Original Article

The effect of hydroalcoholic extract of Oak fruit husks on liver in Rat (Wistar)

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Abstract

Introduction:
The revitalizing properties of medicinal herbs have long been an interest of human beings. Oak (Quercus castaneifolia) is a tree that is widely used in herbal medicine in addition to boasting some nutritional values. Given that some medicinal plants have certain side effects, the present study investigates the effect of the hydroalcoholic extract of oak fruit husk on liver function in rats.

Materials and Methods:
In the present study, 50 Wistar rats weighing approximately 200 ± 15 g were taken and housed under standard conditions. After determining the lethal dose of the hydroalcoholic extract of oak fruit husk and consequently its preparation, 30, 60 and 120 mg/kg/w.bt doses of the extract were selected and injected intraperitoneally to the treatment rats on a daily basis. The rats were divided into five groups, including the negative control group (receiving no treatments), the sham control group (receiving oak fruit husk solvent) and three treatment groups. After 21 days, the blood samples of rats were collected and the serums were biochemically analyzed. Data obtained were then analyzed in SPPS-17 software using the ANOVA test and the groups were compared with each other using Duncan's post hoc test.

Results:
According to results obtained, the serum levels of all three liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) had significantly increased in the treatment groups compared to the negative and sham control groups ($p<0.01$). In the treatment group, with the maximum dose of the extract, protein, cholesterol and LDH had significantly decreased.

Conclusion:
The results of the study showed that the hydroalcoholic extract of oak fruit husk disrupts liver function, and this disruption appears to be due to the doses used. It is therefore recommended to remove the oak fruit husk from the acorn prior to consumption.

Keywords: Liver, Oak, Rat

Introduction

In both disease treatment and prevention, medicinal herbs are particularly valuable in providing community health. Oak tree (Quercus castanifolia), with a long life (sometimes up to 2000 years) and extensive countrywide spread has long been important in providing food for human and livestock in addition to its valuable medicinal properties (1). Because of phenolic and tannin compounds, oak fruit has lipid peroxidation inhibition and antioxidant properties (2, 3). Moreover, it

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also has been used as food, livestock fodder, and in handicraft and leather industries (4). Oak fruit contains large amounts of sodium and magnesium, and thus plays an important role in regulating blood pressure. It also has applications in treatment of cancer, and has high nutritional value, and contains a variety of vitamins and carbohydrates (5).

Many medical properties have also been reported for the husk of oak fruit. Studies have been conducted on the effect of oak fruit husk in controlling minor oral mucosal ulcers, healing coetaneous wounds (6), treatment of diarrhea and sterilization of gastrointestinal system (7), and inhibitory effect on proliferation of HSV (6).

All tannins have a series of common properties, including the ability to coagulate albumins, heavy metal, and alkaloids; they dissolve in water and show antidiarrheal properties. Thus, they may be used to reduce irritations and pain, and their brew can be used to relieve oral inflammations, common cold, bronchitis, topical bleeding, and burns. The extraordinary properties of this substance have led to its wide applications in industry and in production of strong glues (8).

There are compounds such as phlob-atannin, phlobaphene, and flavonoids in oak fruit and husk. Phlobatannins are a mixture of phenols such as pyrogallol and algic acids. Phenolic compounds are among the most important sources of natural antioxidants, and contain large amounts of tannins, pectin mucilage, and quercin (quercetin) malic (9).

Liver is the most important organ involved in metabolism, and its dysfunction causes physiological, anatomical disorders, and diseases. Hepatocytes contain large amounts of enzymes, and as biological catalysts, enzymes control all enzymatic reaction of the body. Thus, any quantitative and qualitative changes in various enzymes can be indicative of health or illness. Because of liver damage, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) can leak into plasma and enter blood in large amounts, and therefore, they are considered as liver damage index (10).

There are large amounts of AST in skeletal and cardiac muscles, and thus it is not considered a specific liver damage index. However, ALT is a more sensitive and specific liver cellular damage index (11). Compared to other enzymes, both of these enzymes are more sensitive to hepatotoxicity and its histopathological changes, and can also be assessed in shorter time. Although ALP also exists in most tissues, it increases due to liver diseases (11).

Many studies have been conducted on antioxidant properties of oak (12, 13, 14, 15, and 16), which have less considered its fruit husk. According to the antimicrobial properties of the fruit husk (12) and its effect on the digestive system (7) on the one hand, and the key metabolic role of the liver on the other, the present study was conducted to investigate potential side-effects of oak fruit husk on liver as the most important organ involved in metabolic activity.

Materials and methods

Fifty heads of adult Wistar rats weighing 200±15 grams were procured and housed in standard laboratory conditions for two weeks to adapt to the new environment.

Preparation of hydroalcoholic oak extract:

To prepare the extract, husk was separated and ground into powder. Then, Soxhlet method (17) was used for extraction, in which 200cc of water and ethanol solution was added for every 10 grams of husk powder in Soxhlet. Using a rotavapor, solvent was separated from extract. After 48 hours, the solution was filtered and kept at 30-35 °C to evaporate its water and alcohol. Then, the resulting condensed liquid was refrigerated (17).

Next, the lethal dose of oak fruit husk extract was determined, and accordingly, 3

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doses 30, 60, and 120 mg/kg body weight of rats were chosen as minimum, medium, and maximum doses.

Rats were randomly divided into 5 equal groups as follows: control group (without any treatment), sham control (receiving intraperitoneal injection of equal water and alcohol solution as husk solvent, and treatment groups 1, 2, and 3 receiving daily injections of 30, 60, and 120 mg/kg/b.wt respectively over 21 days. After treatment course, rats were anesthetized and blood samples were taken from them using insulin syringes. Blood samples were centrifuged at 3000rpm for 10 minutes, and serum was separated and sent to laboratory for chemical analysis. ALT, AST and ALP were analyzed according to the International Federation of Clinical Chemistry (IFCC) method using special kit from Pars Azmoon Company and Biowave spectroph-otometer. Additionally, melondialdehyde, protein, and HDL were also measured using colorimetric methods. Data from chemical analysis of serum were analyzed in SPSS software using ANOVA test. To compare groups, Duncan's post hoc test was used.

Results

The results showed a significant increase ($P<0.01$) in ALT activity in groups treated with minimum and medium doses (figure 1), AST in groups treated with minimum and maximum doses (figure 2), and ALK in all 3 treatment group (figure 3), and also at serum MDA level (figure 6) in control and sham groups. In comparison, a significant reduction was found in serum total protein (figure 4) in treatment group with maximum dose compared to control and sham groups. In relation to serum cholesterol level (figure 5), although a significant increase was observed in treatment group with a medium dose compared to treatment group with a maximum dose, no significant difference was found between treatment groups and control.

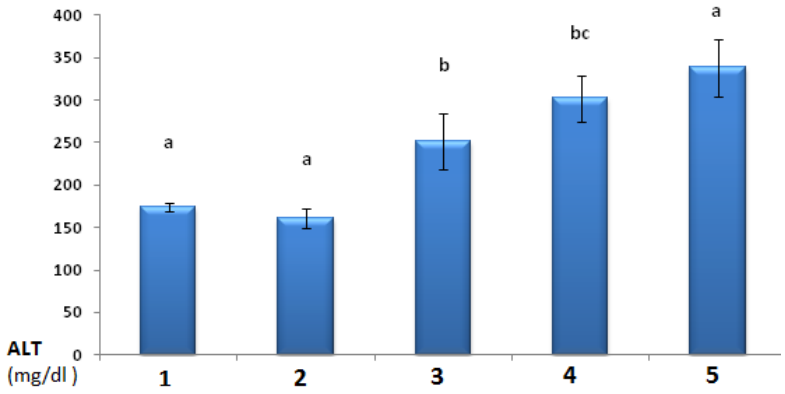

Figure 1: Comparing ALT in study groups

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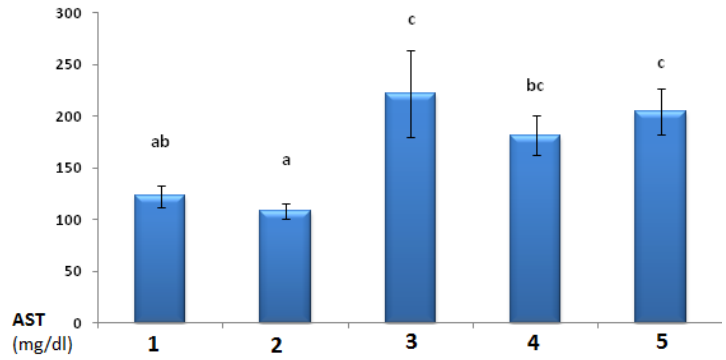

Figure 2: Comparing AST in study groups

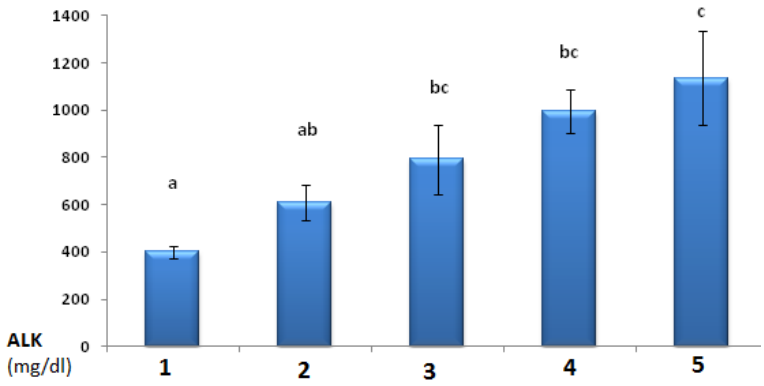

Figure 3: Comparing ALK in study groups

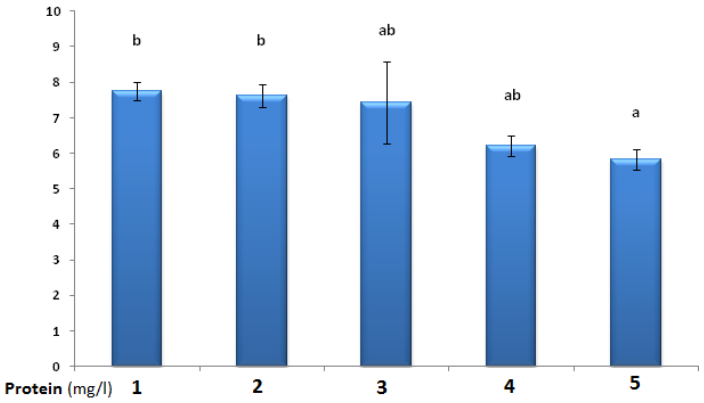

Figure 4: Comparing total protein in study groups

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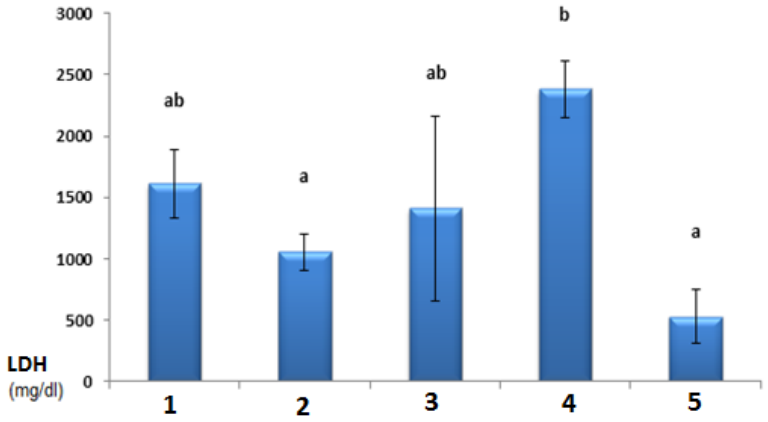

Figure 5: Comparing cholesterol in study groups

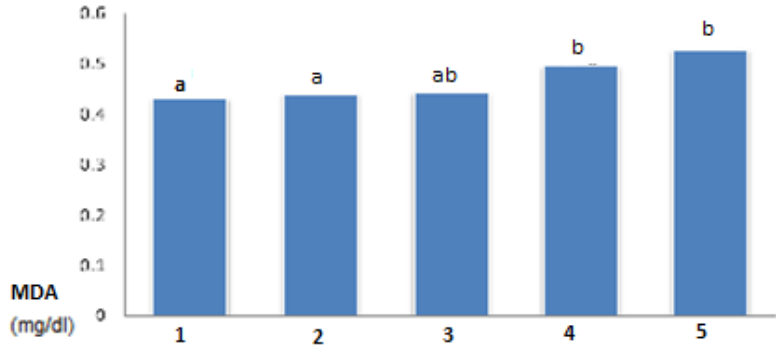

Figure 6: Comparing malondialdehyde in study groups

Discussion

Although many studies have confirmed benefits of oak fruit and cited its antioxidant properties due to the presence of flavonoid compounds (6, 7, and 12), in the present study, an increase in above enzymes (figures 1-3) confirms liver damage (18). The disagreement between results of this and other studies may be due to the difference in the amount of compounds in oak fruit and in its husk, the amount of extract used, administration method, duration of use, and even type of animal tested (9). The compounds in different parts of oak fruit, including skin, leaf, and husk were studied by Haydari et al (9). There is also evidence that external use of oak is more beneficial than its internal use (19).

One of the compounds influential in the incidence of above cases is tannin in oak, which may have entered animal body in large amounts due to short duration of study (21 days). Furthermore, despite its rapid absorption, administration method (injection) is always associated with stress in subjects, which may have caused the difference in results. Compared to previous studies (20), the present study results may be due to large amounts of tannin. Severe poisoning and even death have been reported due to intake of large amounts of tannin (about 5%) (21). Accordingly, when using oak fruit, local people in Kohgiloyeh-Boyerahmad province take the inner skin off. Moreover, oak fruit without tannin has no adverse effects and its use in broilers diet is 20% more economical (20).

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Considering the antioxidant role of oak skin due to gallic and phenolic acid compounds (4, 8), which also exist in the husk, and the disagreement with the present study results confirm the effect of compounds such as tannic acid and lagic acid and their different amounts in skin and husk (22). Tannin, bitter quercin, malic acid, pectin, resin and large amounts of calcium oxalate (especially in the skin) can affect liver tissue and function. To determine the effect of each of these compounds, accurate spectrophotometeric methods are needed.

Turkman et al. (14) determined tannic acid in the chestnut oak tree skin 14% and in the fruit 12%. Tannin in the husk forms a complex with metallic salts, zinc sulfate, heavy metals and alkaline, and by absorbing surrounding liquids and secretions, it is used as an antidiarrheal substance, but severe digestive problems and nausea have been reported due to its intake in large amounts (12).

Increased alkaline aminotransferase (figure 3) indicates toxic effect of husk extract on liver or other tissues, including heart and muscles. In this study, since this enzyme also enters blood from other tissues, serum level of this enzyme showed an increase in all treatments. ALP has been reported to mainly originate from the liver in adults, while in growing children, bone cells are the most important source of this enzyme (11, 18).

Aminotransferase catalyzes chemical reactions in cells, and transfers their amino group from a donor to a receptor molecule. There are large amounts of ALT in liver cytosol that catalyzes alanine conversion into pyruvate and glutamate, and is more specific to liver. Since ALT leaks into plasma and enters blood due to liver damage, its increase in blood is a key indicator of damage to liver cells. Although AST and ALT also exist in other tissues, their increased serum level somewhat indicates liver damage (11, 18).

An increase in above enzymes, and especially MDA (figure 6) confirms destructive effect of the husk extract, especially on the cell membrane. In agreement with previous findings, this result shows impaired transfer of ions and various materials and concentration gradient on both sides of membrane, together with transfer of chemical messengers by membrane receptors, which cause leakage of above enzymes from liver cytoplasm into blood stream (18).

Occasionally, in metabolism of materials, resulting metabolites have different properties from the original compound (15). Thus, despite its antioxidant compounds, hydroalcoholic extract of the husk may produce hepatotoxic metabolites, with subsequent effects found in this study. According to Taran et al. study, the extract being hydroalcoholic may also affect the results (16). The majority of substances and toxins have time and dose-dependent effects, and the target tissue and other factors also have a role in this respect (11).

Albumin that is mainly produced by the liver is precipitated by tannin (13). Albumin binds to metals, and thus prevents formation of active oxygen species and chain reactions in creating free radicals. Binding of tannin to metals and precipitation of albumin leads to reduced albumin, and reduced serum total protein can be explained by the ratio of direct total protein to albumin (19). Albumin level is affected by various factors including diet, hormonal factors, and especially gastrointestinal activity. Moreover, reduced liver activity can also affect this result. Reduced total protein at maximum dose confirms the notion that toxic effect of metabolites at lower doses is partially neutralized by the induced antioxidant effect of the extract (6, 20). Incomplete protein synthesis in the liver, incomplete intestinal absorption and loss of protein due to liver dysfunction can also affect albumin reduction results. Reduced albumin has also been reported in liver cirrhosis (18). As well as increasing lipid peroxidation, these factors can also reduce

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High Density Lipoproteins (HDL). Although inhibitory effect of hydroalcoholic extract of oak fruit skin on serum LDL oxidation in vitro showed that difference in method, especially administration of extract and physiological status of the subject can also cause difference in results (19).

In the present study, increased NDA (figure 6) along with increased liver enzymes (figures 1-3) at some doses of husk extract showed partial liver dysfunction due to the extract. MDA, the final product of lipid peroxidation by active species of oxygen (ROS), is the most important indicator of oxidative stress (11) which terminates cell cycle and increases apoptosis, and cell death by producing free radicals.

Along with growing use of medicinal plants, it is also essential to consider their potential side effects. Although herbal medicines have fewer side effects than chemical ones, some may also affect chemical drugs (21). Although oak fruit is not part of the routine human diet, it is considered a source of food for livestock in some parts of the country, including in Kohgiloyeh-Boyerahmad province, and despite economic benefits, side effects of the husk should also be considered. Thus, it is recommended that a study be conducted on long term use of aqueous extract of oak fruit husk compared to oral methods in rats as similar mammals, so that effective factors can be eliminated and oak may be used to optimize livestock fodder.

Conclusion

According to the present study results, despite containing antioxidants and beneficial properties, oak fruit husk also contains substances that may adversely affect body organs. It is therefore recommended that oak fruit not be overused, and its husk be removed before use.

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