EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICE ON LIPID AND BONE METABOLISM IN OVARIECTOMIZED RATS

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ABSTRACT

INTRODUCTION: The ovariectomized (OVX) rat is a gold-standard model to mimic the changes in the female organism during menopause. *Aronia melanocarpa* fruits are very rich in polyphenols and exert many beneficial effects in animal models and clinical trials.

AIM: The aim of the present study was to investigate the effects of *Aronia melanocarpa* fruit juice (AMFJ) on lipid metabolism and bone mineral density (BMD) in OVX rats.

MATERIALS AND METHODS: Four groups of female Wistar rats were formed, each consisting of 14 animals—sham operated (SO), OVX (treated with distilled water), as well as OVX+AMFJ$_5$, and OVX+AMFJ$_{10}$ (OVX treated with 5 and 10 mL/kg AMFJ, respectively). The animal treatment began 2 weeks after the operation. After a three-month treatment period, body weight (BW), total fat (TF) and retroperitoneal fat (RPF), as well as serum total cholesterol were measured. The indices TF/BW and RPF/BW were calculated. Femur BMD was determined as well. The statistical analysis used was one-way ANOVA.

RESULTS: Compared to SO animals, OVX rats showed a statistically significant increase in TF ($p<0.05$), RPF ($p<0.01$), TF/BW ($p<0.05$), and RPF/BW ($p<0.01$). Plasma cholesterol levels of OVX animals were increased and their BMD was decreased if compared to SO rats, but these changes were not statistically significant. Compared to OVX rats, AMFJ did not affect lipid accumulation and cholesterol levels but at the dose of 10 mL/kg significantly increased BMD ($p<0.05$ vs. OVX).

CONCLUSION: *Aronia melanocarpa* fruit juice was not able to prevent the fat accumulation in OVX rats, but managed to restore their BMD.

Keywords: *Aronia melanocarpa, polyphenols, lipid metabolism, bone mineral density, ovariectomy, rats*

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INTRODUCTION

Menopause is a physiological condition resulting from the decline in ovarian estrogen production. This hormonal deficit has some serious consequences for female health. Postmenopausal estrogen deficit often results in dyslipidemia (decreased HDL cholesterol, as well as increased total cholesterol and LDL cholesterol) and increased bone turnover with osteoporosis and increased fracture risk (1). Menopause also increases the risk of obesity and often causes abdominal fat accumulation (2). The ovariectomized (OVX) rat is a gold-standard animal model that mimics the clinical findings in menopause (3).

Aronia melanocarpa (Michx) Elliot, called also black chokeberry, belongs to the *Rosaceae* plant family. Its fruits are extremely rich in polyphenols, especially proanthocyanidins, flavonoids (anthocyanins, quercetin glycosides) and phenolic acids (chlorogenic and neochlorogenic) (4–6).

Aronia melanocarpa hot water extracts were shown to improve the lipid profile and bone mineral density (BMD) in OVX rats (7). Other polyphenols and polyphenol-rich plant products such as blueberry powder (8), grape proanthocyanidins (9), chlorogenic acid (10,11), and quercetin (12) were able to increase BMD in rats with different osteoporosis models (induced by OVX, glucocorticoids or retinoic acid).

AIM

The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on fat accumulation, plasma cholesterol levels, and femur BMD in rats with OVX-induced estrogen deficit.

MATERIALS AND METHODS

Aronia Melanocarpa Fruit Juice

Aronia melanocarpa Elliot fruits were grown in the Balkan Mountains in Bulgaria. To prepare AMFJ, they were crushed and squeezed. Then, the juice was filtered, preserved with potassium sorbate (1.0 g/L), and stored at 0°C until the experiment (6). The contents of phenolic substances in AMFJ and the method of their determination are given in Table 1.

Table 1. Aronia melanocarpa fruit juice (AMFJ) ingredients and methods of their determination; GAE—gallic acid equivalent, HPLC—high-performance liquid chromatography; the content of the juice was determined by the authors (6)

Ingredient	Content	Method of Determination
Total phenols	5461 GAE/L	Folin-Ciocalteu procedure (13)
Total proanthocyanidins	3122.5 mg/L	Gravimetrically according to the procedure described by Howell et al. (14)
Cyanidin 3-galactoside	143.7 mg/L	HPLC
Cyanidin 3-arabinoside	61.7 mg/L	HPLC
Cyanidin 3-glucoside	4.4 mg/L	HPLC
Cyanidin 3-xyloside	11.6 mg/L	HPLC
Chlorogenic acid	585 mg/L	HPLC
Neochlorogenic acid	830 mg/L	HPLC

Animals and Operation

Female rats were allocated into four groups consisting of 14 animals each—sham-operated (SO) and three groups of OVX rats: OVX, OVX+AMFJ$_5$, and OVX+AMFJ$_{10}$—receiving 5 and 10 mL/kg AMFJ, respectively. Rats were four months old at the beginning of the experiment and sexually naïve. On the day of the operation, anesthesia with ketamine 30 mg/kg and xylazine 30 mg/kg was performed and animals were fixed. The abdominal hair was removed and the abdomen was disinfected with iodine. After that a midline incision was performed. The abdominal cavity was sewed back immediately in SO rats. In OVX rats, the ovaries were isolated with consecutive clamping of uterine tubes and tying a thread around the oviduct and its blood vessels. After this, the abdominal wall was closed, and prophylaxis with cefazolin 200 mg/kg i.p. was performed. There was a recovery period of two weeks after the operation. Af-

ter that, the daily oral treatment began. The experimental substances were applied to rats, using an orogastric tube. Distilled water (10 mL/kg) was given to SO and OVX rats. OVX+AMFJ$_5$ and OVX+AMFJ$_{10}$ animals were treated respectively with AMFJ 5 mL/kg (diluted with water to a volume of 10 mL/kg) and AMFJ 10 mL/kg. Animal care and all experiments were in conformity with national laws and policies as well as with the international guidelines (EU Directive, 2010/63/EU for animal experiments).

Three months after the operation, several behavioral tests were performed. On the last day, animal body weight (BW) was measured. Then, after diethyl ether anesthesia rats were sacrificed and blood was collected from the sublingual veins to prepare serum for biochemical investigations. Then fat deposits (retroperitoneal, mesenteric and perigonadal) were dissected and measured as total fat (TF). Retroperitoneal fat (RPF) was separately weighed. The indices TF/BW and RPF/BW were calculated. Femurs were removed, cleared from all soft tissues and frozen.

Biochemical Measurements

Serum levels of total cholesterol were measured spectrophotometrically (spectrophotometer CE2021, Cecil Instruments Ltd, UK). The analysis was performed using kits from Biomaxima (Poland) according to manufacturer's instructions.

X-Ray Absorptiometry (DXA)

Femur BMD was measured by dual energy X-ray absorptiometry (DXA) using a computer program for small subjects.

Statistical Analyses

Statistical analyses were performed by one-way analysis of variance (one-way ANOVA) with post-hoc Dunnett's multiple comparison test. Data were presented as mean ±SEM. A value of $p<0.05$ was considered significant. GraphPad Prism 5 statistical software was used.

RESULTS

Lipid Metabolism

Data regarding lipid metabolism are presented in Table 2. All OVX animals (both treated with distilled water and AMFJ) had significantly higher fat deposits if compared to SO rats ($F_{3,50}=3.92$, $p<0.05$ for TF; $F_{3,49}=5.39$, $p<0.01$ for RPF; $F_{3,51}=3.50$, $p<0.05$ for TF/BW index and $F_{3,49}=5.78$, $p<0.01$ for RPF/BW index). *Aronia melanocarpa* fruit juice-treated groups had fat deposits which were not significantly different than those of OVX animals.

The mean cholesterol level of OVX group was higher than that of SO animals with no statistical significance. Compared to OVX group, the cholesterol levels of AMFJ-treated groups were also not significantly different.

*Table 2. Total (TF, g), retroperitoneal fat (RPF, g), TF/BW and RPF/BW indices, total plasma cholesterol (TC, mmol/L); *p < 0.05, **p < 0.01 vs. SO; BW—body weight*

	TF (g)	RPF (g)	TF/BW	RPF/BW	TC (mmol/L)
SO	9.4 ± 1.2	1.6 ± 0.2	0.04 ± 0.005	0.006 ± 0.001	2.1 ± 0.1
OVX	15.4 ± 1.8*	3.3 ± 0.4**	0.05 ± 0.005*	0.012 ± 0.001**	2.4 ± 0.1
OVX + AMFJ$_5$	14.2 ± 1.5*	3.1 ± 0.4**	0.05 ± 0.005	0.011 ± 0.001**	2.3 ± 0.1
OVX + AMFJ$_{10}$	15.0 ± 1.3*	3.2 ± 0.3**	0.05 ± 0.004*	0.012 ± 0.001**	2.3 ± 0.1

Bone Mineral Density

The mean BMD of rats belonging to SO, OVX, OVX+AMFJ$_5$, and OVX+AMFJ$_{10}$ groups were respectively 0.273±0.014 g/cm^2, 0.263±0.002 g/cm^2, 0.270±0.010 g/cm^2, and 0.299±0.012 g/cm^2 (Fig. 1). As is obvious, BMD of OVX rats was lower than that of SO group (with no statistical significance). *Aronia melanocarpa* fruit juice treatment increased BMD of the treated groups ($F_{2,10}=3.85$, $p=0.05$). Dunnett's multiple comparison test showed a significantly increased BMD in OVX + AMFJ$_{10}$ group ($p<0.05$ vs. OVX) (Fig. 1).

DISCUSSION

Ovariectomy causes estrogen deficit that mimics the menopause. Typical findings in OVX rats are increased fat accumulation and decreased BMD (8,15).

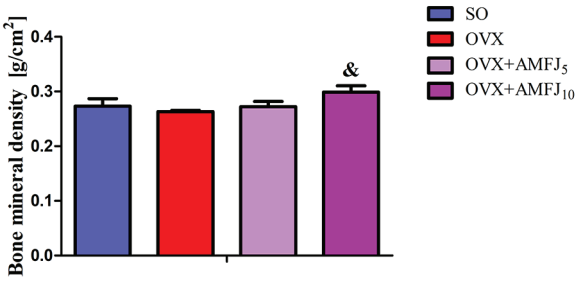

Fig. 1. Femur bone mineral density (BMD, g/cm²); SO—sham-operated rats; OVX—ovariectomized rats; OVX+AMFJ$_5$—OVX rats treated with AMFJ 5 mL/kg; OVX+AMFJ$_{10}$—OVX rats treated with AMFJ 10 mL/kg; & p<0.05 vs. OVX.

In this experiment, OVX rats showed increased fat deposits in comparison with SO animals. This was demonstrated by increased TF and RPF, as well as TF/BW and RPF/BW indices. It might be a result of increased leptin levels leading to leptin insensitivity (16,17). There are data that berries rich in cyanidin-3-glucoside are able to prevent the obesogenic effect of OVX-induced estrogen deficit (18). The *Aronia melanocarpa* fruit juice used in this experiment is very rich in anthocyanins presented by cyanidin-3-glycosides. In several experiments, *Aronia* prevented diet-induced obesity in mice (19,20). In our study, AMFJ did not succeed in antagonizing the effect of ovariectomy on body weight. These results are consistent with the findings of other authors regarding berry fruits (21).

We found that total cholesterol level of OVX animals was insignificantly higher than that of SO rats. This is consistent with other authors' findings (22). In rats estrogen deficit does not always lead to a significant increase in plasma total cholesterol levels. This is probably due to difference in the composition and regulation of the production of plasma lipids, especially of HDL cholesterol (23). *Aronia melanocarpa* fruit juice did not significantly affect cholesterol levels, although there are data that *Aronia* fruits is able to improve lipid metabolism in diet-induced obesity (19,24,25).

In our experiment, a slight, statistically non-significant decrease in BMD was observed in OVX compared to SO rats. This finding is consistent with some other results (8,26). Other experiments performed with Sprague-Dawley rats (27,28) were able to demonstrate a significant reduction of BMD 3 months after ovariectomy. We found that AMFJ was able to increase BMD in OVX rats, which coincides with the results of Kang et al. (7). This beneficial effect of AMFJ is most probably due to its high polyphenolic content. Polyphenols are found to affect bone metabolism by several mechanisms: inhibition of bone resorption by antioxidant and anti-inflammatory action, stimulation of osteoblastogenesis, and inhibition of osteoclastogenesis, as well as by osteoimmunologic mechanisms (29).

CONCLUSION

Aronia melanocarpa fruit juice was not able to reverse estrogen-deficit-induced obesity in OVX rats. It increased BMD of OVX rats, probably due to its high polyphenolic content.

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