

## Investigating on Protective Effects of Barley and Oat Seeds Extracts on Pathological Changes of Blood Factors in Female Rats Fed with High-Fat Diet

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### ABSTRACT

Cardiovascular diseases are now considered as the first cause of death in industrialized countries. Over the past few decades in most countries, the use of alternative treatments and especially herbal therapy and dietary supplements are increased by people to improve a variety of diseases such as high blood cholesterol. So the main goal of the study is investigating on protective effects of barley and oat seeds extracts on some blood parameters (HDL, LDL, and glucose) in female rats fed with a high-fat diet. In the study, a number of 50 female Wistar rats weighing approximately  $20 \pm 300$  g, were bought from Jahrom's animals house. Then they were divided into 5 groups with 10 rats per each, including control group (no material received), blank group (received 1% cholesterol by weight of consumed food), experimental 1 (daily received 1% cholesterol by weight of consumed food, and 125 mg/kg.BW dose of barley extract), experimental 2 (daily received 1% cholesterol by weight of consumed food, as well as 125 mg/kg.BW dose of oat extract), experimental 3 (daily received 1% cholesterol by weight of consumed food, as well as 62.5 mg/kg.BW dose of oat extract). At the end of the experiment animals were bled and HDL, LDL and glucose factors were evaluated. Pathological examinations of blood factors showed that LDL level in the experimental group that simultaneously received barley and oat had significant reduction than the group that received cholesterol alone. Also LDL level had significant increase in the experimental group receiving oats only compared to the group receiving barley and oat simultaneously ( $P < 0.05/0$ ). HDL concentration showed also a significant decrease in all the experimental groups and the amount of glucose showed significant reduction in the experimental groups receiving barley + oat, and barley and oat only in compared to the control group ( $P < 0.05/0$ ). Given to the results can state that barley and oat extracts reduce the risk of cardiovascular diseases due to having vitamins and effective ingredients. The simultaneous use of barley and oat extracts is recommended

**Key words:** Barley, Oat, Pathological Changes, Blood Factors, Rat.

### INTRODUCTION

Now cardiovascular diseases are considered as the first cause of death in

industrialized countries. Reports indicate that the disease caused death of 950 thousand people in the United States in 1998 and the country has sustained about 118 billion dollars for the disease

in 2000<sup>1</sup>. Increase of blood lipids especially cholesterol proposes as a significant factor in intensification of these diseases<sup>2</sup>. Over the past few decades, the amount of using alternative therapies especially herbal therapy and dietary supplements have been increased by people in most countries to improve variety of diseases such as high blood lipids. One of the major problems facing doctors and also consumers of medicinal plants is lack of sufficient information about drugs' safety and their impacts on diseases. Fortunately, during the past 30 years extensive researches have been done on the effectiveness of medicinal plants used in traditional medicine that proves the efficiency or inefficiency of them<sup>3</sup>.

Most of barley species especially agricultural barleys which are planted in different parts of the world are from *Sativum* species<sup>4</sup>. According to Vavilov theory the origin and location of primary distribution of barley have been in Syria (*H. Spontaneum*) and Tibet in central Asia (*H. Agriocrithon*) includes awn barley with short awn and naked seeds<sup>4</sup>. *Hordium* genus includes two species. This group has large seeds barleys that are entirely one-year old and have 2-14n chromosomes and include: (*H. stivium*) *H. vulgare*, their main axis is fragile and includes two- and six-row types and they are cultivable<sup>4</sup>. *H. agriocrithon* is a kind of barley and obtained from central Asia regions, it has fragile main axis, spikes with awn, naked seeds and include two and six-rows and is cultivable<sup>4</sup>.

Barley seeds with low glycemic index<sup>5, 6</sup> and high fiber content<sup>6</sup>, water soluble fibers<sup>7</sup> such as  $\beta$ -glucan and high content of chromium and magnesium<sup>5</sup> are suitable grains for preventing and treating diabetes and blood fat changes<sup>8, 9, 10</sup>. Researches that were performed on barley seeds expressed that the plant has anti-diabetic effects. It was also stated that the use of barley malt extract and consumption of a diet containing barley cause reduction of glucose in diabetic animals and also consumption of barley seeds reduces glucose in diabetic people<sup>9, 11</sup>. Studies on healthy humans showed that the use of dietary containing barley seeds as well as consumption of  $\beta$ -glucan supplements produced from barley results in reduction of glucose<sup>12</sup>.

Among cereals, oat is economically located in the fourth row after wheat, rice and corn and because during the growing period its water requirement is about 500mm, its agriculture is not encouraged in Iran and are cultivated in trace amounts in the North and the West of the country<sup>4</sup>.

Oats contain averagely about 7% fats, 5.13 % nitrogen and 60% hydrocarbons. Fatty acids consist of palmitic acid 10%, oleic acid 50 to 60 % and linoleic acid 15 to 30 %<sup>13-14</sup>.

Cultivated oats varieties had a modest amount of  $\beta$ -glucan and oil in the past. While we have now modified varieties of oats which contains 18% oil and this amount is twice the amount of oil in old plant varieties, another group of modified varieties of oat has too numerous  $\beta$ -glucan which is called  $\beta$ -glucan (15). Studies have found that in diabetic patients who consuming diet high in oat and other carbohydrates fibers, the amount of consuming insulin will be half in their body. And some who had consumed a lower dose of insulin, completely cut off the drug. As a result, fibers reduce the rate of carbohydrates absorption and prevent a sudden increase of blood glucose (16-18).

Given the importance and influences of barley and oats as well as the importance of prevention and control of cardiovascular diseases, and also since till now no research has been done about barley and oat effects on pathological changes of blood factors including HDL, LDL and glucose, so the main purpose of the present study is to investigate the effect of barley and oat extract on the mentioned parameters and on reduce the risk of cardiovascular disease on hypercholesterolemic rats.

## METHODS

The study is conducted in vitro and completely random; and all ethical principles for use of laboratory animals have been observed. A total number of 30 adult female Wistar rats weighing  $200 \pm 300$  and 100-120 days of age were obtained from Jahrom's research center. The rats were kept for 21 days under laboratory conditions including a temperature of  $2 \pm 21^{\circ}\text{C}$  and a cycle of 12 hours of

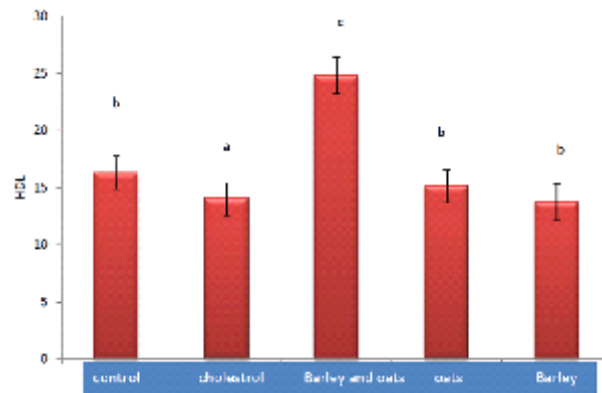
light and 12 hours of darkness in Jahrom's animals' house of Islamic Azad University. The rats were nourished by Pellete and water was provided for them by especial glass bottles. Their cages were disinfected with 70% alcohol 3 times in a week.

To prepare the extract, barley was purchased from market and then was milled well and powdered completely. For preparing aqueous extracts, water distillation system was used. The amount of 100 g of barley and oat seeds was dissolved separately in 1000 ml of distilled water. Then heated in 30-40°C for two hours and the extract was separated by filtration. The remaining residue on the filter was completely dried in an oven and the amount of

dissolved powder was determined. In continues, additional water was concentrated in a rotary device and was kept in dark containers for the next test (19). Preparation method of pressed seeds contain cholesterol is so that Pellete was powdered by a mill. Then 10 kg of powder was weighed and mixed with 100 g of cholesterol. The resulting powder by using of water conversed to a dough-like compound and was brought in the form of plate and then dried in the sun. Each group of animals included 10 female rats. That includes:

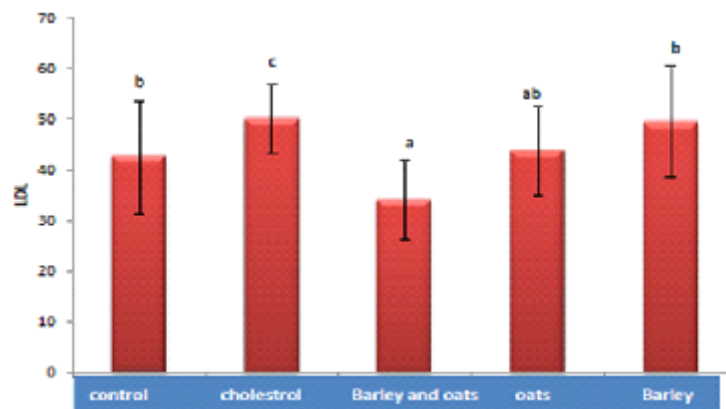
**Control group:** This group had normal diet and received no material

**Blank group:** This group had a high-fat diet



The amount of LDL in the experimental group that simultaneously received barley and oat shows significant reduction compared to the group that only received cholesterol and the control group (Chart 2). Also the experimental group that only received oat shows a significant increase than the experimental group which simultaneously received barley and oat ( $P < 0.05$ ).

**Chart 1: Changes related to HDL concentration in different groups**



Pathological glucose level in the experimental groups received barley + oat, barley and oat only shows significant reduction than the control group ( $P < 0.05$ ) (Chart 3).

**Chart 2: Changes related to LDL concentration in different groups**

(received 1% cholesterol by weight of consumed food)

**Experimental group 1:** Daily received 1% cholesterol by weight of consumed food, and 125 mg/kg.BW dose of barley extract by intraperitoneally injection.

**Experimental group 2:** Daily received 1% cholesterol by weight of consumed food, as well as 125 mg/kg.BW dose of oat extract by intraperitoneally injection.

**Experimental group 3:** Daily received 1% cholesterol by weight of consumed food, as well as 62.5 mg/kg.BW dose of oat extract by intraperitoneally injection.

After completing a period of 21-day, and weighing all the rats then they became anesthetized by ether and 5ml of blood were taken from their hearts by syringe. After separation of blood serum, pathological concentration of HDL, LDL and glucose factors were measured using enzymatic colorimetric method (PAP-GPO) to determine a single point by photometric method, in a laboratory of University of Medical Science of Jahrom. ANOVA analysis followed by t-test and Duncan test were used for multiple comparisons between different groups.  $P < 0.05$  was considered as a significant level. The SPSS software version 18 was used for data analysis and performance of statistical tests.

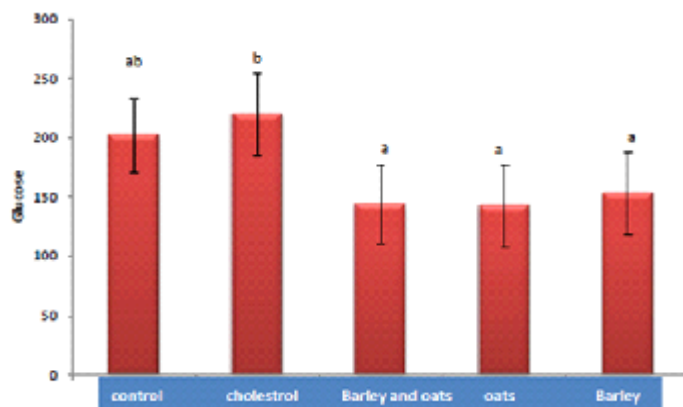
The pathological results of blood factors in the Chart (1) shows that concentration of HDL in the experimental group that received barley and oat has significant increase than the control group

and the other experimental groups show significant reduction than the control group ( $P < 0.05$ ).

## DISCUSSION

Deposition of plaques containing cholesterol and fatty materials with fibrosis tissue and calcium salt deposits in intima and/or inner walls of large and medium arteries in various limbs can cause atherosclerosis<sup>20</sup>. Atherosclerosis is a complex process that cannot determine a major risk factor for it because different factors and parameters are involved with each other<sup>21</sup>. Some important risk factors are including dyslipidemia, glycosylation events, and all events that increase oxidative shock<sup>22-24</sup>. It is suggested in the past researches that high level of total cholesterol and LDL has been identified as an important factor for coronary heart disease<sup>25</sup>. There is evidence that increase of low-density lipoproteins such as LDL and triglycerides also can cause person to be talented to atherosclerosis<sup>26</sup>. A susceptible stage in creation of coronary artery atherosclerosis is oxidation of fatty acids and LDL cholesterol in artery walls. Free radicals produced by metabolism of smooth muscle cells of vascular wall are capable of LDL oxidation<sup>27</sup>.

LDL oxidation is a process caused by free radicals which during of it unsaturated fatty acids available in cell membrane become peroxide and production of aldehydes with high reactivity (malondialdehyde) and conjugated dienes increase<sup>28</sup>. In this study, the pathological



\*The presence of a joint letter does not represent a significant reduction compared to the control group.

**Chart 3: Changes related to glucose concentration in different groups**

concentration of LDL in the group that received oat increased than the group that simultaneously received barley and oat, indicating the positive effect of simultaneous using of both extracts. Also, the pathological concentration of LDL in the group receiving barley and oat extracts had significant reduction than the group receiving cholesterol. In an review about effects of yeast and  $\beta$ -glucan plant on lipid profile and Secom of probiotic bacteria using rats having high cholesterol diet showed that beta-glucan extract derived from barley lowered more cholesterol and LDL levels and increased HDL level. While beta-glucan derived from yeast less lowers cholesterol so beta-glucan anti-fat effects depend on derived source<sup>29</sup>. Researches also suggest that dietary fibers available in oat flour and bran rapidly reduce 20% of blood harmful cholesterol (LDL) level, and increase 15% of good cholesterol (HDL) level<sup>18</sup>.

Reports suggested that consumption of vitamins probably by reducing free radicals reduce risk of cardiovascular diseases and in hyperlipidemic patients improve vascular endothelial function<sup>30</sup>. Available vitamin C in herbal compounds as an antioxidant reduces the peroxidation of lipids and oxidative degradation of vessels. Using vitamin C and diets rich of antioxidant vitamins also leads to health protection and reduces risk of heart diseases<sup>31</sup>. It seems according to the results that vitamin C through two main mechanisms cause favorable changes at HDL and LDL level: By 1. Applying the antioxidant effect that cause reduction of LDL oxidation and increase of its identification by its receptors. 2. Applying competitive effect (due to structural similarity) with glucose in process of HDL and LDL Glycation leads to increase LDL catabolism and reduce HDL excretion<sup>32</sup>. What is certain is that oat extract contains different vitamins such as vitamin C that by using the above mechanisms leads to reduction of blood lipids<sup>33, 34</sup>.

According to Chart 1, the pathological concentration of HDL in the experimental groups receiving oat and barley alone and also simultaneously decreased and the most effect was related to simultaneous use of extracts. Previous studies have found that high-density lipoprotein has beneficial effects on activity of hyperlipidemic patients and thus reduction of its serum level will follow lipid

problems in patients and people with coronary artery disease<sup>35, 36</sup>. As mentioned above the high-density lipoprotein level in the experimental group which simultaneously received oat and barley extracts had a significant increase compared to the control group. The increase should be considered useful since this kind of cholesterol causes secretion of harmful cholesterol out of arteries and it is useful to prevent from heart attack and cerebral stroke, so it is better that cholesterol and HDL levels to be high in blood<sup>37</sup>. What is certain is that the beneficial effects of extracts are probably due to effective ingredients and soluble vitamins within it that are mentioned before.

Studies found that oat extract contains 10% palmitic acid, 50 to 60% oleic acid and 15 to 30% linoleic acid (33, 34) and also found that unsaturated fatty acids reduce pathological LDL which is an atherogenic material<sup>38</sup>. Therefore, in this study, the pathological concentration of LDL in the experimental group that simultaneously received the both extracts shows the positive effect of them.

According to the Chart (3) a significant decrease was observed in the groups receiving barley and oat alone and simultaneously. The history of use of barley seeds for feeding and treatment of diseases<sup>39</sup> including diabetes<sup>40, 41</sup> shows that this extract is effective in treatment of diabetes and low blood sugar. It is stated in the past that barley seed is the case that its role in treatment of diabetes was mentioned in ancient texts of Iran's traditional medicine. In study of *Hordeum vulgare* barley seed extract effect on concentration of fasting plasma glucose of streptozotocin-induced diabetic rats stated that water extract of barley seed was significantly reduced the level of serum glucose after four weeks in diabetic rats and after a week in non-diabetic rats. In non-diabetic rats the effect was not sustained after the first week. It is clarified in studies that in diabetic patients, who have high-fiber diet of oat and other carbohydrates, level of consumption of insulin receives to half and some people, who take less insulin, cut the drug consumption. Thus, fibers rapidly reduce absorption of carbohydrates and prevent upsurge in glucose<sup>16, 18</sup>. So the results of researches are in agreement with the current study. In addition, simultaneous use

of barley and oat extracts causes more reduce in glucose level, which is quite logical.

### CONCLUSION

The results showed that barley and oat extracts reduce pathological concentration of LDL

and glucose, and increase pathological concentration of HDL with regard to the effective ingredients within the extracts and simultaneous use of both extracts is recommended.

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