

Original Research Article

Effects of Persian leek (*Allium ampeloprasum*) on hepatic lipids and the expression of proinflammatory gene in hamsters fed a high-fat/ high-cholesterol diet

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Abstract

Objective: Persian leek is one of the most widely used herbal foods among Iranians. In this study, effects of oral administration of Persian leek on plasma and liver lipids were examined in hamster.

Materials and Methods: Male Syrian hamsters were randomly divided into three groups: control (standard diet), high fat control (high-fat/high-cholesterol diet), Persian leek (high-fat/high-cholesterol diet + 1% per weight of diet from dried powdered Persian leek) for 14 weeks.

Results: High fat diet increased plasma and liver lipids as compared to standard diet. Adding Persian leek to the high-fat/high-cholesterol diet resulted in no significant changes in the concentration of the plasma lipids or liver cholesterol. However, liver triglycerides (TG), plasma Alanine aminotransferase and gene expression of tumor necrosis factor- α were decreased in hamsters fed high-fat diet containing Persian leek as compared to high-fat diet only.

Conclusion: Persian leek might be considered as a herbal food that can reduce liver TG accumulation induced by high fat diets.

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Introduction

Dyslipidemia is a well-known risk factor for cardiovascular diseases (Luisi, 2000). High-fat diet is one of the factors that influence plasma lipids and cause dyslipidemia. In addition, fat accumulation, especially triglyceride and cholesterol, in the liver can be caused by high-fat and high-cholesterol diets. Fat accumulation can consequently cause non-

alcoholic fatty liver disease (NAFLD) (Kotronen and Yki-Jarvinen, 2008). Fat accumulation in the liver caused by high-fat diet, increases the expression of inflammatory cytokines such as tumor necrosis factor- α (TNF- α) in the liver (Park et al., 2010) and these cytokines play a role in inducing liver damage (Braunersreuther et al., 2012).

Research on herbs which offer enhanced health or reduced risk of disease

Leek and liver fat

is an increasing interest. *Allium ampeloprasum* persicum or Persian leek (also called Tareh) a member of onion family (Alliaceae) is native to Middle East and Iran. Unlike onion or garlic, Persian leek does not develop real bulb or produce cloves. It is one of the most widely used herbal foods among Iranians with about 5000 tons being produced every year (Mousavi et al., 2006). The linear green leaves have mild onion flavor and are eaten raw. It contains various bioactive compound including organosulfur compounds (Mnayer et al., 2014) and flavonoids such as kaempferol (Fattorusso et al., 2001; Bernaert et al., 2012). Leek has been found to have antioxidant and liver-protecting properties (Molay Kumar Roy et al., 2007; Nasir, 2012).

A large number of studies have shown that members of onion family, such as garlic and onion, have hypolipidemic effect and could potentially improve some risk factors related to cardiovascular disease or NAFLD (Liu and Yeh, 2001; Kim et al., 2008; Vidyavati et al., 2010; El-Din et al., 2014). However, to our knowledge, only one study has evaluated hypolipidemic effects of leek (Movahedian et al., 2006). In the present study, effects of oral administration of Persian leek on the lipids of plasma and liver were examined. Since Golden hamster's lipoprotein metabolism has the highest similarity to that of humans (Nistor et al., 1987; Bhatena et al., 2011), these animals were used in the current study.

Materials and Methods

Animals and diets

Twenty one male Syrian hamsters weighing 90 to 110 g were obtained from Pasteur institute (Tehran, Iran). The animals of each group were housed at 22 ± 2°C with a 12hr-12hr light-dark cycle. Animals had free access to food and water. The study was approved by the ethical committees of National Nutrition and Food

Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

After one week of adaptation to the environment, animals were weighed and randomly divided into study groups (7 hamsters per group): a standard diet (AIN 93M) (Reeves et al., 1993), a high-fat/high-cholesterol diet and high-fat/high-cholesterol with 1% Persian leek diet for 14 weeks (Table 1). For measurement of food consumption, animals were kept in separate cages and allowed to eat *ad libitum* and the leftover was weighed.

Table 1. Composition of diets

Component	Standard	High fat	Persian leek
Casein (g/Kg)	140	200	200
L-Cysteine (g/Kg)	1.8	3	3
Corn starch (g/Kg)	468.2	392	392
Sugar (g/Kg)	100	140	140
Dextrin(g/Kg)	155	-	-
Cellulose (g/Kg)	50	50	40
Minerals (g/Kg)	35	35	35
Vitamins (g/Kg)	10	10	10
Butter (g/Kg)	-	150	150
Cholesterol (g/Kg)	-	20	20
Soybean oil(g/Kg)	40	-	-
Persian leek powder (g/Kg)	-	-	10
Total weight	1000	1000	1000
Energy (Kcal/g diet)	3.82	4.47	4.47
Carbohydrates (g/Kg)	723.2	532	532
% Energy	75	48	48
Proteins (g/Kg)	40	203	203
% Energy	10	18	18
lipids(g/Kg)	141.8	170	170
% Energy	15	34	34

At the end of the study, the animals were subjected to fasting for 6 hr and then, animals were anaesthetized with an intraperitoneal injection of 83.3 mg/kg of ketamine hydrochloride and 3.3 mg/kg of xlyazine. The blood sample was taken using a heparinized syringe from the heart of the anesthetized animals. A specific part of the liver was removed and then, washed with cold normal saline. The extracted liver and plasma samples were stored at -80°C until analysis.

Levels of cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) as well as alanine

aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma were measured by colorimetric-enzymatic kits.

Liver Lipids

In order to extract the liver's total lipids, 100 mg of the frozen liver tissue was dissolved in 2 ml of chloroform-methanol solution (2:1 ratio). After a complete homogenization process, the above-mentioned mixture was shaken at room temperature on a rocker for 1 hr. Then, the solution was centrifuged for 20 min and 1 ml of the supernatant (the extracted lipids) was taken and mixed with 1 ml of triton X-100 solution (1% in chloroform). Then, the combined solution was dried under a stream of nitrogen. To each tube of the dried lipids, 0.5 ml of deionized water was added and the tube was placed in a water bath at 37°C for 15 min. Afterwards, triglyceride and cholesterol levels of the solutions were measured by colorimetric-enzymatic method using commercial kits (Carr *et al.*, 1993; Abidi *et al.*, 2006).

In a separate liver sample from each animal, activity of hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase enzyme was determined indirectly by measuring the ratio of HMG CoA to mevalonate. HMG CoA and mevalonate concentrations were determined after the reactions with hydrochloride amine hydroxyl in different pH conditions using colorimetric method (Rao and Ramakrishnan, 1975).

Expression of TNF- α and CYP7A1 genes in the liver

Total RNA of the liver tissue was extracted using RNeasy mini kit (Qiagen, Germany). Maxima H Minus First Strand cDNA Synthesis kits (Thermo Scientific) was used for cDNA synthesis. Real-time PCR was performed on a BioRad MiniOpticon device using Maxima SYBR-green PCR Master Mix (Thermo Scientific). Sequences of primers used in this study were as follow: TNF- α forward:

5'-CCCAAAGGGAAGAGAAGTTC-3',
reverse: 5-CCACTTGGTGGTTTGCTACA-3' (Lowanitchapat *et al.*, 2010);
cytochrome P450 7A1 (CYP7A1) forward: 5'-ACTGCTAAGGAGGATTCCTACTCT-3',
reverse: 5'CTCATCCAGGTATCGATCATATT-3' (Hung *et al.*, 2012). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was amplified as the house keeping gene, forward 5'-GACCCCTTCATTGACCTCAACTAC-3';
reverse: 5'-GCCAGTAGACTCCACAACATACTC-3' (Duncan *et al.*, 2013). Thermal cycler conditions included holds for 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 60 sec at 60°C and 30 sec at 72°C. A melting curve analysis was plotted for each reaction with 55–95°C ramp. Normalized Ct or Δ Ct (Ct gene –Ct house-keeping) was calculated and the Δ Ct data were analyzed statistically.

Statistical analysis

Values are expressed as mean \pm SD. Since our hypothesis was evaluation of hypolipidemic effects of inclusion of leek in high-fat diet, results from leek group were compared with high-fat group. In addition, to show hyperlipidemic effects of high-fat diets, results of high-fat group were compared with standard diet. To compare the quantitative variables with normal distribution between the two groups, the independent t-test was used, while for the quantitative variables without normal distribution, the non-parametric Mann Whitney test was applied. Statistical significance was accepted at $p < 0.05$. All statistical analyses were carried out using SPSS software (version 20).

Results

Food intake was 6-7.5 g/day and no significant difference was observed among groups in terms of average consumed food. In addition, weight of the animals were 98-106 g and did not differ among the groups.

Leek and liver fat

When compared to standard diet, high-fat/high-cholesterol diet resulted in a significant increase in the concentration of the plasma AST ($p<0.05$), ALT ($p<0.01$) and lipids including total cholesterol ($p<0.01$), LDL-C ($p<0.01$) and TG ($p<0.01$) (Table 2). Levels of liver TG ($p<0.01$), cholesterol ($p<0.01$) and ratio of HMG CoA to mevalonate ($p<0.05$) were also significantly increased (Table 3).

Adding Persian leek to the high-fat/high-cholesterol diet resulted in no significant changes in the concentration of the plasma lipids. Plasma AST levels did not significantly decrease in Persian leek-treated group as compared with the high-fat group. However, plasma concentration of ALT was significantly lower than that of the high-fat group ($p=0.01$).

Table 2. Effect of diets on plasma parameters

Parameter	Standard diet	High fat	Persian leek
TC(mg/dL)	81.30 ± 19.69	140.79 ± 26.68 **	141.75 ± 31.29
LDL(mg/dL)	18.02 ± 5.01	33.07 ± 10.17 **	41.64 ± 6.85
HDL(mg/dL)	61.04 ± 10.28	70.92 ± 8.28	74.33 ± 9.38
TG(mg/dL)	71.83 ± 27.54	144.71 ± 25.61 **	128.25 ± 50.76
ALT(IU/L)	44.62 ± 14.67	129.70 ± 33.54 **	78.05 ± 19.84 †
AST(IU/L)	47.5 ± 15.75	99.03 ± 54.19*	74.75 ± 16.38

* $p<0.05$ compared with standard diet, ** $p<0.01$ compared with standard diet, † $p<0.05$ compared with high-fat diet. TC= total cholesterol, LDL=low-density lipoprotein, HDL=high-density lipoprotein, TG= triglycerides, ALT= alanine aminotransferase, AST= aspartate aminotransferase.

Table 3. Effect of diets on liver parameters

Parameter	Standard diet	High fat	Persian leek
Hepatic TC(mg/dL)	24.25 ± 3.77	84.42 ± 23.24 **	78 ± 13.47
Hepatic TG(mg/dL)	45 ± 17.2	90.57 ± 27.96 **	63.57 ± 12.39 †
HMG CoA / mevalonate ratio	2.04 ± 0.2	2.61 ± 0.47 *	2.84 ± 1.26

* $p<0.05$ compared with standard diet, ** $p<0.01$ compared with standard diet, † $p<0.05$ compared with high-fat diet

Adding Persian leek to the high-fat/high-cholesterol diet did not result in any difference among the groups in terms of liver cholesterol and ratio of HMG CoA to mevalonate. However, levels of liver TG were significantly lower in the high-fat group ($p=0.03$). Adding Persian leek to the high-fat/high-cholesterol diet reduced mRNA expression of TNF- α in the liver (Figure 1). However, the expression of CYP7A1 did not differ between the two groups.

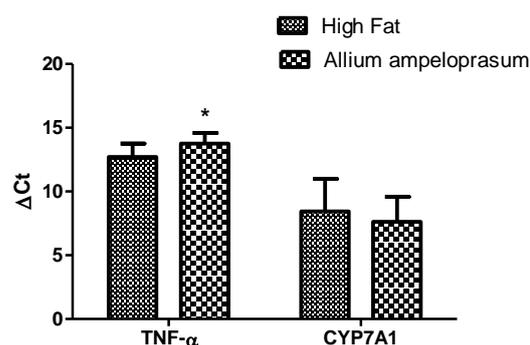


Figure 1. Expression levels of TNF- α and CYP7A1 mRNA in the liver. Δ Ct was calculated using GAPDH as the house keeping gene.

Discussion

In this study, diet-induced hyperlipidemia was not modified by Persian leek. No previous study has evaluated hypolipidemic effects of Persian leek consumption. However, in rabbits, different doses of leek (*Allium porrum* L.) extract decreased plasma cholesterol and LDL-C with no effects on TG and HDL-C levels (Movahedian *et al.*, 2006). In the current study, 1% (w/w) dried leek was added to the animals diet which is much less than the amount used by Movahedian *et al.* to produce the extract (Movahedian *et al.*, 2006).

High-fat/high-cholesterol diet increased liver fats including triglyceride and cholesterol, and elevated plasma levels of ALT and AST as compared to the standard diet. Ratio of HMG CoA to mevalonate was increased with high-fat/high-cholesterol diet as compared to the standard diet, indicating reduced HMG-CoA reductase activity (the rate limiting enzyme in cholesterol biosynthesis) and endogenous cholesterol biosynthesis. Adding Persian leek in the high-fat diet had no significant impact on liver cholesterol and ratio of HMG CoA to mevalonate. In addition, mRNA expression of 7-alpha-hydroxylase enzyme (CYP7A1), a key enzyme in the cholesterol excretion pathway and its conversion into bile acids, did not change. Nonetheless, Persian leek significantly reduced TG levels in the liver. One of the bioactive compound present in Persian leek is d-limonene which constitutes about 27% of the essential oils (Karimi *et al.*, 2013). Dietary d-limonene has been shown to reduce fat accumulation (cholesterol and TG) in the liver of animals (Victor Antony Santiago *et al.*, 2012) and sterol regulatory element-binding protein-1 and polipoprotein E gene expression, with potential influence on liver fat (Jing *et al.*, 2013). Leek is also rich in ferulic acid (Bernaert *et al.*, 2013), which may reduce TG accumulation in the liver presumably through increase in TG excretion in feces

and decrease in the gene expression of fatty acid synthase (Adluri *et al.*, 2008; Jin Son *et al.*, 2010). Organosulfur compounds present in leek may also reduce liver TG production (Liu and Yeh, 2001).

In the present study, liver TNF- α gene expression significantly decreased in Persian leek-treated animals. Active ingredients in the Persian leek such as kaempferol may presumably be effective in reducing TNF- α expression (Kong *et al.*, 2013). TNF- α is an inflammatory mediator secreted by several inflammatory cell types, and also by many other tissues. In the liver, TNF- α is secreted directly by hepatocytes and Kupffer cells and several studies have shown that TNF- α is a key factor in the development of NAFLD (Braunersreuther *et al.*, 2012). Pathways activated by TNF- α could lead to hepatocyte apoptosis (Ding and Yin, 2004) and inhibition of TNF- α production can be effective in reducing liver damage (Iimuro *et al.*, 1997; Yu *et al.*, 2012). Adding Persian leek to the diet also led to a significant decrease in plasma ALT which increases in liver damage. Antioxidant activities of leek (Bernaert *et al.*, 2012) may also decrease liver cell damage and ALT elevation. In previous studies, kaempferol which is abundant in leek (Bernaert *et al.*, 2013) as well as d-limonene, ferulic acid and organosulfur compounds showed antioxidant properties, reduced liver cell damage and prevented serum ALT and AST elevations (Wu *et al.*, 2001; Jin Son *et al.*, 2010; Victor Antony Santiago *et al.*, 2012; Wang *et al.*, 2014). In addition, leek extract reduced liver toxicity of carbon tetrachloride and plasma levels of liver enzymes in rats (Nasir, 2012).

In conclusion, considering the useful effects of Persian leek in reducing liver fats, TNF- α expression and plasma ALT, it might be considered as a herb with the potential of reducing liver TG accumulation induced by high fat diets.

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Conflict of interest

The authors declare that there is no conflict of interest.

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