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Antihypercholesterolemic Effects of Beet (*Beta vulgaris* L.) Root Waste Extract on Hypercholesterolemic Rats and its Antioxidant Potential Properties

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Abstract: The methanolic extract of beet (*Beta vulgaris* L.) root waste was investigated for its possible antihypercholesterolemic in cholesterol rich diet-induced hypercholesterolemia in male adult albino rats and its antioxidant potential. Different *in vitro* assays used for determining antioxidant potential of extracts of pulp wastes were: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and total antioxidant capacity. Thirty five adult male albino rats were randomly divided into two main groups, the first main group (control negative: 7 rats each) fed on basal diet only, while the second main group (28 rat) fed on hypercholesterolemic diet (basal diet, 1% cholesterol, 0.25% bile salt and 15% beef tallow) for 1 month to raise the lipid profile level. After that, these hypercholesterolemic rats divided into four subgroups as follow: the first subgroup (control positive) fed on hypercholesterolemic diet only, while the other three subgroups fed on hypercholesterolemic diet and administered orally with beet root waste extract (BRWE) at a dose of 200, 400 and 600 mg/kg/day, respectively. Lipid profile was measured in serum of rats. The results indicated that, administration with extract of beet root waste at the doses of 200, 400 and 600 mg/kg b.w for 30 day showed a significant ($p<0.05$) decrease in total cholesterol, triglycerides, LDL-c and caused also a significant ($p<0.05$) increase in HDL-c level. Total phenols were analyzed in the extract of BRWE and the results showed a high content of total phenols and antioxidant capacity. The DPPH scavenging activity IC50 values of the methanolic extracts were (253 µg/mL).These findings indicate that the extract 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 waste. So, these results pave the way for utilization of bio-wastes from the food industry.

Key words: Beta vulgaris, beet root Pulp, hypercholesterolemia, lipid profile, antioxidant capacity, DPPH, rats

INTRODUCTION

Residue from the processing of fruits and vegetables, traditionally considered as an environmental problem, are being increasingly recognized as sources for obtaining high phenolic products. The polyphenolics from waste materials, being derived from agro-industrial production, may be used as functional food ingredients and as natural antioxidants (Zhou *et al*., 2009). Nowadays, attention has been addressed towards byproducts or wastes from large scale industrial operations and agricultural waste materials based on their availability, high efficiency, easy handling and low cost (Sud *et al*., 2008).

Sugar-beet (*Beta vulgaris* L.) pulp, the remaining byproduct of beet juices and sugars extracted from the root, is widely used in animal feed as a source of dietary, it is also used as a biosorption matrix.

Hypercholesterolemia is a well known risk factor in the development of atherosclerosis and subsequent coronary heart disease. Cardiovascular diseases represent the primary cause of mortality in the United States, Europe and most parts of Asia (Khoo *et al*., 2003). There are strong evidences that hypercholesterolemia increases the production of reactive oxygen species (Gokkusu and Mostafazadeh, 2003), which may play an important role in the pathogenesis and progression of cardiovascular diseases (Wu *et al*., 2002).

Evidence from the cohort studies supported the view that a sufficient intake of fruits and vegetables is inversely associated with the risk of chronic diseases (Dyun and Pivonka, 2000; Dauchet *et al*., 2006) and a number of possible mechanisms have been proposed with antioxidant nutrients through lowering oxidative stress. Therefore, much attention has been focused on natural antioxidants in fruits and vegetables (Ames *et al*., 1995). The effect of diet supplemented with 5% and 15% cellulose or with 15% fiber isolated from red beet (Beta vulgaris var. rubra) on the development of hypercholesterolemia and chemically induced colon carcinoma was studied by (Bobek *et al*., 2000) in male Wistar rats. Fibrous matter isolated from red beet contained 89% fiber, in which 9% was in water soluble form. Red beet fiber diet (and not the increased cellulose intake) caused a reduction of serum cholesterol and triacylglycerol levels (by 30 and 40%,

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respectively) and a significant increase in the fraction of cholesterol carried in HDL. Also the results demonstrated an increase in the activities of superoxide dismutase and catalase in erythrocytes and in colon and activities of glutathione peroxidase and glutathione-S-transferase in liver.

Shyamala and Jamuna (2010) studied the chemical composition and antioxidant potential of pulp waste from carrot (Daucuscarota) and beetroot (*Beta vulgaris*). The results indicated that protein content was high in beetroot (13.23 mg/100 g) and low in carrot (6.21 mg/100 g). Total polyphenols were higher in methanol extracts of samples (220-250 mg/100 g) compared to ethanol and aqueous extracts. The antioxidant activity determined by the DPPH method exhibited 40% and 78% activity in methanol extracts of carrot and beetroot pulp waste (20 mg), respectively.

A considerable amount of vegetable pulp is left after filtration of juice or sugar production and is available as a byproduct of this beverage industry. There are very few systematic studies carried out on possible utilization of such waste material. So that, the study was undertaken with the objective of analyzing the waste of beetroot extract for its potential antioxidant activity. Also, investigate the hypercholesterolemic effect of the waste of beet root extract on rats fed on hypercholesterolemic diet.

MATERIALS AND METHODS

Materials: Red beet root (*Beta vulgaris* L.) was obtained from the local market.

Rats: Thirty five adult male albino rats of Sprague Dawley strain each weighing 200±5 g b.wt. were used in the study. The rats were obtained from the Laboratory Animal Colony, Helwan, Egypt. The animals were housed under hygienic conditions in plastic cages which contain wood shavings. The animals were kept at a room temperature of 25±2°C with relative humidity of 50-60% and on 12h light/12 h dark cycle. The rats were provided free access of basal diet and water. The rats were allowed to acclimatize to the laboratory environment for 7 days before start of the experiment.

Kits for biochemical analysis were purchased from Sigma Company, Cairo, Egypt.

Methods: Preparation of extract from beetroot waste: Beetroot (*Beta vulgaris* L.) about 1kg was obtained from the local market, cleaned, peeled and processed for obtaining juice using an electric blender. The pulp left after juice extraction was 366 g. A portion (5 g) of red beetroot-byproducts was homogenized with 70% (v/v) aqueous methanol at 10000 rpm for 4 min with a model AM-3 Ace homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan). The homogenate was centrifuged at 400 x g for 15 min and the supernatant was collected. The obtained precipitate was homogenize again in 40 mL of 70% (v/v) aqueous methanol and centrifuged. The combined supernatant was evaporated using rotary evaporator (Laborata 4001, Heidolph WB, Germany, serial no. 069800367) at 40°C. The methanol-free residue was 9.36 g obtained from 1 kg red beet root.

Determination of total phenols: Total phenols (TP) were determined colorimetrically using Foline Ciocalteu reagent (Pasko *et al*., 2009). The standard curve was prepared with gallic acid. Final results were given as gallic acid equivalents (GAE).

Evaluation of total antioxidant capacity: An amount of 0.1 mL of sample solution containing methanol extract was combined in an Eppendorf tube with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube was capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the solvent solution was measured at 695 nm against a blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. The antioxidant capacity was expressed as equivalents of ascorbic acid (Prieto *et al*., 1999).

Determination of DPPH radical scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH, scavenging activity) was carried out according to the method described by Mensor *et al*. (2001). Sample stock solutions (1.0 mg/mL) were diluted to different concentrations of 450, 300 and 150 µg/mL, in ethanol. One mL of a 0.3 m MDPPH solution was added to 2.5 mL solution of plant extract at different concentrations and standard. After 20 min incubation period at room temperature in the dark, the absorbance of the resulting mixture was measured at 515 nm. The percentage Antioxidant Activity (AA%) was calculated using the expression below:

$$AA(\%) = \frac{A\ control - A\ sample}{A\ control} \times 100$$

where, A is the absorbance at 515 nm. DPPH solution (1.0 mL, 0.3 mM) plus ethanol (2.5 mL) was used as a negative control. The EC50 values were calculated by linear regression of plots where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of antioxidant activity from three separate tests.

Experimental animal: The basal diet (g/kg diet) consisted of: 140 g casein (≥85% protein), 100 g sucrose, 47 g corn oil, 50 g cellulose, 35 g mineral

mixture, 10 g vitamin mixture, 1.8 g L-cystine, 2.5 g choline bitartrate and the remainder is corn starch up to 100%. Diets were formulated according to (Reeves, *et al*., 1993). After the adaptation period, thirty five rat were randomly divided into two main groups, the first main group (control negative: 7 rats each) fed on basal diet only, while the second main group (28 rat) fed on hypercholesterolemic diet (basal diet, 1% cholesterol, 0.25% bile salt and 15% beef tallow according to (Zulet *et al*., 1999) for 1 month to raise the lipid profile level. After that, these hypercholesterolemic rats divided into four subgroups as follow: the first subgroup (control positive) fed on hypercholesterolemic diet only, the second subgroup fed on hypercholesterolemic diet and administered orally with BRWE at a dose of 200 mg/kg/day, the third subgroup fed on hypercholesterolemic diet and administered orally with BRWE at a dose of 400 mg/kg/day and the fourth subgroup fed on hypercholesterolemic diet and administered orally with BRWE at a dose of 600 mg/kg/day. At the end of the experimental period (4 weeks)rats were fasted over night before sacrificing, blood was collected then centrifuged. Serum was separated and stored at -20°C until analysis.

Estimation of the lipid profile: Blood samples were collected from overnight fasted rats. Serum total cholesterol (TC) (Richmond, 1973), triglycerides (TG) (Wahlefeld, 1974), high density lipoprotein (HDL-c) (Albers *et al*., 1983), were analyzed according to the reported methods. Meanwhile, low density lipoprotein (LDL-c) was calculated according to (Fridewald *et al*., 1972) using the following equation:

$$LDL\text{-}c = TC - (HDL\text{-}c + VLDL\text{-}c)$$

where, Also very low density lipoprotein (VLDL-c) was estimated according to the equation of (Fridewald *et al*., 1972), as follow:

$$VLDL\text{-}c = \frac{TG}{5}$$

Statistical analysis: Results were expressed as mean±SE. All data from the experiment were examined statistically by one-way analysis of variance with computerized SPSS package program (SPSS 16.00 software for Windows) by ANOVA test to test the variations among groups and Post Hoc test (Duncan's test) was used to compare group means. A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The data in Table 1 illustrated that, the total phenolic content in the beet root waste extract was 218.7 mg/g GAE. This finding is in the line with (Shyamala and Jamuna, 2010) who found that, the polyphenols content of methanolic extract beetroot pulp was (220 mg/100 g). Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants (or) plant products. Coronary artery disease patients had lower serum levels of TAC, retinol, HDL-c, albumin and total protein. The authors' observed an inverse association between TAC and the number of damaged vessels (Nojiri *et al*., 2001). Confirming previous data from patients submitted to coronary angioplasty which had presented lower TAC and higher lipid peroxidation plasma levels (Buffon *et al*., 2000).

The role of antioxidant nutrients in fighting against oxidative stress is well-established in a great number of diseases including cancer, cardiovascular and neurological pathologies (Ferrari and Torres, 2003; Ferrari, 2004). Researchers had materially proved that following consumption of diets rich in fruits and vegetables caused an increase in serum TAC (Cao *et al*., 1998; Tyssandier *et al*., 2004). In our study as shown in Table 1 the beet root waste extract was higher in TAC 14.928 m mole ascorbic acid/g. this agree with (Shyamala and Jamuna, 2010) who mentioned that, the antioxidant activity of the methanolic extract of beet pulp was 15.784m mole ascorbic acid/g.

However, depending on the previous studies, it could be say that, consumption of food supplemented with red beet root waste extract potentially increased total antioxidant capacity. In a similar concern, adherence to Mediterranean dietary practices was positively associated with TAC levels and negatively associated with oxidation of the atherogenic LDL-c (Pitsavos *et al*., 2005). These higher TAC values of foods in Mediterranean diet could in part explain why adherence to this diet reduces mortality risk (Trichopoulou *et al*., 2005). Red bet root waste extracts (rich in polyphenolics),with have higher TAC, were able to suppress LDL-c, TG and Coronary heart diseases.

In our study, the antioxidant activities of red beet root pulp extracts was evaluated using $DPPH^{\cdot}$ free radical-scavenging assays. This method is recommended by many authors (Sanchez-Moreno *et al*., 1998; Shahidi *et al*., 2006; Klimczak *et al*., 2007; Villanio *et al*., 2007) as easy and accurate assays for measuring the antioxidant activity of red beet root pulp waste extracts. The effect of antioxidants on DPPH radicals scavenging was thought to result from their hydrogen donating ability. $DPPH^{\cdot}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. It is visually noticeable as a discoloration from purple to yellow. Figure 1 shows the dose response curve for the radical

Table 1: Total phenols and total antioxidant capacity of the beet root waste extract

Parameters	Beet root waste extract
Total phenols	218.7 (mg gallic acid equivalent/100 g)
Total antioxidant capacity (TAC)	14.928 (m mole ascorbic acid/g)

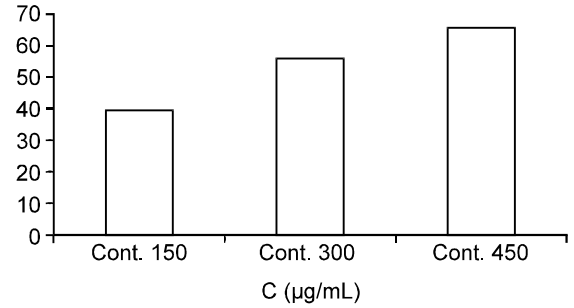

Fig. 1: Antioxidant activity (AA%) of different concentrations of methanol beet root waste extracts on DPPH radicals

scavenging effect of the methanol red beet root pulp extracts. The scavenging of DPPH radicals increased with increasing extract concentration.

EC50 is inversely related to the antioxidant capacity of a compound, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower EC50 value the higher antioxidant activity of a compound (Villanio *et al*., 2007). The EC50 value of red beet root pulp waste extracts (253 µg/mL) Beet root waste extracts has a higher antioxidant activity contained higher concentration of phenolic compounds.

The results presented in Table (2 and 3) showed the effect of beet root waste extract on serum lipid profile in hypercholesterolemic rats. Rats fed with cholesterol rich diet developed hypercholesterolemia and hyperlipidemia significantly ($p<0.05$) by increasing total cholesterol, triglycerides, VLDL-c, LDL-c levels and caused a significant ($p<0.05$) decrease in HDL-c levels as compared with negative control group. Treatment with beet root waste extract at three tested levels (200, 400 and 600 mg/kg b.w.) along with the cholesterol rich diet showed a significant ($p<0.05$) decrease in total cholesterol and triglycerides and caused a significant ($p<0.05$) increase in the level of HDL-c as compared with positive control group. Fortunately, rats treated with beet root waste extract at a dose of (600 mg/kg b.w.) have higher HDL-c and lower TG level as compared with group fed on normal di*et al*one.

The present study examined the anti-hypercholesterolemic possibility of methanol beet root waste extracts at a dose of (200, 400 and 500 mg/kg b.w.) 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

Table 2: Effect of beet root waste extraction on serum TC and TG in hypercholesterolemic rats

	TC	TG
Parameters groups	---------- mg/dL ----------	
Control (-)	107.52±1.70^{e}	79.53±3.79^{bc}
Control (+)	211.17±1.84^{a}	125.22±2.38^{a}
200 mg/kg/day	198.33±3.79^{b}	88.00±2.58^{b}
400 mg/kg/day	178.75±2.68^{c}	82.75±2.62^{bc}
600 mg/kg/day	165.00±3.00^{d}	75.50±2.50^{c}

*Values were expressed as Means±SE. Values at the same column with different litters are significant at $p<0.05$

Table 3: Effect of beet root waste extraction on serum lipioproteins in hypercholesterolemic rats

	HDL-c	VLDL-c	LDL-c	LDL/HDL
Groups	---------- mg/dL ----------			
Control (-)	48.62±1.48^{e}	15.90±0.75^{bc}	42.97±2.79^{e}	0.88±0.08^{d}
Control (+)	34.75±2.05^{d}	25.00±0.47^{a}	151.75±3.16^{a}	4.42±0.32^{a}
Beet (1)	58.25±2.13^{b}	16.60±0.51^{b}	122.65±4.21^{b}	2.11±0.14^{b}
Beet (2)	64.75±2.05^{a}	16.55±0.52^{bc}	97.45±4.11^{c}	1.51±0.10^{c}
Beet (3)	69.50±0.95^{a}	15.10±0.47^{c}	80.40±3.34^{d}	1.15±0.05^{cd}

*Values were expressed as means±SE. Values at the same column with different litters are significant at $p<0.05$

HDL-C in rats. Our results are in agreement with earlier studies of (Ashraf *et al*., 2005; AL-Dosari *et al*., 2011) and provide an experimental model for dietary hyperlipedemia (Grundy and Denke, 1991). In addition, Sheyla *et al*. (2005) showed that high fat content diet, which used to induce hypercholesterolemia, leads to lower ingestion by the animals and induces malnutrition. Clinical studies indicated that hypercholesterolemia is an essential risk factor for coronary artery disease, where LDL-c plays a major role in the athetosis and pathogenesis of vascular diseases (Trubelja *et al*., 2005). Gupta *et al*. (1994, 1995) reported that higher LDL-c level is related with greater deposition of cholesterol in artery and aorta thereby increasing risk for coronary artery disease, whereas low HDL-c is the prevalent lipoprotein abnormality. In the current investigation treatment with BRWE significantly decreased the levels of TC and TG and increased the levels of HDL-c suggesting a cardioprotective and lipid lowering potential of BRWE. This lipid lowering potential of beet root may be due to phenolic content, total antioxidant capacity and free radical-scavenging which were found to BRWE in our screening. These findings are in accordance with the earlier studies demonstrating the effect of beta vulgaris on hypercholesterolemia (AL-Dosari *et al*., 2011). Also, red beet roots have higher content of flavonoids and/or saponins which have ability to effect on cholesterol metabolism (Hostettman and Marston, 1995).

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 TC and HDL-c, simultaneously (Wilson, 1990), thus BRWE could

prove to be a more effective therapy due to its ability to significantly increase HDL-c while lowering total cholesterol.

Conclusion: The present study provides a preliminary scientific basis for hypolipidemic effects of beetroot pulp waste that has been extensively used in a folkloric nutrition therapy. Further studies are however required to reveal the possible supplemented the beetroot waste extract in the food product and pave the way for utilization of bio-wastes from the food industry.

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