

Effect of *Kelussia odoratissima* Mozaffarian on Lipid and Glucose Profile

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Background: Flavonoids are a group of polyphenolic compounds with antioxidant properties that help reduce cardiovascular risk factors. *Kelussia odoratissima* is a flavonoid-containing plant, the effect of which was analyzed on decreasing blood lipid levels in mice.

Objectives: The present study was carried out with the aim of determining the anti-hyperlipidemia effects of this herb on mice.

Materials and Methods: In this preclinical study, 70 Balb/c mice were divided to five 14-member groups and received normal diet, normal diet containing 5% olive oil, high cholesterol (2% cholesterol and 5% olive oil) diet and high cholesterol diet accompanied by *Kelussia odoratissima* 10% or 20% hydro-alcoholic extract. After two weeks, blood samples were taken and cholesterol, triglyceride, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), and fasting blood sugar (FBS) were measured.

Results: In this animal model, the levels of all serum lipids including cholesterol, HDL, VLDL, LDL, triglyceride and FBS had a significant decrease two weeks after the beginning of the study. Moreover, at the same time, in the group that received 20% hydro-alcoholic extract, the serum levels of cholesterol, HDL and LDL had a significant decrease ($P < 0.05$).

Conclusions: *Kelussia odoratissima* might have a desirable effect on serum lipid profile and might have beneficial effects on hyperlipidemic patients.

Keywords: Hyperlipidemia; Mice; Human; Triglyceride; Cholesterol

1. Background

The relationship between the level of cholesterol and the risk of cardiac diseases was determined in 1948 for the first time. In 1990, interventional studies showed that decrease in the cholesterol level by diet, drugs or surgery reduces the risk of coronary heart disease (CHD). However, taking HMG-CoA (hydroxy-methyl-glutaryl-CoA reductase) reductase inhibitors reduce the amount of LDL- and CHD-related mortality by up to 30% (1, 2). Among the major consequences of hyperlipidemia, acute pancreatitis and atherosclerosis can be mentioned, and controlling the level of TG can be effective for its control (1-5). However, currently many drugs are used to cure hyperlipidemia; all of them have some side effects (6-9). On the other hand, medicinal plants have generally less side effects and are accepted by people as an alternative treatment since ancient times (10-18). Furthermore, recent trends in treating and controlling diseases tend to favor natural antioxidant compounds more than synthetic ones (19-27).

Researches carried out on medicinal herbs have showed hopeful results regarding the treatment of various diseases such as hyperlipidemia (2, 28, 29), diabetes (30-38), hypertension (39-41) and renal toxicity (26, 42-47).

Kelussia odoratissima Mozaffarian is a medicinal plant

that is believed by the people of Chaharmahal and Bakhtiari province to have effective hypolipidemic effects (48). *Kelussia odoratissima* is one of the genera of the Umbelliferae (Apiaceae) family and has only one species, *Kelussia odoratissima* that is found only in Iran (49). It is a wild perennial aromatic, rebus, glabrous and erect herb that grows to the height of 120 to 200 centimeters. This sweet-smelling, self-growing monotypic medicinal plant is endemic and is found in a restricted area in west of Iran. Its flowers are hermaphrodites and have a diameter of one to two millimeters. The plant is propagated annually through seeds in natural conditions, during late summer. The aerial parts of *Kelussia odoratissima* have high nutritional value due to the existence of minerals and vitamins (50). The leaves of this plant are edible and are also used as a wild herb for flavoring and as a drug for curing dyspepsia and rheumatism by the natives of Chaharmahal and Bakhtiari province (51). As mentioned before, *Kelussia odoratissima* is found in some parts of the Zagros Mountain ranges at the height of 2500 meters above sea level, including Isfahan and Lorestan provinces, and is called "Karafs-e-Bakhtiari" by natives (50-52). In addition, it is traditionally used as a medicinal plant to

treat hypertension, inflammation, ulcers and cardiovascular diseases (53).

There is limited literature on the pharmacological properties of this plant, yet recent studies have shown that the hydro-alcoholic extract of *Kelussia odoratissima* is sedative (54), which may be due to its bioactive phthalides (55). Shojaei Asadiyeh et al. in 2011 showed that major ingredients of the aerial parts of *Kelussia odoratissima* are Z-ligustilide and 3-N-butylphthalide (50). Another study also showed that the aerial parts of *Kelussia odoratissima* contain 23 different valuable components and the major compound is Z-ligustilide (56). Ligustilide and Butylphthalide are the major components of *Kelussia odoratissima* that have a positive impact on the nervous system, blood pressure and serum cholesterol level (57, 58).

2. Objectives

Despite the natives' beliefs in the antioxidant effects of *Kelussia odoratissima* and its components, few research projects have been performed on this species. Therefore, the present study was carried out with the aim of determining the anti-hyperlipidemia effects of this herb on mice.

3. Materials and Methods

In this pre-clinical study, after obtaining the approval of the ethics committee of the medical university of Shahrekord, 70 Balb/C mice were purchased from the Iran Pasteur institute with a weight range of 24 - 30 grams, and were kept in the animal house of Shahrekord medical university for seven days for acclimatization. Then, they were weighted and divided to five 14-member groups. The first group (control) received normal food; the second group received normal food containing 5% olive oil; the third group was fed a high cholesterol diet (pellets containing 2% cholesterol and 5% olive oil); the fourth group received a high cholesterol diet accompanied by *Kelussia odoratissima* 10% hydro-alcoholic extract, and finally the fifth group received high cholesterol diet accompanied by *Kelussia odoratissima* 20% hydro-alcoholic extract for two weeks. At the end of two weeks, after fourteen hours of fasting, blood samples were taken and lipid factors such as cholesterol, Triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL) and fasting blood sugar (FBS) were measured by the enzymatic method and spectrophotometry (Pars Azmoon Co.).

3.1. Herbal Preparation and Extraction

Kelussia odoratissima that grows at the heights of Zardkooh Bakhtiari was collected and recognized by a botanist, and was registered with the code of 245 in the Herbarium unit of the medical plant research center of Shahrekord university of medical sciences.

The percolation technique was used for extraction. The aerial parts of the plant were soaked in 80% methanol and then dried and powdered. Afterwards, they were

transferred to a "percolator" and extraction was done at the temperature of 15 - 20°C with the help of 80% methanol. Before adding the powder to the percolator, a piece of "glass wool" was placed at its bottom.

Furthermore, 1000 gr of dried plant powder was placed in a beaker, and 80% methanol was added with amounts to cover the surface of the powder (in a way in that a 3.5 cm layer of methanol covered the surface of the powder). The mixture was added to the percolator after 24 hours and a filter paper was placed over it. After adding 80% methanol, the tap of percolator was set in a way that the rate of solvent flow was two to three drops per minute. The distillation of the extract was done under vacuum at a low temperature by a "rotary operator". The condensed extract was transferred to a "watch glass" and placed in the oven at 40 degrees centigrade for 72 hours to dry completely (59-61).

Considering that the sample volume was less than 30 mice (n = 14) in this research and the lipid observations did not follow normal distribution, non-parametric Kruskal-Wallis test was used for data analysis (62).

4. Results

The results showed that lipid factors including cholesterol, LDL, VLDL, HDL and FBS were significantly different in the intervention groups (extracts 10% and 20%) in comparison with the case (normal diet) and high cholesterol groups (P < 0.05). The effect of the extract in decreasing cholesterol and LDL in the 10% group was more than the 20% group (P < 0.05). The mean cholesterol, TG, VLDL and FBS levels were more in the two groups of olive oil and high cholesterol in comparison with the case group (normal food) (P < 0.05). The level of cholesterol was more in the high cholesterol compared to the olive oil group (P < 0.05). The reduction rate of TG, VLDL and FBS was statistically significant in the group receiving the 10% extract in comparison with the high cholesterol group (P < 0.05); however, this reduction was not significant in the group that received the 20% extract compared to the high cholesterol group (P > 0.05). The two groups that received the 10% and 20% extracts did not have statistical significant differences in the reduction rate of TG and VLDL (P > 0.05).

The mean HDL in the high cholesterol and olive oil groups showed a significant reduction in comparison with the case group (P < 0.05), yet the difference between the high cholesterol and olive oil group was not statistically significant (P > 0.05; Table 1).

The mean LDL/HDL ratio had a significant increase in the olive oil and high cholesterol groups in comparison with the case group and this increase was more significant in the high cholesterol group than the olive oil group. The mean LDL/HDL ratio had a significant decrease in groups that received the 10% and 20% extract compared to the olive oil and high cholesterol groups and this decrease was more significant in the group that received the 10% extract in comparison with the group that received the 20% extract of *Kelussia odoratissima* (P < 0.05; Table 1).

Table 1. The Mean Level of Lipid and Blood Glucose of Mice in the Studied Groups at the End of the Two Weeks of Intervention ^{a,b,c}

Group	Factor						
	FBS	Cholesterol	VLDL	Triglyceride	LDL	HDL	LDL/HDL
Control (Group 1)	144.71 ± 15.12	145.71 ± 19.97	39.54 ± 10.69	197.71 ± 53.49	52.59 ± 17.80	53.57 ± 14.80	1.09 ± 0.57
Group 2 ^d	244.41 ± 201.67	299.23 ± 184.89	53.92 ± 16.16	269.64 ± 80.84	296.76 ± 265.2	9.46 ± 9.56	42.14 ± 50.33
Group 3 ^{d,e}	203.00 ± 73.34	282.5 ± 82.23	57.75 ± 16.57	288.78 ± 82.89	444.82 ± 337.36	9.14 ± 0.53	49.13 ± 37.67
Group 4 ^f	107.21 ± 17.31	114.46 ± 69.08	45.42 ± 25.35	277.14 ± 126.76	64.96 ± 56.13	7.35 ± 2.09	8.92 ± 7.17
Group 5 ^g	225.76 ± 57.33	134.07 ± 28.40	45.98 ± 48.56	299.92 ± 70.02	78.93 ± 27.60	8.07 ± 1.75	10.82 ± 7.08

^a Abbreviations: FBS, fasting blood sugar; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

^b Data are based on mg/dL and in the form of mean ± SD.

^c Group 1: the case group receiving normal food without any drug and extract; Group 2: receiving normal food including olive oil; Group 3: receiving high cholesterol food and olive oil; Group 4: receiving high cholesterol and hydro-alcoholic extract of *Kelussia odoratissima* Mozaffarian 10%; Group 5: receiving high cholesterol food and *Kelussia odoratissima* Mozaffarian 20%.

^d P < 0.05 in comparison with "Group 1" in all factors.

^e P < 0.05 in comparison with "Group 2" in all biochemical factors except cholesterol.

^f P < 0.05 in comparison with "Group 1" in FBS (fasting blood sugar), cholesterol, HDL (high density lipoprotein) and LDL/HDL, and in comparison with "Group 2" in all factors except VLDL and TG, and in comparison with "Group 3" in all factors.

^g P < 0.05 in comparison with "Group 1" in FBS, LDL (low density lipoprotein), HDL and LDL/HDL, and in comparison with "Group 2" in all factors except VLDL (very low density lipoprotein) and TG (triglyceride), and in comparison with "Group 3" in cholesterol, LDL HDL and LDL/HDL, and in comparison with "Group 4" in FBS and cholesterol.

During the study, the weight of mice increased in all groups except the group that received the 10% extract, yet this increase was not significant ($P > 0.05$).

5. Discussion

In this animal study, the use of *Kelussia odoratissima* 10% hydro-alcoholic extract caused a significant reduction in mean HDL, cholesterol, TG, VLDL, LDL/HDL ratio and FBS at the end of the second week in hyperlipidemic mice. However, for the 20% extract, the mean levels of cholesterol, LDL/HDL ratio and LDL were significantly reduced ($P < 0.05$). Moreover, in all groups except the group receiving *Kelussia odoratissima* 10% extract, the weight of animals increased.

Different studies have shown that *Kelussia odoratissima* has flavonoid combinations (63). Fraction analysis of the total extract of the plant showed the routine existence of 3, 4, 7-trihydroxyflavone, caffeic acid and phthalide (63-65). Since the mentioned flavonoids are all in the form of aglycone, they have fast intestinal absorption due to their specific structure (66). Therefore, the lipid decrease was expected after the use of *Kelussia odoratissima* Mozaffarian. Antioxidant compounds decrease harmful lipids through inhibition of cholesterol biosynthesis. Cholesterol synthesis regulation is normally done by HMG-CoA. The conversion reaction of HMG-CoA to mevalonate takes place by the mediation of HMG-CoA reductase and NADPH (Nicotinic amide adenine dinucleotide phosphate) (67). The beneficial effect of *Kelussia odoratissima* was observed with reducing lipids in hypercholesterolemic mice in the laboratory.

The decrease of HDL in the animal test can be due to the increased cholesterol and LDL due to consuming high-cholesterol food. The LDL/HDL ratio decreased in animal tests. This ratio is highly significant in predicting the rate

of coronary diseases. This relationship can be justified with the roles suggested for LDL in transferring cholesterol to tissues and the role of HDL in the reverse transfer of cholesterol (68).

In this study, taking the extract of *Kelussia odoratissima* caused a significant decrease in lipid factors in mice. Therefore, performing human studies on the effect of normal antioxidants and especially *Kelussia odoratissima* on patients with lipid and glucose of hyperlipidemia seems necessary. It should be noted that the exact mechanism of of garlic extract is not clear. If antioxidant property of the plant is a main factor, hence other plants with antioxidant activity (69,70) may have the same effect, which worth examining.

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