intermediate compounds and eventually excreted in the urine after 24 hours, but it will induce harmful effects on liver tissue [23]. The effect of different compounds with natural origin, such as honey and Nigella sativa on liver inflammation as well as

other parts of the body after exposure to thioacetamide has examined and acceptable results have obtained. For example, in the late nineteenth century, it has proved that honey quickly repair the damage increases. The effect of alone honey in various disorders have been considered by many researchers.

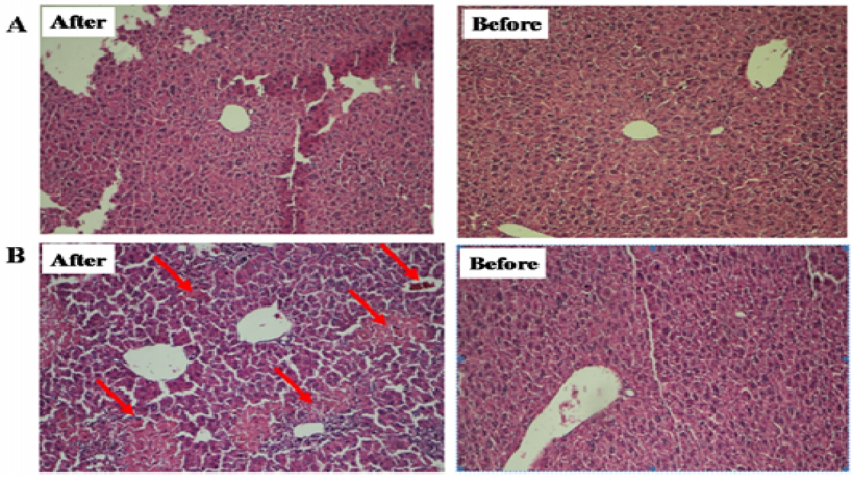

Figure 1. The liver tissue in control (A) and injection control groups (B).

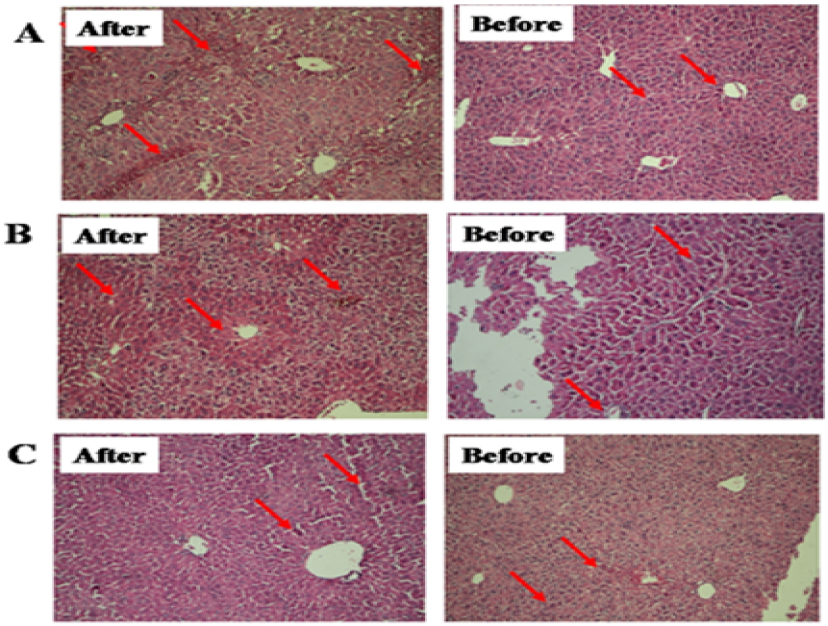

Figure 2. The effects of different concentration of black seeds on liver tissue (A: 125 mg/kg, B: 250 mg/kg and C: 500 mg/kg).

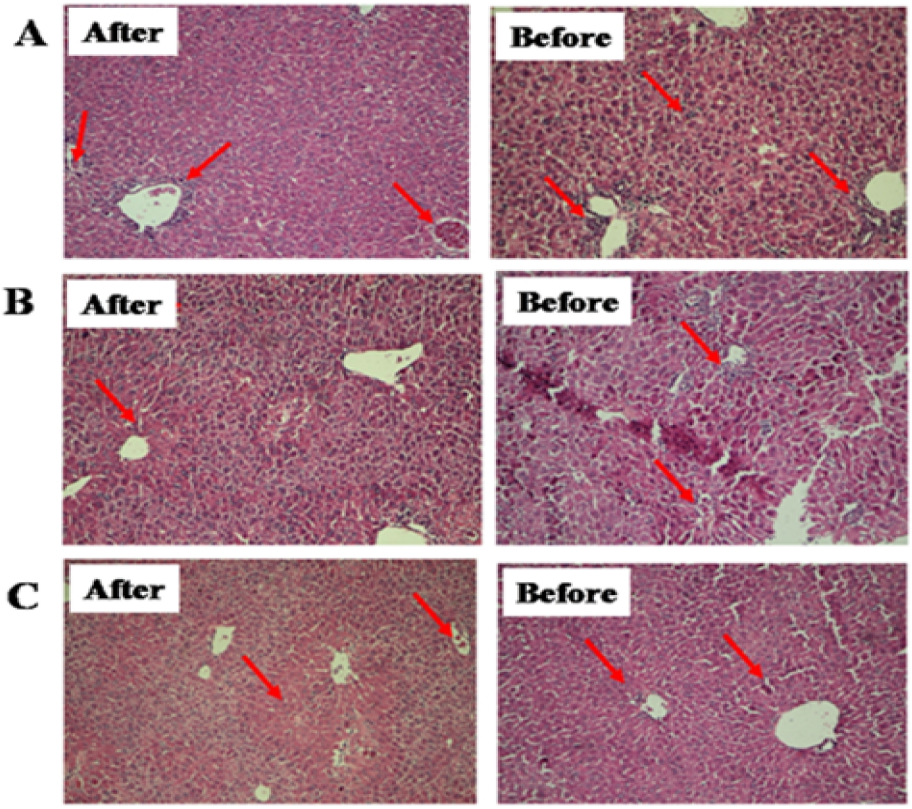

Figure 3. The effects of different concentration of honey on liver tissue (A:125 mg/kg, B: 250 mg/kg and C: 500 mg/kg).

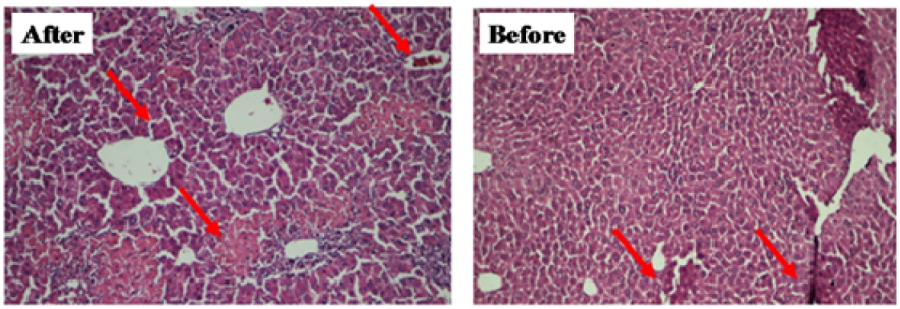

Figure 4. The effects of combination of black seeds and honey on liver tissue.

For example, the results of Shannon et al. in 2002 showed that honey lead to formation of granulated tissue and epithelial tissue in wound healing [24]. The beneficial effect of alone Nigella sativa has been demonstrated in various studies by measuring various factors. Alkobazy and Elli have proved the protective role of nigella oil against the harmful effects of carbon tetrachloride on liver cells. Carbon tetrachloride is a chemical compound that leads to liver necrosis by increasing the level of liver enzymes and bilirubin. The results of aforementioned study showed that treatment with nigella has an important role in reducing hepatic necrosis [25]. The findings of the this study in

combination treatment mode were in line with our study explored liver tissue changes in a way that in combination treatment mode the observed damage severity was less after thioacetamide injection in comparison with single mode showing honey and nigella moderating role in damaged caused by thioacetamide. The impact of other plant compounds, their use suggested in traditional medicine, on the liver tissue, and various factors are examined in various studies and good results are obtained that are in line with our study. In a study conducted by the Madani and colleagues the protective effect of polyphenolic extracts of chicory and *Silybum marianum* (as a positive control) against oxidative damage of hepatic cells, induced by thioacetamide, was explored on rats. The obtained results of this study indicated the protective effect of the extract on liver cells [24]. Investigating the hepatoprotective effect of jujube fruit extract on rats, Ebrahimi et al. concluded that Jujube poses protective property against carcinogens and toxic factors on the liver cells. This effect is related to flavonoids. Mirzaee and colleagues in a study in 1390 conclude that alcoholic extract of grape and fruit pair of oak have liver protective impact against the effects of free radicals due to their flavonoid compounds [26]. Bayat et al. concluded that pistachio gum extract posed protective effect on liver against carbon tetrachloride which may be related to flavonoids found in pistachios gum [27]. The *Silybum marianum* extract with dose of 50 mg/kg is able to prevent hepatic necrosis development caused by carbon tetrachloride injection [28]. In another study, it was shown that alcoholic extract of fumaria can reduce necrosis, fatty changes, and hepatocyte swelling. This plant may have collecting free radicals property or may be able to monitor their production due to Thymoquinone and alkaloids [28]. Honey known as biological antioxidants poses important properties such as anti-microbial and anti-inflammatory. Honey phenolics increase its antioxidant potency. In addition, other compounds such as catalase, fructose, glucose, minerals like magnesium, potassium, calcium, vitamin C, and various types of vitamin B confirm the antioxidant properties of honey [29]. According to obtained data from current study, the use of honey and nigella individually or in combination were suggested in traditional medicine to treat liver diseases. Histological changes revealed that the combination mode of nigella extract and honey had positive effect on reducing sever effects of thioacetamide injection on lever tissue.

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