STUDIES CONCERNING ANTIOXIDANT AND HYPOGLYCAEMIC ACTIVITY OF *ARONIA MELANOCARPA* FRUITS

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Abstract

The juice from *Aronia melanocarpa* berries is a source of phenolic compounds with antioxidant properties: procyanidins, anthocyanins, (–)-epicatechin, chlorogenic acid, neochlorogenic acid. The aim of our work was to investigate *in vivo* the antioxidant action of *A. melanocarpa* on healthy rats that were fed on normal diet supplemented with berries juice for six weeks. Cholesterol, serum triglycerides, total proteins and urea nitrogen were determined. The total antioxidant capacity, thiobarbituric acid - reactive substances, total thiol groups and glutathione (TSH and GSH), nitric oxide production, protein carbonyls, catalase and ceruloplasmin were also assayed in serum drawn from the animals. Our data indicated that the *A. melanocarpa* berry juice intake correlated with a decrease in the oxidative stress markers such as total antioxidant capacity, total thiol groups and glutathione. The usual biochemical parameters, as well as the enzymes catalase and ceruloplasmin were unaffected by the treatment.

Alloxan-induced diabetic rats treated with juice from *A. melanocarpa* berries exhibited a significant decrease of glycaemia levels (42.83% for alloxan-induced diabetic rats and 6.85% for healthy rats).

Rezumat

Sucul obţinut din fructele de *Aronia melanocarpa* reprezintă o sursă de compuşi fenolici cu proprietăţi antioxidante: proantociani, antociani, (–)-epicatechină, acid clorogenic, acid neoclorogenic. Scopul studiului a fost să evalueze acţiunea antioxidantă *in vivo* a plantei *A. melanocarpa* asupra şobolanilor sănătoşi care, timp de 10 zile, au primit o dietă normală suplimentată cu suc obţinut din fructele acestei plante. Au fost analizaţi câţiva parametri biochimici uzuali din serul animalelor: colesterol, trigliceride serice, proteine totale şi uree. Din serul animalelor au mai fost determinaţi următorii parametri:

capacitatea antioxidantă totală, indexul de peroxidare lipidică, tiolii totali şi neproteici (TSH şi GSH), concentraţia anionului nitrit, carbonilii proteici, activitatea catalazei şi concentraţia ceruloplasminei. Rezultatele obţinute au arătat că suplimentarea cu suc de *A. melanocarpa* a dietei normale s-a corelat cu o scădere a valorii unor markeri de stres oxidativ: capacitatea antioxidantă totală, tiolii totali şi neproteici. Parametrii biochimici, ceruloplasmina şi activitatea catalazei, n-au fost influenţaţi de suplimentarea alimentaţiei obişnuite cu suc de *Aronia melanocarpa*.

Şobolanii cu diabet indus cu aloxan trataţi cu suc de fructe de *A. melanocarpa* au prezentat o scădere considerabilă a glicemiei (42,83% pentru şobolanii cu diabet indus cu aloxan şi 6,85% pentru şobolanii sănătoşi).

Keywords: *Aronia melanocarpa*, antioxidant activity, hypoglycemic activity.

Introduction

Aronia melanocarpa (Michx) Elliott (chokeberry) belongs to the *Rosaceae* family and originated from the eastern parts of North America and East Canada. Its fruits are harvested between August and September [15]. The juice from *A. melanocarpa* berries is an important source of phenolic compounds with antioxidant properties: procyanidins, anthocyanins, (–)-epicatechin, chlorogenic acid, neochlorogenic acid [20]. The studies regarding the metabolism of the phenolic compounds highlighted their contribution to the health potential benefits and the chemopreventive effects of berry phytochemicals were recently reviewed [13, 15].

There are many studies which demonstrated the antioxidant activity of the chokeberry fruits *in vitro* [20] by various methods: the inhibition of methyl linoleate oxidation, oxygen radical absorbance capacity (ORAC), trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), as well as the scavenger activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [15].

The ability of the chokeberry juice to protect from *in vitro* oxidative damage neutrophils from obese and non-obese individuals was demonstrated by Zielinska-Przyjemska [32].

A series of works on the *A. melanocarpa* berries described their *in vivo* antioxidant activity, using various biomarkers: the ability of chokeberry anthocyanins to decrease lipid peroxidation [5] and enhance the activity of antioxidant enzymes [14], the antiradical capacity using DPPH [16], the erythrocyte superoxide dismutase activity when the pro-oxidative and hypercholesterolemia diet was supplemented with black chokeberry extract [31] and the thiobarbituric acid-reactive substances (TBARS) [29].

Recently, it was shown that chronic administration of chokeberry juice improves vascular function in men with mild hypercholesterolemia by increasing NO release from endothelial cells [22].

The oral administration, for six weeks, of chokeberry juice to diabetic rats (with streptozotocin-induced diabetes) produced a decrease of glycaemia [29]. The influence of *A. melanocarpa* Elliot on alloxan induced diabetes in rabbits was also studied [10].

Aronia juice had the ability to lower fasting glucose level in human patients with non-insulin dependent diabetes who received the juice daily over a period of three months. In the same study the *Aronia* juice showed a positive effect on HbA1c-glycated haemoglobin, total cholesterol and lipid levels [25].

There is evidence of the effectiveness of *Aronia* products in the treatment of many different pathological conditions (diabetes, cancer, dyslipidemia, hypertension, inflammation, microbial infections) which are characterized by uncontrolled oxidative processes [13].

The aim of our work was to investigate the *in vivo* antioxidant action of *A. melanocarpa* on healthy rats that were fed on normal diet supplemented with berry juice, and to evaluate the influence of berry juice on alloxan-induced diabetic rats.

Materials and Methods

Plant material and characterization

A. melanocarpa berries from the cultivar of the Pitesti-Maracineni Research Institute for Fruit Growing (Romania) were harvested in September 2009. Berry samples were triturated and homogenized with cooling mill and an ultrasonic bath and were subsequently filtered on sterile cotton wool. The sonication was performed using an ultrasonic cleaning bath (Bransonic 1510, operating at 40 kHz frequency). The filter cake was filtered on cellulose membrane (pore size 0.22μm). The quantification of ascorbic acid [17], total anthocyanins [27], carotene [4] and polyphenols [26] in the berry juice was performed as described in literature. The antioxidant activity of the chokeberry juice was determined by the 2,2'-azinobis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS) method [1].

Experimental design

Male Wistar albino rats weighing 200-220 g (two months old) were used. All the animals used in the study were kept in standard laboratory conditions and all the experiments were performed according to European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government dated February 2nd, 2002. The rats were fed on normal diet, supplemented or not with berry juice. The animals were housed in standard boxes with standard laboratory diet and water *ad libitum*.

The rats were divided into four groups, each group consisting in 10 animals. The treatment consisted in oral administration over a six weeks period, as it follows:

- the control group was untreated, receiving 10 mL water /kg body weight (group 1);
- a group was treated with *A. melanocarpa* juice administered daily in 10 mL/kg body weight (group 2);
- a group was treated with Alloxan, 130mg/kg body weight (group 3);
- a group was treated with Alloxan, 130mg/kg body weight, together with juice of *A. melanocarpa* administered daily in 10 mL/kg body weight (group 4).

At the end of the experiments, two hours after the last administration, the animals were anesthetised with chloroform and killed by cervical decapitation.

Blood was collected and serum was separated out [18] for analysis of the biochemical parameters and oxidative stress markers described below.

Biochemical parameters

Blood glucose concentration (BGC), serum lipids (SL), blood cholesterol (total cholesterol (TC), LDL-cholesterol and HDL-cholesterol), serum triglycerides (ST), blood urea nitrogen (BUN), proteins, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured using commercial kits [18].

Oxidative stress markers

Protein carbonyls and AOPP (advanced oxidation protein products) concentrations were determined, using previously described methods, with minor modifications [8]. Analysis of nitrite was performed using Green's method [6] and the lipid peroxidation index was determined by the thiobarbituric-reactive substances assay (TBARS) [11, 21].

Ceruloplasmin concentration (CPA) [23] and catalase activity [2, 30] were assessed as described, while the total thiol groups (TSH) and glutathione (GSH) were performed using Ellman reagent [33] and Janero method [9], respectively.

The method for total antioxidant capacity (TEAC) was previously described [24].

Statistical analysis

The analysis was performed in triplicate for each parameter; values represent the average values ± SEM. Data for the antioxidant response induced by *A. melanocarpa* berry juice on healthy rats were obtained in triplicate and were expressed as mean ± SEM and statistically assessed by Graph Pad Instat 3. Data for the hypoglycaemic effect were expressed as

mean ± SEM and statistically assessed by one-way analysis of variance (ANOVA). The difference between juice-treated animals and controls was evaluated by Student's t-test. The differences were considered to be statistically significant for $p < 0.05$.

Results and Discussion

The presence and the amount of the main antioxidants were determined in the *A. melanocarpa* berry juices that were used in this study. It was found that they consistently contained ascorbic acid, total polyphenols, carotenes and total anthocyanins (Table I). The total antioxidant capacity of *A. melanocarpa* berry juice was 30.24±0.014 μmol Trolox/L. The chokeberry juice obtained from the Romanian variety has plenty of antioxidant compounds and these data confirm the therapeutic values of the studied vegetable product [13, 15].

Table I

The composition of *A. melanocarpa* berry juice

No.	Compounds	Detected amount
1	Ascorbic acid	29 g ascorbic acid / L juice
2	Total anthocyanins (as cyanidol equivalent)	1.30 mg / mL juice
3	Carotene compounds (as β-caroten equivalent)	97.8 μg / L juice
4	Total polyphenols (as caffeic acid equivalent)	31.84 g / L juice

Most biochemical parameters (BGC, SL, TC, LDL-cholesterol, HDL-cholesterol, ST, BUN, AST, ALT) were not affected by the administration of chokeberry juice (data not shown). Similarly, some parameters from serum (ceruloplasmine, proteins carbonyl and nitrite) did not differ significantly from the control group (Table II).

Table II

The antioxidant response induced by *A. melanocarpa* berry juice

Parameters	Control group (1)	Treated group (2)
TEAC (mmol Trolox equivalent/L)	3.17±0.04	3.32±0.07
Total thiols (mmol/L)	1.67±0.13	2.46±0.31
Non-proteic thiols (mmol/L)	0.105±0.016	0.141±0.020
Ceruloplasmin (mmol/L)	0.36±0.01	0.38±0.01
Catalase (kU/min/mg proteins)	3.52±0.47	3.07±0.51
AOPP (μmol/L)	0.39±0.02	0.51±0.04
Protein carbonyls (nmol/mg proteins)	0.52±0.11	0.54±0.28
Nitrite (μmol/L)	6.42±0.36	6.62±0.21
TBARS (mmol/L)	15.97±0.52	17.14±0.44

The antioxidant status and biomarkers of healthy rats' serum for control group (group 1) and for group treated with *A. melanocarpa* juice (group 2) are presented in Table II.

In the present study, the antioxidant capacity of the serum was increased by the chokeberry juice in the treated group (healthy rats that were fed on normal diet supplemented with berries juice for six weeks, group 2) compared with the control group (1). These data go in line with recent studies [19].

The significant increases in the levels of serum total- and non-proteic thiols were observed (Table II). Our data about the thiols were confirmed by previous studies about the hepatoprotective activity of chokeberry juice or other plants poliphenols [12]. It has been shown that *Aronia* juice inhibited the decrease of GSH level in the liver of rats exposed to CCl_4 or sulfide-2-chloroethyl-3-chloropropyl (alkylating agent) [13].

The lipid peroxides assay (TBARS) gave unexpected results (17.14 mmol/L for group 2 and 15.97 mmol/L for group 1); in another study, the supplementation of rat diet with black chokeberry juice reduced TBARS levels in gastric mucosa and plasma (produced by indomethacin-induced increase of TBARS levels) [29].

AOPP increased in the serum of treated group (group 2) compared with control group (group 1); this was also unexpected, and there were no data concerning this parameter in rats fed on diet supplemented with black chokeberry juice.

The catalase activity was lower in rats' serum of healthy rats that were fed on normal diet supplemented with berries juice (group 2) than control (3.07±0.51 kU/min/mg proteins, 3.52±0.47 kU/min/mg proteins respectively).

There is little information concerning any toxic effects or unwanted pharmacological effects of *A. melanocarpa* berries, juice or extracts [15]. In addition, it is known that the plant phenols inhibit lipid peroxidation, but these compounds also produce pro-oxidant effects under certain circumstances *in vitro*. This activity could accelerate the oxidative damage by producing O_2^- and H_2O_2 or by reducing metal ions (or by binding ions like Fe^{3+} or Cu^{2+}) [7].

As expected, the glycaemia of alloxan-induced diabetic rats (group 3) and of control group (group 1) were 220.4mg/dL and 103.5 mg/dL respectively (Table III).

Table III

The hypoglycaemic effect of *A. melanocarpa* berry juice on alloxan-induced diabetic rats

No.	Groups	Treatment	Glycaemia (mg/dL)		
			X±SEM	Effect % (versus control group)	t-student *p*
1.	Control group	Distilled water 10mL/kg body weight	103.5±11.2	-	-
2.	Healthy rats treated with juice	10mL juice/kg body weight	96.4±8.7	-6.85	0.5683
3.	Alloxan-induced diabetic rats	Alloxan 130mg/kg body weight	220.4±23.5	+112.9	0.0231
4.	Alloxan-induced diabetic rats treated with juice	Alloxan 130mg/kg body weight + juice 10mL/kg body weight	136.8±14.6	- 42.83	0.0156
	ANOVA	$F = 12.8132$ $P = 1.2381\ E^{-8}$			

X±SEM = average ± SEM

Alloxan-induced diabetic rats which were fed with diets supplemented with *Aronia melanocarpa* berry extracts (group 4) showed an increase of blood glucose concentration (Figure 1) compared to the group 3 (without diets supplemented with *Aronia* extract). It is well-known that the polyphenolic fractions from plants can display insulin-like effects by reducing blood glucose levels after food intake [3].

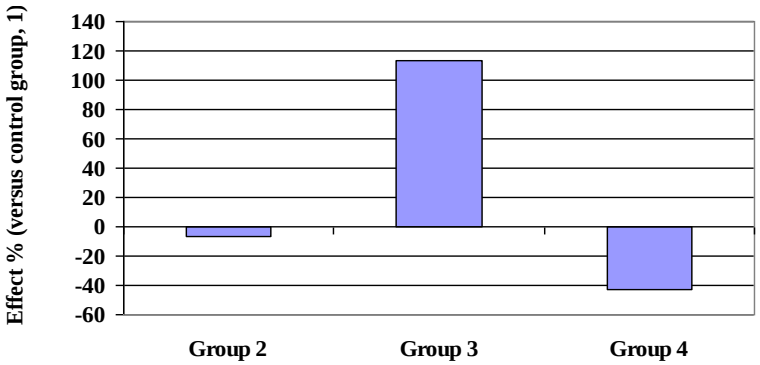

Figure 1.

The hypoglycaemic effect of *Aronia melanocarpa*. (group 1: control group, group 2: healthy rats treated with juice, group 3: alloxan-induced diabetic rats, group 4: alloxan-induced diabetic rats treated with juice)

The antidiabetic activity of *A. melanocarpa* could be explained by lowering mucosal maltase and sucrase activities in the small intestine or by reducing the oxidative stress, as well as by stimulating the glucose uptake by increased insulin secretion [13].

Conclusions

Our data indicated that the *A. melanocarpa* berry juice induced a decrease in the oxidative stress markers: total antioxidant capacity, total thiol groups and glutathione. Most biochemical parameters were unaffected by the administration of *Aronia* juice. The enzymes, catalase and ceruloplasmin, of the treated group (group 2) did not differ significantly from the control group (group 1). Alloxan-induced diabetic rats treated with juice from *A. melanocarpa* berries exhibited a significant decrease of glycaemia (42.83% for alloxan-induced diabetic rats and 6.85% for healthy rats).

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