



## The study of the effects hydro-alcoholic extract of *Eryngium billardieri* on lipid profiles levels and liver and renal functions tests in hypercholesterolemic rats

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### ABSTRACT

We aimed to study the effects of alcoholic extract of *Eryngium billardieri* on lipid profiles levels and liver function tests in hypercholesterolemic rats. In this study, 35 male wistar rats in 5 groups (n =7) were selected: Control group with normal diet, sham group with fat diet, and three experimental groups containing animals with fat diet that daily received a minimum dose of 100 mg/kg, an average dose of 200mg/kg and a maximum dose of 300mg/kg of hydro alcoholic extract of *Eryngium billardieri* over a period of 21 days, respectively by gavage feeding. Amounts of lipid and lipoprotein profiles, with low and high density, as well as indicators of liver function such as alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), albumin, and Serum total protein and renal function tests, such as creatinine and blood urea nitrogen (BUN), in blood samples were taken. Results Serum ALT and ALP in the experimental group receiving the minimum dose of the extract were significantly reduced compared to the sham group ( $P \leq 0.05$ ). The groups which received the extract showed significant decrease in cholesterol, triglycerides and LDL. While in all groups receiving the extract no significant changes in BUN and creatinine were seen compared to the sham group. Conclusions: These results suggest that the extract of *Eryngium billardieri* which has alkaloid and antioxidant compounds can improve liver function in hypercholesterolaemic rats without leaving side effects on their renal functions.

**Keywords:** *Eryngium Billardieri*, Liver, Kidney, Cholesterol, Rats, *Eryngium* (Zul)

### INTRODUCTION

Fats may accumulate in liver for many reasons and especially in the form of triacylglycerol, causing fatty liver [1]. Fatty liver is one of the major causes of chronic liver disease in children and adults. Fatty infiltration of the liver is associated with its increased echogenicity and depends on infiltration rate of fat in liver [2, 3]. On the other hand, the kidneys play an important role in the removal of metabolic waste products from the blood and excrete chemicals in urine, too. In addition, kidneys have an undeniable role in regulating blood pressure and body homeostasis; hence, it is mandatory to ensure their health and proper function in drug testing [4].

*Eryngium* (in Persian زو ل) with scientific name of *Eryngium billardieri* belongs to the order of Apiales in the genus of Apiaceae (Umbelliferae) (Figure 1). There are about 250 species. The genus has a cosmopolitan distribution, and 9 species of this prickly herbaceous plant have scattered throughout Iran [5-7]. *Eryngium billardieri* has diuretic and laxative properties and the seed and the root tinctures are used as to treat kidney stones, infections, skin diseases and tumors. It is also used as a herbal snakebite antidote, and a cure for liver disease, infertility and toxicity [8-9].

*Eryngium billardieri* plant has anti-inflammatory activity, anti-microbial and antioxidant properties as well as the ability to inhibit lipid peroxidation in rat liver [8]. In most species, the roots and leaves are used. Its anti-diabetic properties have been shown in some studies [10]. Genus *Eryngium* contains tannin, sucrose, saponins, alkaloids [9] acetylene, flavonoids, coumarone and tri-terpenes, sesquiterpene, monoterpene [10, 11] Kampferol, Chlorogenic acid, caffeic acid, lutein and  $\beta$ -carotene. Many of these compounds are anti-inflammatory and antioxidant [12]. Despite the active compounds in this plant, which are probably effective in the function of liver, kidneys and in controlling blood fat, and its long term use in traditional medicine to treat liver and kidney diseases, few laboratory studies are available in this field. The purpose of the present study was to investigate the effects of alcoholic extract of *Eryngium billardieri* on renal and liver function tests in hypercholesterolemic rats.



**Fig1.** The aerial parts of *Eryngium billardieri*

#### EXPERIMENTAL SECTION

The present experimental study was carried out on 35 male Wistar rats. The entire process was in compliance with the codes of ethics and guidelines for working with laboratory animals approved by the Islamic Republic of Iran Ministry of Health and Medical Education (MOHME). The animals were raised in a lab in the University of Medical Sciences and were studied in PNU Abadeh branch (Fars Province, Iran). Animals were first settled at 22 to 26° C and 12 hours light and 12 hours darkness, then were randomly classified into 5 groups (n=7), as follows: control group which received no solvents or drug treatment during the experimental period and had a normal diet; sham group, with rats which were daily given cholesterol 0.2 ml solvent (normal saline) as gavage during the experimental period; experimental group 1 were hypercholesterolemic rats which daily received 100 mg/kg (minimum dose) of alcoholic *Eryngium billardieri* extract by gavage feeding; experimental group 2 were hypercholesterolemia rats which daily received 200 mg/kg (medium dose) of *Eryngium billardieri* extract in oral gavage; experimental group 3 consisted of hypercholesterolemic rats which were daily given 300 mg/kg (maximum dose) of *Eryngium billardieri* extract. During the experimental period (21 days) the extract and the solvent were administered at 9 AM by gavage feeding. In this period, the control group and the experimental groups were all treated with high cholesterol diet. At the end of the period the rats were mildly anesthetized with ether and blood samples were taken from their hearts to measure the level of biochemical factors in plasma. After centrifugation of the blood at the rate of 3000 rpm, the plasma was separated and transferred to the laboratory for measurement of factors.

**Method of Preparing 2% High-Cholesterol Food:**

Twenty grams of pure Merck cholesterol powder (Fluke Chemika) was dissolved with 5 ml of warm olive oil and mixed well with a kilogram of rat food. To prevent deterioration, we tried to keep the food for only two days in the refrigerator at the temperature of 4 °C [13-15].

**Extraction method:**

At first, the plant was examined and confirmed by a botanist with its herbarium specimen (code: 091-002-006) in the department of Botany and Plant of PNU Abadeh and a sample of it was archived there. To prepare the alcoholic extract of *Eryngium billardieri*, after providing the aerial parts and removing impurities, 600 g of the plant was ground and mixed with ethyl alcohol 90 % at the ratio of 1 to 5. After 24 hours it was placed on the stirring device. Then the extraction was leached by the filter paper and funnel. Ethyl alcohol 70% was added to the obtained slag and was placed on the stirring device for another 24 hours and then was added to the first extract. Then, the whole extract was distilled in the vacuum distillation unit at 60 °C and 70% rotations until the remaining volume was one-fifth of the initial one. The remainder was poured into Petri and dried in an Avon (Finetech, Korea) at 50 °C. The obtained extract (about 10 g per 100 g of crushed plant) was mixed with normal saline to obtain different concentrations of mg/Kg body weight [13].

**Method of Measurement:**

Alanine amino transferase (ALT) and aspartate aminotransferase (AST) were measured with phosphate buffer DGKC; alkaline phosphatase was measured using P-Nitrophenyl phosphate AMP; albumin by Bromocresol green, total protein with the method of Biuret reaction end point (by using a commercial kit test from Pars Azmoon, Tehran, Pars). D. Steele monoxime and enzymatical cholesterol oxidase were used to measure Blood urea nitrogen (BUN) and cholesterol respectively [16]. Serum cholesterol and Triglycerides were determined colorimetrically by means of a kit made by Darmankav Company (Iran). Lipoproteins were measured by using a combination of ultracentrifugation and precipitation methods and kits by Darmankav Company (Iran). HDL cholesterol HDL (High Density Lipoprotein) was measured by precipitation method. Firstly, precipitation reagent was added to the serum lipoprotein HDL in order to integrate all components. Then these compounds were precipitated by centrifugation for 10 minutes. After that HDL cholesterol was measured enzymatically. LDL cholesterol (Low-Density Lipoprotein) was calculated by Friedewald's formula. The means obtained (Mean  $\pm$  SEM) were statistically analyzed using one way ANOVAs and Tukey test. All statistical analyses were performed by using SPSS version 17 ( $P \leq 0.05$ ).

**RESULTS**

The findings as shown in Table 1 indicate that: for ALT: The sham group showed a significant increase compared to controls. In the first experimental group there was a significant decrease compared to the sham group. There was no significant difference between experimental groups ( $P= 0.00$ )

For AST: Its rate for the sham group did not show significant changes compared to the control group. There was no significant difference between the experimental group receiving the extract and the sham group ( $P= 0.00$ ).

For ALP: the rate in the sham group shows significant increase compared to the control group. There was also a significant decrease in the first experimental group compared to the sham group ( $P=0.004$ ).

For cholesterol: The rate in the sham group shows significant increase than in the control group. In experimental groups 1 and 2 there was a significant decrease in cholesterol compared to the sham group. ( $P= 0.01$ ).

For triglyceride: The rate in the sham group shows significant increase compared to the control group. All experimental groups receiving the extract showed significant decrease compared to the sham group ( $P= 0.00$ ).

For LDL: There is no significant difference between sham and control groups. The first experimental group showed a significant decrease compared to the sham group ( $P= 0.01$ ).

For HDL: The control group did not show significant changes compared to the sham group. Between the experimental groups receiving the extract and the sham group no significant difference was found ( $P=0.11$ ).

For albumin: The control group did not show any significant changes compared to the sham group. Between the experimental groups receiving the extract and the sham group no significant difference was found ( $P=0.13$ ).

**Table1. Effect of different doses of the extract of *Eryngium billardieri* on indicators of liver and renal functions in hypercholesterolemic rats**

Parameters	Control	Sham	Experimental Group1 (100 mg/kg)	Experimental Group 2 (200 mg/kg)	Experimental Group3 (300 mg/kg)
Cholesterol (mg/dl)	69.87±2.93	86.57±1.93 *	69.2±5.46 $\pi$	68.33±3.9 $\pi$	72.50±6.31
Triglyceride (mg/dl)	109±4.88	157±13.3 *	55±7.83 $\pi$	62.00±4.9 $\pi$	65.33±3.3 $\pi$
LDL (mg/dl)	27±1.87	32±2.12	20.50±2.02 $\pi$	23.16±2.31	26.83±2.46
HDL (mg/dl)	19.83±1.13	21.50±1	16.83±1.11	18.33±1.52	18±1.43
ALT (IU/L)	33.16±2.41	54.33±4.93 *	37.16±3.08 $\pi$	65±3.04	62±5.89
AST (IU/L)	176±13.99	187±6.62	182±14.07	239±20.5	236±12.64
ALP (IU/L)	614±33.87	941±71.08 *	588±42.98 $\pi$	770±74.71	839±95.05
Albumin (mg/dl)	45.6±0.8	4.50±0.1	4.26±0.06	4.14±0.12	4.56±0.06
Total-Protein (mg/dl)	7.46±0.1	7.05±0.1	7.05±0.16	7.25±0.17	7.40±0.20
BUN (mg/dl)	19.5±1.54	19.50±1.8	23.50±1.52	21.66±1.60	23.83±0.90
Creatinine (mg/dl)	0.5±0.01	0.51±0.01	0.53±0.03	0.54±0.04	0.54±0.02

Mark\* is used to show significant level as compared to the control group

Mark  $\pi$  is used to show significant level as compared to the sham group.

ALT: Alanin Amino Transferase

AST: Aspartat Amino Transferase

ALP: Allalin Phosphatase

BUN: Blood Nitrogen Urea

LDL: Low-Density Lipoprotein

HDL: High Density Lipoprotein

For total protein: The control group did not show significant changes compared to the sham group. Between the experimental groups receiving the extract and the sham group no significant difference was found ( $P=0.20$ ).

For BUN: The control group did not show significant changes compared to the sham group. Between the experimental groups receiving the extract and the sham group no significant difference was found ( $P=0.10$ ).

For creatinine: The control group did not show significant changes compared to the sham group. Between the experimental groups receiving the extract and the sham group no significant difference was found ( $P= 0.85$ ).

## DISCUSSION

The results of this study showed that the amounts of lipid profiles and liver enzymes increased in the sham group, while the levels of these factors decreased in the groups receiving the extract of *Eryngium billardieri*. None of the renal function tests in groups that received the extract showed significant differences (Table 1). About the increase in lipid profiles and liver enzymes in the control group we can say that dietary fat may lead to the accumulation of lipids, mainly triacylglycerols in the liver [1]. Excessive accumulation of these substances is considered as a pathological state. When lipid accumulation in the liver becomes chronic, fibrous changes that occur in cells may progress to cirrhosis and liver functions may be disrupted. Sometimes fatty liver disease is associated with increased free fatty acid levels in plasma which is the result of fat transfer from adipose or the hydrolysis of the triacylglycerol in lipoproteins in extra hepatic tissues.

The production of VLDL cannot compensate for the entry and esterification of free fatty acids into the liver and thus triacylglycerol accumulates and creates fatty liver. This happens when feeding occurs with a high-fat diet or in case of lack of food. Finally, following this particular disorder, the levels of liver enzymes, especially ALT increase [1, 2]. Mayer and Coles also believe that the increased serum alkaline phosphatase and ALT in liver are caused by fat metamorphosis and liver lipidosis [17-19], which clearly explains what, was observed in the sham group treated with cholesterol.

As mentioned before, the extract reduces lipid profiles levels. It is something that can be anticipated, because the fat in food (high fat diet) stimulates the secretion of bile from the liver. So, some of the cholesterol is used to synthesize bile acids. On the other hand, other compounds contained in the extract such as tannins, reduce fat absorption and increase bowel movements [20,21]. Some compounds in the extract, like alkaloids may also inhibit cholesterol

synthesis [22]. This herb is also used to treat infertility since some of the cholesterol may also be used to make steroid hormones [9]. All these factors show that the extract is effective in blood fat control probably through inhibition of cholesterol synthesis, increased consumption of it and decreased fat absorption in the intestines.

Previous studies have shown that this herb has anti-diabetic properties [17]. On the other hand, many herbal antioxidant compounds have insulin-like effects and increase glucose uptake in peripheral tissues [23, 24]. One of the main actions of insulin on adipose tissue is to control hormone-sensitive lipase activity which is followed not only by the release of free fatty acids, but also by decrease in the release of glycerol. Adipose tissue is much more susceptible to insulin than other tissues and is one of the main places in the body for insulin action [1].

Hyperlipidemia may also stimulate the production of free radicals [25]. Active radicals such as hydroxyl, superoxide anions are able to remove hydrogen atoms from the side chain of saturated fatty acids in biological membranes and to cause lipid peroxidation damage. Enzymatic and non-enzymatic antioxidants mammalian cells have the ability to stop the free radicals from sticking to them. Vitamin E, beta-carotene and vitamin C are among the non-enzymatic antioxidants [26]. Because the herb contains carotene and other antioxidant compounds such as tannins, sucrose, saponins, alkaloids [9] acetylene, flavonoids, coumarin and triterpene, sesquiterpene, monoterpene [10,11] and also, due to the lower blood fat and liver enzymes after taking the extract, it appears that *Eryngium billardieri* improves liver function and is effective in blood lipid therapy. However, it is somewhat difficult to draw definitive conclusions because very few studies have been conducted on the effects of the herb.

Furthermore, an increase in body fat, increases leptin level. Stimulating the inflammation increases leptin secretion and humoral and cellular responses increase. In addition, adipocytes secrete a variety of protein signals which may consist of a number of cytokines, such as TNF- $\alpha$ , IL-6, and absorbing proteins. Leptin by stimulating the secretion of TNF- $\alpha$  and 6 IL- of mononuclear cells causes inflammation in cytokines (increased TNF- $\alpha$  is possibly followed by liver necrosis which results in an increase in liver enzymes) [26]. Therefore, the strong anti-inflammatory properties that are listed for the plant are possibly functioning through reducing liver enzymes.

Alkaline phosphatase is a transpeptidase which increases in bone and liver diseases. Research has shown that phenolic compounds in herbs can help prevent the liver from toxic effects of drug and reduce the secretion of glutamic-pyruvic transaminase, and alkaline phosphatase into the blood [27, 28].

Triacylglycerol synthesis by liver is an immediate stimulus for the production and secretion of VLDL. One source of fatty acids for the production of triacylglycerol is the consumption of fatty foods during which liver lipogenesis is inhibited. Imbalance in the manufacture and transport of triacylglycerol can cause fatty liver or damage to it. This damage is characterized by elevated levels of liver enzymes, particularly ALT, and this is indeed what was seen in the hypercholesterolemic sham group. But following the intake of the extract, hypercholesterolemic groups showed a decrease in blood lipids and liver enzymes which could be due to the inhibition of lipid peroxidation in rat liver. Studies on some other plants, which belong to the species of *Eryngium* like the genus *Creticum*, have also showed this property.

On the other hand, tannins, terpenoids and flavonoids in the extract have antioxidant and anti-inflammatory properties and help the liver to protect against free radicals and factors that cause hepatotoxicity [10].

The chlorogenic acid in the composition of this plant has anti-radical activity and causes loss of oxygen free radicals. The extract of the plant *E. foetidum* contains kaempferol, chlorogenic acid, caffeic acid, lutein, and  $\beta$ -carotene, all of which have anti-inflammatory and antioxidant activities.

Recently,  $\beta$ -Carotene has received considerable attention as a chain-breaking antioxidant and as being able to interact with free radicals and quench singlet oxygen, [27-29]. It has shown ability to act as peroxy radical scavenger and to remove oxygen free radicals.

The results of this study indicated that plasma creatinine levels and BUN, showed no significant change in the sham group compared to controls. Also, those values had no significant effects in groups receiving different doses of the extract.

The measurement of creatinine and urea are among the routine tests to determine kidney disorders. However, in determining glomerular filtration rate (GFR) Plasma BUN level is a less specific index than plasma creatinine. Nevertheless, a decrease in GFR is always associated with increase in both creatinine and BUN levels.

In this study, one can conclude that hypercholesterolaemia and concomitant intake of *Eryngium billardieri* extract in different doses have no side effects on renal function.

According to a study that Zahedi and colleagues conducted on valerian and borage, different doses of these medications caused no increase in BUN and creatinine (compared to sham group). Therefore, the extracts of these herbs have no toxic effects on rat kidneys [29]. This is consistent with the results of present study; so, it can be concluded that this extract has no toxic effects on the kidney of rats, either.

### CONCLUSION

Since the levels of liver enzymes and cholesterol and other lipid parameters in the groups receiving the extract reduced, it can be concluded that as the extract of *Eryngium billardieri* has antioxidant and anti-radical compounds, it can improve liver function in hypercholesterolemic rats without adverse side effects on renal function. In addition, compounds like tannins and alkaloids that are present in the extract, inhibit fat synthesis, reduce intestinal fat absorption and increase excretion. Thus, this plant can also be important in the treatment of lipid disorders, and its extract can be a suitable drug candidate for the treatment of hyperlipidemia and its symptoms.

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