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Effect of the Hydroalcoholic Extract of Winter Cherry Fruits (*Physalis alkekengi*) on Serum Lipid Profile and Paraoxonase Activity of Healthy Male Rats

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Abstract

Background: Paraoxonase enzyme is attached to HDL and is involved in the maturation of this lipoprotein. This enzyme is activated by several antioxidants. These antioxidants exist in Winter cherry (*Physalis alkekengi*). In fact, it contains a variety of antioxidants.
Objectives: The objective of this study is to investigate the effects of a hydro-alcoholic extract of winter cherry fruits on serum lipid profile and paraoxanase1 (Pon1) activity in the rat.
Methods: In this randomized experimental study, hydroalcoholic extract of *Physalis alkekengi* fruits was obtained by a percolation method. Four groups of male Sprague Dawley rats (260 +/- 5 g), each containing 7 animals, were housed separately and fed standard rat chow and water ad libitum. Experiments were performed for 28 days on each group. Group1 (control 1) received only the standard diet, with no hydroalcoholic extract. Group 2 (control 2) received 1mL of water by gavage in addition to the standard diet. Groups 3 (experiment 1) and 4 (experiment2) received, respectively, 200 and 400 mg /kg b.w. of the extract in 1 mL of water through gavage. At the end of the experimental period, animals were fasted overnight and lipid profile and paraoxonase activity were determined and compared statistically by one way ANOVA and Tukey post hoc tests, using SPSS version 11.5
Results: Triglyceride, total cholesterol and LDL cholesterol were decreased dose dependently by the extract. HDL cholesterol and serum paraoxonase activities were increased significantly by Physalis extract.
Conclusions: The hydroalcoholic extract of *Physalis alkekengi*, possibly through the presence of antioxidants, increases paraoxonase activity and this enzyme, in turn, augments the level of HDL in serum.

Keywords: Fruit, Hydroalcoholic Extract, Serum, Lipid Profile, Paraoxonase 1 (Aryldialkyl Phosphatase) Enzyme Activity, *Physalis alkekengi*

1. Background

In addition to the effects of the aqueous extract of winter cherry fruits (*Physalis alkekengi*) on the reduction of the activity of several estrogen induced enzymes [1-6], winter cherry fruits have also demonstrated free radical scavenging and lipid peroxidation inhibitory properties [7]. Using a 70% methanolic extract of the fruit, Rashwan [8] reported the protective effects of a 600 mg/kg body weight (b.w.) of this extract on paracetamol induced liver injury as demonstrated by a significant increase in food consumption, weight gain, serum total protein and high density lipoprotein cholesterol (HDL-c). They also observed a decrease in malondialdehyde (MDA) and an increase in several antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GpX). Significant decreases in serum low density lipoprotein cholesterol (LDL-c), triglycerides and very low density lipoprotein cholesterol (VLDLP-c) were also observed compared to the paracetamol treated rats [8].

Paraoxonase-1 (PON-1) is one of the antioxidant enzymes which strongly prevents oxidation, hydrolyzes long chain fatty acids in phospholipids and by such an action inhibits LDL oxidation [9]. PON-1 is synthesized in the liver and is secreted in the serum where it is attached to HDL through its hydrophobic residues [10, 11]. We had previously demonstrated that several flavonoids are able to significantly increase the activity of serum paraoxonase in male rats [12]. Medina-Medrano et al. [13] evaluated the phenolic constituents and the antioxidant properties of different parts of wild Physalis and presented a detailed review of the previous literature on the antioxidant properties of several other Physalis species including those of *Physalis alkekengi*. Therefore based on the presence of antioxidants in the calyces and fruits of this species and based on our own findings that several antioxidants increase PON-1 activity, we decided to study, for the first time, the effect of the hydroalcoholic extract of *Physalis alkekengi* fruits on serum paraoxonase activity of healthy male rats and to observe if

there is going to be a corresponding significant increase in serum HDL-c followed by a significant diminution of total cholesterol, LDL-c and triglycerides upon such a treatment.

2. Methods

2.1. Reagents

Ethanol (96%) was purchased from Ghadir Co. (Teheran, Iran). Tris- hydrochloride and calcium chloride were obtained from Merck (Darmsttadt, Germany). Paraoxon (diethyl paranitrophenyl phosphate) was secured from Sigma Chemical Co. (St. Louis, MO). Triglyceride and cholesterol kits were from Pars Azemoon (Teheran, Iran), Protein Kit was from Zist Chimie (Teheran, Iran), and LDL and HDL kits were obtained from Pishtaz Teb (Teheran, Iran).

2.2. Preparation of Hydroalcoholic Extract

Dried winter cherry fruits (*Physalis alkekengi*) were obtained from local herbalists, identified as previously mentioned [1] and was further confirmed by the Research Center of the Agriculture School of Shiraz University (Shiraz, Iran). The fruits were cleared from the dried sepals and calyces, washed with distilled water, dried at 70°C and pulverized. Five hundred milliliters of 70% ethanol was added to 100 g samples of pulverized winter cherry fruits in a percolator and left for 72 hours. The percolation was done as mentioned by Majekodunmi [14].

The extract produced was concentrated in a rotary evaporator at 35°C at a speed of 60 revolutions per minutes. The final mass of the viscous extract obtained from 100 g of the pulverized fruits was 4.6 g.

2.3. Experimental Animals

Animal experiments were according to the ethics committee of our university. Adult male Sprague Dawley rats obtained from Shiraz University of Medical Sciences Animal quarters were acclimatized to the conditions of the animal house at 25°C and a light/dark cycle of 12 hours, while receiving standard rat chow (Pars Dam, Tehran, Iran) and water ad libitum. At the end of this period, the rats were weighed, 4 groups of seven rats each weighing 260 +/- 5 g, were randomly assigned to different cages. Experiments were performed for 28 days on each group. Group1 (control 1) received standard diet and water ad libitum, without any ethanolic extract. Group2 (control 2) were treated as in group1, except that, in addition, they also received 1 mL of water per day through stomach tube. Group 3 received every day, through gavage, 200 mg of the the hydroalcoholic extract per kg b.w. in 1 mL water, while group 4 received , every day, 400 mg of the hydroalcoholic extract per kg b.w. in 1 mL water through gavage. Rats were weighed every week. At the end of the 28th day, the rats were fasted overnight; blood was taken from the heart and the animals were sacrificed. Serum prepared from blood samples were kept at -70°C for measuring the lipid profile and assaying for paraoxonase1 activity.

2.4. Paraoxonase Assay

The hydrolytic activity of serum Pon-1 enzyme was measured by the production of para-nitrophenol from diethyl p- nitrophenyl phosphate (paraoxan) according to the method of Beltowski et al. [15]. Briefly, to 460 μL of 100 mM Tris-HCl buffer containing 2 mM $CaCl_2$ (pH 8.0), 40 μL of serum and 500 μL of 4 mM paraoxon were added and the increase in absorbance at 412 nm was recorded after 2 minutes at 25°C against a blank containing the buffer instead of the serum. Using an extinction coefficient of 0.0182 L.μmole^{-1}.cm^{-1} for p-nitrophenol, enzyme units (U) were calculated in terms of nmoles of p-nitrophenol produced per min. per ml of the serum. Serum protein was measured by a kit (Zist Chimie, Teheran, Iran) based on Biuret method [16]. The specific activity of serum paraoxanase enzyme was reported as U/mg serum protein.

2.5. Measurement of Serum Triglycerides

Using a kit (Pars Azemoon, Teheran, Iran), serum triglycerides was measured by Prestige 24i autoanalyzer (Japan). According to this method [17], serum triglycerides are hydrolyzed by lipoprotein lipase. Glycerol produced is converted to dihydroxy acetone phosphate and H_2O_2 in the presence of glycerol kinase and glycerol-phosphate oxidase. Hydrogen peroxide produced reacts with 4-aminoantipyrine to form a quinine imine dye which is measured spectrophotometrically at 546 nm.

2.6. Measurement of Serum Total Cholestrol

Using Pars Azemoon kit (Teheran, Iran), serum total cholesterol level was also measured by Prestige 24i autoanalyzer (Japan). In this method, cholesterol esters are first hydrolyzed by cholesterol esterase followed by oxidation of the free cholesterol by cholesterol oxidase which results in the formation of cholestenone and H_2O_2. Hydrogen peroxide reacts with 4-aminoantipyrine and phenol to produce quinine imine dye which is measured specrophotometrically at 546 nm [17].

2.7. Measurement of HDL Cholesterol

Pishtaz Teb kits (Teheran, Iran) were used for the enzymatic- colorimetric method of measuring HDL cholesterol [17]. According to this method, compounds with high

affinity for LDL, VLDL and chylomicrons are added to prevent their participation in the reaction. A surfactant is then added which selectively accelerates the reaction of cholesterol esterase and cholesterol oxidase with HDL cholesterol to produce H_2O_2 which reacts as in previous section with 4-aminoantipyrine and produces the colored quinine imine dye which is measured at 600 nm.

2.8. Measurement of LDL Cholesterol

LDL cholesterol is also measured by a kit (Pishtaz Teb, Teheran, Iran) in which two different detergents are used. The first detergent blocks the reaction of HDL, VLDL and chylomicrons, while the second detergent accelerates the enzymatic reaction on LDL cholesterol to produce a colored quinine imine which is measured spectrophotometrically at 600 nm [18].

2.9. Statistical Analysis

Data were statistically analyzed by one way ANOVA and Tukey post hoc test using SPSS software (version 11.5; SPSS, Chicago, IL., USA). $P < 0.05$ was considered to be significant.

3. Results

The effects of gavage feeding of two doses of the hydroalcoholic extract of *Physalis alkekengi* fruits to rats on weight gain, and serum lipid profile after 4 weeks are shown in Table 1. As noticed, the 2 doses of the extract had no significant effect on weight gain as compared with the 2 control groups. However, Physalis extract decreased serum triglyceride and LDL cholesterol levels dose dependently. Total cholesterol decreased gradually by the lower dose and this reduction reached a significant level when feeding 400 mg/kg b.w. of the extract per day. The reverse was true of the serum HDL cholesterol which was gradually increased at the lower dose and reached a significantly higher value compared to both control groups at the higher concentration of the extract.

The effects of 4 weeks of gavage feeding of 2 different levels of the extract on serum paraoxonase activity, serum protein content and the enzyme specific activity are shown in Table 2. As shown, both paraoxonase enzyme activities and specific activities were raised significantly upon feeding 200 mg/kg b.w. per day of the winter cherry extract. No further significant increase was noted in these two parameters at the higher level of the extract. Total serum protein level was decreased significantly upon feeding the lower dose, and no further significant decrease was noted upon doubling the dose.

4. Discussion

Apparently, feeding the hydroalcoholic extract of winter cherry fruits at the two levels produced no toxic effects as noted by lack of significant differences among gain in weight of the control groups and the animals fed two different doses of the extract (Table 1). The present study also demonstrates that the hydroalcoholic extract of *Physalis alkekengi* significantly decreases serum triglycerides, total cholesterol and LDL cholesterol, while increasing HDL cholesterol (Table 1) and paraoxonase activity (Table 2). Zarei et al. [19] noticed a significant decrease in serum total cholesterol and LDL cholesterol upon feeding 0.1, 0.2, and 0.3 g of the hydroalcoholic extract of winter cherry fruits per kg b.w. to a group of rats on a high fat diet. However, they were not able to report any significant effect of the extract on serum triglycerides and HDL cholesterol. In the present study, we noticed a significant increase in HDL cholesterol upon gavaging 400 mg per kg b.w. of the extract for 28 days (Table 1). Our data on the effect of the hydroalcoholic extract of *Physalis alkekengi* fruits on serum lipid profile agrees well with those of Na et al. [20], who noticed the same trend of changes in serum lipid profile, when feeding single doses of 10, 20, and 30 g of Physalis per kg b.w. to hyperlipidemic rats.

The increase in the activity of serum paraoxonase by the hydroalcoholic extract of *Physalis alkekengi* fruit could be due to its high antioxidant capacity as demonstrated by both Rashwan [8] and Medina- Mendrano et al. [13]. Also it could be due to the fairly high content of calcium (9 mg per 100 g raw fruit), since paraoxonase is a Ca- dependent enzyme and upon activation by Ca^{++}, it will increase serum HDL and decrease total cholesterol [21].

We suggest that this preliminary work to be followed by using several higher doses of the extract to find out if the increase in the activity and the specific activity of the serum paraoxonase follow a dose dependent pattern.

Table 1. Effects of 4 Weeks of Gavage Feeding of the Hydroalcholic Extract of *Physalis alkakengi* Fruits on Body Weight and Serum Lipid Profiles of Adult Healthy Male Rats[a,b]

Experimental Group	N	Body Weight 1st day, g	Body Weight 28th day, g	TG, mg/dL	Total Cholesterol, mg/dL	LDL-C, mg/dL	HDL-C, mg/dL
Control 1 (-extract)	7	262 ± 6a	322 ± 11a	98.9 ± 3.2a	65.6 ± 1.0a	21.7 ± 0.9a	24.2 ± 1.1a
Control 2 (-extract + 1 ml H_2O)	7	265 ± 6a	305 ± 5a	97.8 ± 3.0a	65.1 ± 1.5a	20.5 ± 0.7a	25.5 ± 1.1a
Experiment 1 (200 mg extract per ml H_2O)	7	260 ± 6a	302 ± 7a	79.8 ± 2.5b	61.1 ± 2.4ab	17.4 ± 0.9b	27.8 ± 1.5ab
Experiment 2 (400 mg extract/mL H_2O)	7	254 ± 8a	304 ± 2a	67.3 ± 3.4c	57.9 ± 1.0b	13.4 ± 0.4c	31.0 ± 2.0b
Anova p values		0.183	0.144	0.006	0.001	0.001	0.001

Abbreviations: HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; N, number of animals in each group; TG, triglycerides.
[a]Values are expressed as mean ± SEM.
[b]In each column, values with different letters (a, b, c,…) are significantly different from the control values.

Table 2. The Effects of 4 Weeks of Gavage Feeding of the Hydroalcoholic Extract of *Physalis alkekengi* Fruits on Serum Paraoxonase Activity, Serum Protein and Paraoxonase Specific Activity of Adult Healthy Male Rats[a,b]

Experimental Group	N	Paraoxanase Activity, U/mL	Serum Protein, mg/mL	Paraoxonase Specific Activity, U/mg Protein
Control 1 (-extract)	7	107.9 ± 8.7a	72.5 ± 0.9a	1.5 ± 0.1a
Control 2 (-extract + 1 mL H_2O)	7	112.4 ± 8.3a	71.6 ± 1.1a	1.6 ± 0.1a
Experiment 1 (+ 200 mg/mL extract)	7	182.4 ± 3.0b	66.1 ± 1.0b	2.8 ± 0.1b
Experiment 2 (+ 400 mg/mL extract)	7	186.9 ± 4.0b	64.6 ± 1.3b	2.9 ± 0.1b
Anova P values		< 0.001	< 0.001	0.001

Abbreviations: N, number of animals in each group; U, enzyme unit.
[a]Values are expressed as mean ± SEM.
[b]In each column, values with different letters (a, b, c,…) are significantly different from the control values.

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Footnotes

Authors' Contribution: Dr. Mahmood Vessal designed the experiments and wrote the paper, while Ms. Roya Keshtkaran performed the experiments.

Conflict of Interest: No conflict of interest

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