EFFECT OF *BETA VULGARIS* L. ON CHOLESTEROL RICH DIET-INDUCED HYPERCHOLESTEROLEMIA IN RATS

MOHAMMED AL-DOSARI[1], SALEH ALQASOUMI[1], MAJID AHMAD[2], MOHAMMED AL-YAHYA[1], M. NAZAM ANSARI[2], SYED RAFATULLAH[1*]

[1]*Department of Pharmacognosy and Medicinal, Aromatic & Poisonous Plants Research Center (MAPPRC), College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia*
[2]*Department of Pharmacology, College of Pharmacy, Alkharj University, Alkharj, Saudi Arabia*
[*] *corresponding author: srafat@ksu.edu.sa*

Abstract

The lyophilized aqueous extract of *Beta vulgaris* L. (beet root) (BVE) was investigated for its possible antihypercholesterolemic and antioxidant potential in cholesterol rich diet-induced hypercholesterolemia in Wistar albino rats. Hypercholesterolemia was induced in rats by feeding 1% cholesterol rich diet for 10 weeks. Lipid profile and glucose were estimated in serum. Malondialdehyde (MDA) and non-protein sulfhydryls (NP-SH) levels were measured in liver and heart. Hypercholesterolemic rats showed a significant increase in total cholesterol and triglycerides and a significant decrease in high-density lipoprotein-cholesterol (HDL-C) levels. BVE at the doses of 250 and 500 mg/kg body weight for 70 consecutive days showed a significant decrease in total cholesterol and triglycerides and significant increase in HDL-C. Furthermore, hypercholesterolemic rats showed free radical generation (lipid peroxidation), evident by a significant increase in MDA level and a significant reduction in NP-SH content in both liver and heart homogenates. BVE treatment significantly decreased MDA level and significantly replenished the reduced NP-SH content in both liver and heart tissue. The acute toxicity test of BVE showed no mortality or morbidity in rats. The findings indicate that BVE has a significant antihypercholesterolemic and antioxidant potential and/or free radical scavenging properties in hypercholesterolemic, rats possibly exerted by the phytoconstituents present in the beet root.

Rezumat

Studiul experimental evaluează acţiunea antihipercolesterolemiantă şi antioxidantă a extractului apos liofilizat al rădăcinii plantei *Beta vulgaris* (*Chenopodiaceae*). Studiul a fost realizat pe şobolani albi de laborator, cărora li s-a indus experimental hipercolesterolemia. A fost evaluat profilul lipidic şi glucidic al animalelor, concentraţia serică a malonildialdehidei. De asemenea, au fost evaluate (în ţesutul hepatic şi cardiac) grupările sulfhidril non-proteice. Rezultatele obţinute indică proprietăţile antihipercolesterolemiante şi antioxidante, datorate fitoconstituenţilor prezenţi în rădăcina plantei studiate.

Keywords: *Beta vulgaris,* beet root, hypercholesterolemia, lipid profile, antioxidant

Introduction

Vegetables are edible plants or part of the plants and they may be aromatic, bitter or tasteless. The nutrients content of different types of vegetables vary considerably and they do not represent a major source of carbohydrates compared to starchy foods which form the bulk of food eaten, but contain vitamins, essential amino acids as well as minerals and antioxidants.

Recent findings indicated that some of the vegetables and herbs, in addition to their lipid-lowering ability, can also reduce the production of reactive oxygen species (ROS) and increase the resistance of plasma lipoprotein to oxidation that may contribute to their effectiveness in preventing atherosclerotic disease [18,21,26]. Hypercholesterolemia is a well known risk factor in the development of atherosclerosis and subsequent coronary heart disease (CHD). Cardiovascular diseases represent the primary cause of mortality in the United States, Europe and most parts of Asia [2,17]. There are strong evidences that hypercholesterolemia increases the production of ROS [10,24], which may play an important role in the pathogenesis and/or progression of cardiovascular diseases [8,35].

Beta vulgaris L. (*Chenopodiaceae*), popularly known as Beet root, a native of the coasts of Mediterranean, is extensively cultivated in Europe, America and many parts of Asia. It has been used for centuries as a traditional natural coloring agent in many cuisines. Medicinally, the roots and leaves of the beet have been employed as a folk remedy to treat a wide variety of ailments including immune system stimulation, liver and kidney diseases. It is also employed as a special diet in the treatment of cancer [5]. The seeds are cooling and diaphoretic and the root is a nutrient [6]. In a preliminary study, aqueous and ethanolic extracts of *Beta vulgaris* have been reported to possess free radical-scavenging activity, reducing the radical cations and phase II enzyme-inducing activities in murine hepatoma cell *in vitro* [23]. Beet root extract has also been reported to be one of the useful means to prevent lung and skin cancers [16]. Furthermore, it was reported that the phenolic amides isolated from the seeds of *Beta vulgaris* produce the inhibitory effect on lipopolysaccharide-induced nitric oxide production in experimental isolated tissues in a dose dependent manner [32].

The present study was designed to assess whether the BVE could exert any protective action against cholesterol rich diet-induced hypercholesterolemia in rats, in order to substantiate the claims of its folkloric use to reduce cholesterol level.

Materials and Methods

Plant Material and Preparation of Dosage Form

The fresh roots of *Beta vulgaris* used in this study were purchased from the local vegetable market of Riyadh, and identified by an experienced taxonomist. A voucher (#210309) specimen was deposited in the Medicinal, Aromatic and Poisonous Plants Research Center of the College of Pharmacy from King Saud University, for future reference.

The roots of *Beta vulgaris* were processed in order to obtain juice using an electric blender. After obtaining the juice, it was lyophilized to get the dry powder using Freeze Dry System (LABCONCO, England). The freeze-dried powder was then dissolved in distilled water and used in all experiments.

Phytochemical Screening

A preliminary phytochemical analysis of the Beet root was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, coumarins, sterols and/or triterpenes [9].

Acute Toxicity Test

The acute toxicity of the BVE was evaluated in mice using the up and down procedure [30]. Six female rats (weight: 200-250g) received BVE starting at 2g/kg b.w. orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were further maintained for 13 days under daily observations.

Animals and diet

Healthy male adult Wistar albino rats, weighing between 150–200 g, obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh, were used. They were housed in polyethylene cages in groups of six rats per cage and were kept at a constant temperature (22±2°C), humidity (55%) and 12 h light-dark conditions for 7 days. The animals were provided with Purina chow rat diet and free access to drinking water. The experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Cholesterol supplemented feed

In crushed pellet diet, cholesterol (1%w/w) powder was thoroughly mixed; the pellets were reconstituted with water and dried properly to avoid any fungal contamination.

Experimental design

A systematic study was performed on the adult male rats divided in five groups. Each group comprised 6 animals.

Group 1: Control rats fed with normal pellet diet.
Group 2: Rats fed with cholesterol mixed pellet diet.
Group 3: Rats fed with cholesterol mixed pellet diet plus BVE (250 mg/kg b.w. p.o./day).
Group 4: Rats fed with cholesterol mixed pellet diet plus BVE (500 mg/kg b.w. p.o./day).
Group 5: Rats fed with normal pellet diet along with BVE (500 mg/kg b.w., p.o./day).

Biochemical determinations

Estimation of the lipid profile and glucose

Blood samples were collected from overnight fasted rats. Serum total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-C) levels were determined by commercially available spectrophotometric assay kits (Crescent Diagnostics, Jeddah, Saudi Arabia). The serum glucose was estimated using Reflotron® Plus Analyzer and Roche kits.

Determination of malondialdehyde (MDA)

The method reported by Utley *et al* [31] was followed. The heart and liver tissues were removed and each tissue was homogenized in 0.15 M KCl (at 4°C; Potter-Elvehjem type C homogenizer) to give a 10% w/v homogenate. Aliquots of homogenate (1 mL) were incubated at 37°C for 3 h in a metabolic shaker. Then 1 mL of 10% aqueous trichloroacetic acid was added and mixed. The mixture was then centrifuged at 800 g for 10 min. One mL of the supernatant was removed and mixed with 1 mL of 0.67% thiobarbituric acid in water and placed in a boiling water bath for 10 min. The mixture was cooled and diluted with 1 mL distilled water. The absorbance of the solution was then read at 535 nm. The content of malondialdehyde (nmoles/g wet tissue) was then calculated, by reference to a standard curve of malondialdehyde solution.

Estimation of non-protein sulfhydryls (NP-SH)

Non-protein sulfhydryls were measured according to the method of Sedlak and Lindsay [29]. The heart and liver tissues were homogenized in ice-cold 0.02 mmol/L ethylene diaminetetraacetic acid (EDTA). Aliquots of 5 mL of the homogenates were mixed in 15 mL test tubes with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 3000 rpm. Two milliliters of supernatant were mixed with 4 mL of 0.4 mol/L Tris buffer (pH 8.9). 0.1 mL of 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was measured within 5 min after the addition of DTNB at 412 nm against a reagent blank.

Statistical Analysis

Values are given as arithmetic means ± standard error of the mean (S.E.M.). Data was statistically analyzed by using One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Results and Discussion

Phytochemical Screening

The preliminary qualitative phytochemical screening of the root of *Beta vulgaris* revealed the presence of flavonoids, saponins, sterols and/or triterpenes.

Acute toxicity test

The extract at the dose of 2 g/kg b.w. was found to be safe, as the mice did not show any symptoms of toxicity and mortality during a period of 14 days of observation.

Effect of BVE on serum lipid profile:

Rats fed with cholesterol rich diet developed hypercholesterolemia and hyperlipidemia significantly by increasing total cholesterol, triglycerides levels, and a significant decrease in HDL-C levels as compared with control rats. Treatment with BVE (250 and 500 mg/kg b.w.) along with the cholesterol rich diet showed a significant decrease in total cholesterol, triglycerides, and a significant increase in the level of HDL-C compared with hypercholesterolemic group. The results are presented in table I. However, rats treated with BVE (500 mg/kg b.w.) alone and maintained on

normal diet (*per se*) did not show any change in serum lipid profile compared with the control group.

Table I.

Effect of BVE on serum lipid profile and glucose

Groups	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)
Group 1: Control	72.53 ± 5.61	86.06 ± 2.92	68.33 ± 2.47	37.77 ± 0.36
Group 2: 1% Cholesterol only	87.67 ± 6.92	174.55 ± 3.87 [a]	163.33 ± 2.79 [a]	12.79 ± 0.67 [a]
Group 3: BVE (250 mg/kg b.w.) + 1% Cholesterol	76.02 ± 4.67	129.09 ± 5.53*	122.50 ± 2.50*	20.41 ± 0.44*
Group 4: BVE (500 mg/kg b.w.) + 1% Cholesterol	70.38 ± 2.98	127.27 ± 11.42*	113.33 ± 5.80*	21.42 ± 0.34*
Group 5: BVE (500 mg/kg b.w.)	69.32 ± 3.63	80.60 ± 4.14	64.17 ± 2.63	38.99 ± 0.53

All values represent mean±SEM.

[a] $p < 0.05$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with normal control group.

* $p < 0.05$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with group fed with 1% cholesterol only.

Effect of BVE on serum glucose:

In table I, rats fed with cholesterol rich diet did not show any significant change in serum glucose levels as compared to control rats. Also, no significant changes were recorded in serum glucose levels in treatment groups (BVE 250 and 500 mg/kg b.w.) and normal diet group (*per se*) compared with hypercholesterolemic group.

Effect of BVE on lipid peroxidation in liver and heart tissue:

Rats fed with cholesterol rich diet showed a significant increase ($p<0.05$) in liver and heart MDA level compared to control rats.

Treatment with BVE at both doses (250 and 500 mg/kg b.w.) along with the cholesterol feeding showed a significant ($p<0.05$) decrease in malondialdehyde level. However, rats treated with BVE (500 mg/kg b.w.) and maintained on normal diet (*per se*) did not show any change in liver and heart MDA level compared with the control group (Table II).

Effect of BVE on NP-SH levels in liver and heart tissue:

Rats fed with cholesterol rich diet showed a significant decrease ($p<0.05$) in liver and heart NP-SH content compared to control rats.

Treatment with BVE (250 and 500 mg/kg b.w.) along with the cholesterol feeding showed a significant increase in liver and heart NP-SH content compared with hypercholesterolemic rats (Table III). However, rats treated with BVE (500 mg/kg b.w.) and maintained on normal diet (*per se*) did not show any change in liver and heart NP-SH levels as compared with the control group.

Table II

Effect of BVE administration on MDA levels in liver and heart homogenates

Groups	MDA (nmols MDA/g wet tissue)	
	Liver	Heart
Control	1.87 ± 0.067	1.63 ± 0.043
1% Cholesterol only	5.71 ± 0.48 [a]	5.41 ± 0.63 [a]
BVE (250 mg/kg b.w.) + 1% Cholesterol	2.95 ± 0.21*	2.80 ± 0.17*
BVE (500 mg/kg b.w.) + 1% Cholesterol	2.72 ± 0.059*	2.52 ± 0.05*
BVE (500 mg/kg b.w.)	1.88 ± 0.094	1.72 ± 0.05

All values represent mean±SEM.

[a] p <0.05 (ANOVA, followed by Dunnett's multiple comparison test) as compared with normal control group.

* p <0.05 (ANOVA, followed by Dunnett's multiple comparison test) as compared with group fed with 1% cholesterol only.

Table III

Effect of BVE administration on NP-SH levels in liver and heart homogenates

Groups	NP-SH (nmols/g wet tissue)	
	Liver	Heart
Control	10.86 ± 0.26	9.38 ± 0.33
1% Cholesterol only	3.75 ± 0.18 [a]	4.44 ± 0.18 [a]
BVE (250 mg/kg b.w.) + 1% Cholesterol	8.11 ± 0.18*	7.58 ± 0.28*
BVE (500 mg/kg b.w.) + 1% Cholesterol	8.58 ± 0.30*	8.07 ± 0.25*
BVE (500 mg/kg b.w.)	9.68 ± 0.50	9.69 ± 0.61

All values represent mean±SEM.

[a] p <0.05 (ANOVA, followed by Dunnett's multiple comparison test) as compared with normal control group.

* p <0.05 (ANOVA, followed by Dunnett's multiple comparison test) as compared with group fed with 1% cholesterol only.

Conclusions

The present study examined the possible antihypercholesterolemic, antihyperlipidemic and antioxidant potential of lyophilized aqueous *Beta vulgaris* extract (250 and 500 mg/kg b.w., p.o.) in cholesterol rich diet-induced hypercholesterolemia in rats. Rats fed with the diet rich in

cholesterol resulted in an increase of total cholesterol and triglycerides in serum and decreased circulating HDL-C in rats, besides an increase in malondialdehyde and decreased non-protein sulfhydryl content in liver and heart. These results are in agreement with earlier studies [1,4], and provide an experimental model for dietary hyperlipedemia [12].

Higher plasma LDL-C level is related with greater deposition of cholesterol in artery and aorta thereby increasing risk for coronary artery disease[25], whereas low HDL-C is the prevalent lipoprotein abnormality reported [13,14]. In the current investigation BVE treatment decreased the levels of total cholesterol and triglycerides and increased the levels of HDL-C suggesting a cardioprotective and lipid lowering potential of *Beta vulgaris*. This lipid lowering potential of beet root may be due to flavanoids and/or saponins which were found to be the main constituents of BVE in our preliminary phytochemical screening. These findings are in accordance with the earlier studies demonstrating the effect of flavonoids on cholesterol metabolism [3, 15]. It has also been reported that saponins from some medicinal plants reduced the triglycerides and cholesterol levels in rats [15]. Also, flavonoids are considered as active principles in many medicinal plants [34] and natural products with positive effect on human health [7] and saponins content has been also suggested to reduce heart diseases [20].

Some of the earlier studies have indicated that hypercholesterolemia induces oxidative stress by causing a reduction in the enzymatic antioxidant defense potential of tissues and generation of oxygen free radical like superoxide anions leading to the development of cardiovascular and neurodegenerative diseases [10,22,24,28]. High-cholesterol diet provides a relevant example of endogenous chronic oxidative stress due to the resulting hypercholesterolemia. In hypercholesterolemic diet, liver, the primary organ that metabolises the cholesterol ingested in excess, is affected by oxidative stress. It results from an imbalance between the production of free radicals and the effectiveness of antioxidant defense system [19]. The present study confirms the efficiency of cholesterol-enriched diet to produce a state of oxidative stress with biochemical and biological characteristics of hypercholesterolemia.

In the current investigation, elevated MDA levels were decreased and reduced NP-SH levels were replenished significantly in liver and heart by the treatment with BVE, thereby, enhancing the endogenous hepatic and myocardial antioxidant levels. These findings are in accordance with the earlier studies suggesting the antioxidant potential of *Beta vulgaris* [23,32].

The recorded increment in HDL-C, increased antioxidant activity and reduced lipid accumulation in hypercholesterolemic animals suggest the

usefulness of BVE in the treatment of hyperlipidemia. Also, synthetic hypercholesterolemic drugs lower both the total cholesterol and HDL-C, simultaneously [33]; thus BVE could prove to be a more effective therapy due to its ability to significantly increase HDL-C while lowering total cholesterol. The present study provides a preliminary scientific basis for hypolipidemic effects of *Beta vulgaris*, a plant that has been extensively used ina folkloric medicine. Further studies are however required to reveal the possible molecular mechanism(s) of action.

Acknowledgements

Authors are thankful to Mr. Malik Sawood Ahmed for his technical assistance.

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Manuscript received: April 4th 2010