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ORIGINAL PAPER

Effects of microencapsulated and non-encapsulated aronia extract on serum lipid profile and liver histology in Sprague-Dawley rats fed a high fat diet*

Serim Tuna Koç[1], Süleyman Kök[2], Onur Ersoy[3], Sertaç Atalay[4]

[1] Department of Biotechnology and Genetics, Institute of Science and Technology, Trakya University, Edirne, Türkiye
[2] Department of Genetics and Bioengineering, Faculty of Engineering, Trakya University, Edirne, Türkiye
[3] Department of Pathology Laboratory Techniques, Vocational School of Health Services, Trakya University, Edirne, Türkiye
[4] Central Research Laboratory (NABILTEM), Tekirdağ Namık Kemal University, Tekirdağ, Türkiye

Abstract

The aim of this study was to determine the biofunctional effects of aronia extract and its microencapsulated form on lipid metabolism and liver tissue in rats fed a high-fat diet. In the study, 42 male Sprague-Dawley rats aged 10 weeks were used. The rats were divided into 6 groups, with 7 animals in each experimental group. The experimental groups were as follows: 1 – standard diet control (CON), 2 – high fat diet control (HF), 3 – HF + 400 mg kg^{-1} aronia extract (HF400E), 4 – HF + 200 mg kg^{-1} aronia extract (HF200E), 5 – HF + 400 mg kg^{-1} aronia encapsulated (HF400C), and 6 – HF + 200 mg kg^{-1} aronia encapsulated (HF200C). From the 10th week to 20th week, rats were fed with HF diet for 10 weeks except (CON). At the end of the 20th week, rats fed HF were administered aronia extract and its encapsulated form (200-400 mg kg^{-1}) by oral gavage for 6 weeks. After six weeks of treatment, biochemical analyses were performed in blood and tissue samples of the rats. Liver histology was evaluated. As a result of the study, it was determined that the high-fat diet significantly increased serum ALT, AST, TC, TG and LDL-C levels and hepatic MDA levels, whereas administration of HF400E to hyperlipidaemic rats caused a significant decrease in the levels of these parameters. Furthermore, histopathological analysis of liver sections revealed that the HF400E treatment also protected against liver injury. These results indicated that HF400E improved lipid profiles, inhibited lipid peroxidation and played a protective role against liver injury in hyperlipidaemic rats. The lack of a significant effect of the encapsulation of aronia may be due to the short duration of treatment. Thus, future studies should be carried out for longer periods (>6 weeks) and at higher doses.

Keywords: aronia, high fat diet, microencapsulated, Sprague-Dawley

Serim Tuna Koç, PhD student, Department of Biotechnology and Genetics, Institute of Science and Technology, Trakya University, Türkiye, e-mail: stunakoc@hotmail.com

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INTRODUCTION

Free radicals are substances formed in the body as a result of polluted air inhaled throughout the day, toxic substances in spoilt foods, additives, unhealthy diet and inactivity. These negative external influences cause oxygen atoms to break down and circulate freely in the body, hydrogen atoms to form, and tissue damage to occur. Free radicals formed by natural metabolic pathways in the body are normally eliminated by radical-degrading antioxidant systems. However, a series of pathological events called oxidative stress occurs as a result of the increase in reactive oxygen species for some reasons and the inadequacy of antioxidant mechanisms (Atmaca Aksoy 2009). This causes some damage in the body; as the amount of free radicals increases, first aging accelerates, cell death intensifies, then tissue death as well as damage to the brain vessels occur (Schieber, Chandel 2014). Antioxidant substances are molecules that reduce and block the effects of free radicals in the body and prevent chain reactions that can cause various diseases and premature aging (Duysak et al. 2024).

Owing to their high antioxidant capacity, fruits are used to reduce oxidative stress. In terms of its capacity to scavenge oxygen free radicals, aronia (*Aronia melanocarpa*) has the highest antioxidant activity compared to other fruits (Tolic et al. 2017, Denev et al. 2018, Jurendic, Ščetar 2021). Aronia fruit contains anthocyanins, flavonoids, phenolic acids and polyphenolic substances. The fruits of this plant are the most abundant natural source of anthocyanins (Chen et al. 2023). Many investigations have shown that these compounds have potent anti-inflammatory, antioxidant, lower blood pressure, antimicrobial, and cancer-preventing activities, as well as being able to lower plasma fat levels, benefiting the cardiovascular system (Jurikova et al. 2017, Tolic et al. 2017, Denev et al. 2018, Jurendic, Ščetar 2021, Chen et al. 2023). Aronia is a common herbal medicine in Russia and Eastern Europe, in which it is prescribed for hypertension and anti-atherosclerosis (Ciocoiu et al. 2013, Jurendi'c, Ščetar 2021). Anthocyanins have potential health benefits and are considered interesting natural food colourants. However, their structure, poor bioavailability and sensitivity to environmental conditions such as temperature, light, oxygen and pH fluctuations hinder their practical application. Consequently, encapsulation of these chemicals may be an effective way to increase the amount of bioactive anthocyanins in the intestinal tract, thus enhancing their beneficial effects. Encapsulation is a technology that protects various bioactive chemicals from environmental variables by enclosing them in capsules and at the same time releasing the substances they contain into the environment under certain conditions (Saifullah et al. 2019). Since the coating materials used in encapsulation are polysaccharides that dissolve in basic solutions, they maintain their integrity under acidic gastric conditions and completely dissolve under small intestinal conditions.

Microencapsulation has significant advantages for functional foods; seasonal and endemic fruits such as aronia can be efficiently distributed and consumed throughout the year. Among different techniques for microencapsulation, such as coacervation-phase separation, pan-coating process, solvent evaporation, air suspension, interfacial polymerisation and multi-hole centrifugation process, spray drying is the most common method for liquids containing phenolic compounds (Yousuf et al. 2016). Moreover, the use of natural polymers (maltodextrin and gum arabic) as coating materials increases the stability and oxidative protection of functional components.

The aim of this study was to determine the effects of aronia extract and encapsulated form on serum lipid profile and liver histopathology in male Sprague-Dawley rats fed a high-fat diet.

MATERIALS AND METHODS

Plant material

This study used the Nero variety of aronia (*Aronia melanocarpa* (Michx) or Elliot (black chokeberry) grape fruit species growing in Soğucak village of Vize region, Kırklareli province (41°38′26.1132″ North and 27°39′22.7808″ East) as materials.

Lyophilization (freeze) drying of aronia fruits

Fresh fruits were cleaned, washed and subjected to lyophilization after going through preliminary preparation processes. The lyophilization process was carried out at Tekirdağ Namık Kemal University Central Research Laboratory. Lyophilization process consisted of 2 stages. In the first stage, the fruits were frozen under low temperature (~40°C) and low pressure (~0.5 mbar). After the freezing process, the fruits were dried and the remaining water was evaporated (Christ alpha 2-4 LD plus, Germany).

Extraction process of aronia fruit

The dried material was ground to make fine powder. It was then extracted by acidifying 70% ethanol with 0.01% citric acid at a pH of 4.9. Afterwards, it was then placed in a room at 26°C for about six hours. Next, the extracts were filtered on Whatman 415 paper and concentrated using a vacuum rotary evaporator (Buchi, Switzerland) until all alcoholic residues were removed (Nicouea et al. 2007).

Microencapsulation process of aronia fruit

As coating ingredients, maltodextrin-dextrose equivalent 16.5-19.5 and gum Arabic were used; both were dispersed separately in water until they

reached a solid content of 10%. Maltodextrin and gum Arabic were mixed in a 4:1 (v/v) ratio to create the coating material solution. The coating solution and aril extract were homogenized in a magnetic stirrer at 60°C, at 8000 rpm and for 10 minutes. The homogenate was spray-dried (Büchi B-191 Mini Spray-Dryer) at room temperature, with an intake air temp. of 110°C and a pump flow of 600 mL min^{-1}. The microencapsulated powder was stable when stored in darkness at room temperature until used (Álvarez-Cervantes et al. 2021).

Animals and diets

Forty-two inbred adult male Sprague-Dawley rats (eight weeks of age) were obtained from Trakya University Faculty of Medicine Experimental Animals Unit (Edirne/Turkey). All rats were provided free access to rodent chow and filtered tap water, and were housed in an air-controlled room under standard conditions (temp. 22±2°C; relative humidity, 50± 10%; 12 h light/dark cycle) throughout the study.

Experimental design and treatments

After two weeks of acclimatization, the rats were randomly divided into six dietary groups (n=7 in each group). Rats in the control (CON) group received regular rodent chow, while those in the other five groups were fed with a high-fat diet for ten weeks. At the end of the twentieth week, 200-400 mg kg^{-1} BW aronia extract and its encapsulated form were given via oral gavage to rats fed a high-fat diet for 6 weeks.

Treatment

i) Control (CON) – rats were fed with normal diet (Standard Rodent Diet", 3.1 kcal g^{-1} containing 4.0% fat, 44.2% carbohydrate, 20.0% protein from Arden Research & Experimental, Standard Diet Ankara, Turkey), and gavaged with water.
ii) High fat diet (HF) Control – rats were fed with HF (Arden Research & Experimental Ankara /Turkey, Atherogenic Rodent Diet, 5.54 kcal g^{-1} composed of 35.0% fat, 0.1% cholesterol, 0.04% colic acid 33.0% carbohydrate, 20.0% protein) and gavaged with water.
iii) HF200E – rats were fed with HF and 200 mg kg^{-1} aronia extract was administered by gastric tube once daily as an aqueous suspension.
iv) HF400E – rats were fed with HF and 400 mg kg^{-1} aronia extract was administered by gastric tube once daily as an aqueous suspension.
v) HF200C – rats were fed with HF and 200 mg kg^{-1} encapsuled aronia was administered by gastric tube once daily as an aqueous suspension.
vi) HF400C – rats were fed with HF and 400 mg kg^{-1} encapsuled aronia was administered by gastric tube once daily as an aqueous suspension.

Anthropometrical measurements

Body measurements were taken at the end of the experiment; body weight (g), body length (cm) and liver weight (g) and liver weight index (LWI) were calculated.

Body Mass İndex (BMI, g cm^{-2}) = body weight (g) / (body length)^2 (cm^2) – Tekgül et al. (2012);

Lee Index (g cm^{-3}) = $\sqrt[3]{}$ body weight (g) / nasoanal length (cm) x 100 000 (Wang et al. 2017);

LWI = [Liver weight (g) /Body weight (g)] × 100;

Body weight growth rate changes (g/w) = (FW-IW) / T (López-Espinoza et al. 2015);

FW – Final weight (g);

IW – İnitial weight (g);

T – is the period length (week);

Liver damage score (LDS) – the grading of inflammation and hepatocellular changes in rat liver tissues.

Determination of total polyphenols

The extract then was analysed for total phenolic content using the Folin--Ciocalteu method (Perez et al. 2023). Briefly, a volume of 0.5 mL of the extract (100 μg mL^{-1}) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min. The absorbance was measured at 765 nm. The total phenolic content was determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds was expressed as mg/g gallic acid equivalent (GAE) of the extract.

Antioxidant capacity

The antioxidant capacity of aronia extract and of the standard solution (ascorbic acid) were measured using the 1,1,-diphenyl-2-picrylhydrazyl (DPPH) method (Wang et al. 2019). A mixture of 2 mL of 1.0 mmol L^{-1} DPPH solution in methanol and 1 mL of standard solution of extract solution with different concentrations (10-500 μg mL^{-1}) were prepared. The mixture solution was incubated in dark at 37°C for 20 min. A decrease in absorbance of each solution was measured at 517 nm. Ascorbic acid was used as positive control while DPPH radical solution was taken as blank. The percentage of radical scavenging activity was measured using formula:

DPPH radical-scavenging activity (%) = [(absorbance of control – absorbance of sample)/absorbance of control] × 100.

The concentration of a sample required to neutralize 50% of DPPH (IC50) was determined using the curve of percent inhibitions plotted against the respective concentration.

Chemical composition of aronia by LC-MS

An Agilent 6460 liquid chromatography system (Agilent Technologies, Waldbronn, Germany) was used. Data were collected and processed using MassHunter, an Agilent LC-MS software (Bayram et al. 2020). Table 2 presents all parameters. The concentration of phenolic acids in each sample was determined using a calibration curve created on the same day and analysed during the same analytical run. All calibration curves were generated using the following concentrations: blank (water, methanol, formic acid: v:v:v, 79:20:1), 5, 10, 25, 50, and 100 ng mL^{-1}, and all points were injected three times. The linearity of all phenolic acids was $R2 \geq 0.995$. The samples were analysed in accordance with the sample preparation protocol. Table 1 shows the LOD and LOQ values for phenolic acids (derived over the S/N ratio).

Measurement of blood biochemical parameters and lipid profiles

Biochemical measurements

At the end of the experiment, all rats were injected i.m. Ketamin (Richter Pharma, Avusturya) (90 mg kg^{-1}) + Xylazine (Alfasan International B.V., Hollanda) (10 mg kg^{-1}), and blood samples were obtained via direct cardiac puncture with a 20 G needle. The blood samples were collected in tubes with EDTA (ethylene-diamine-tetra-acetic acid disodium salt). Plasma was separated via centrifugation (Heraeus Labofuge, Hanau, Germany) at 3000× g on 4°C for 15 min, aliquoted, and stored at –80°C for further analysis. Liver tissue samples were dissected, washed with cold neutral buffered formalin (Sigma Aldrich, Taufkirchen, Germany) and frozen at −80°C for further analysis in Trakya University Faculty of Engineering.

Determination of serum total cholesterol, triglycerides and high-density lipoprotein-cholesterol

Serum total cholesterol (TC) and triglyceride (TG) were measured using the equipment Accutrend Plus, and its test strips were used. An enzymatic reflectance photometric assay was used based on the glycerol-phosphate oxidase (GPO) and cholesterol oxidase methods, respectively, following the manufacturer's instructions. Briefly, one drop of fresh venous blood was applied to the reagent area of a test strip and, when prompted, inserted into the test chamber of an instrument that directs light onto the test area. The triglycerides/total cholesterol in the sample reacts with the reagents in the strip pad causing a color change. The amount of light reflected from the colored test area is proportional to the concentration of triglycerides/total cholesterol measured by a photometer and is converted into a digital readout. High-density lipoprotein-cholesterol (HDL) was measured by ELISA using commercial kits (Ref No: 201-11-0255, Sunred Catalogue, China) in accordance with the kit protocol instruction and read by ELISA microplate reader (Thermo Scientific MultiSkan GO, USA).

Determination of serum VLDL-and LDL-cholesterol

Serum LDL- and VLDL-cholesterol were calculated indirectly by the Friedewald's equations (Friedewald et al. 1972).

LDL = total-cholesterol- (HDL + VLDL),

VLDL = triglycerides/5.

Determination of liver tissue AST, ALT and MDA

Serum levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Ref No: 80025 and 80027 Biolabo, Maizy, France) levels were measured using spectrophotometric and colorimetric methods with an automatic chemical analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). Malondialdehyde (MDA) absorbance values were achieved using commercial kits (Ref No: 201-11-0157, Sunred Catalog, China) and read on an ELISA microplate reader (Thermo Scientific MultiSkan GO, USA). The results were computed according to the manufacturer's instructions.

Histological analysis

Liver tissues were removed, fixed with 10% neutral buffered formalin, and embedded in paraffin. The paraffin block samples were cut into 5 μm sections and stained with hematoxylin and eosin (H&E) for histological observation using a light microscope (Olympus BX50; Olympus, Tokyo, Japan).

Ethical clearance

The study has been approved by the Research Ethics Committee of Trakya University (Recommendation number: 2022/03) dated March, 29, 2022).

Statistical analysis

The relationship between the effects of microencapsulated and non--capsulated aronia extract on serum lipid profile and liver histology in Sprague-Dawley rats fed a high-fat diet was evaluated using the Duncan test for multiple comparisons by SPSS18.0. When $P<0.05$, there was a significant difference between the two forms of aronia, and if $P<0.01$, the difference was more significant.

RESULTS

The extract profiles

The concentration of total phenolic compounds is 3.40% and the antioxidant capacity is 51.46 ppm in terms of IC50 (Table 1).

Table 1

Total phenolic content and antioxidant capacity of the aronia extract

Aronia extract profile	Result	Unit	Method
Total phenols	3.40	(%)	Folin-Ciocalteu
Antioxidant capacity	51.46	(Ppm)	DPPH

Contents by LC-MS: Water soluble constituents of aronia extract yielded 22 peaks corresponding to tentatively identifiable compounds by their monoisotopic mass and fragmentation patterns (Table 2).

Table 2

Composition of phenolic compounds in aronia extract

Compound	RT	Response	Final concentration (ng ml^{-1})
Gallic acid	1.736	16 761	4 069.60
Protocatechuic acid	1.922	119 272	74 901.93
2,5-dihydroxybenzoic acid	2.326	4 279	6 423.52
Caffeic acid	3.756	200 861	83 568.61
Chlorogenic acid	3.777	1 597 724	254 455.61
Salicylic acid	3.859	289	37.65
Catechin	4.014	3 131	2 280.51
p-coumaric acid	4.112	2 099	3 691.60
Rutin	4.062	300 347	29 902.36
Hesperidin	4.054	80 677	11 067.99
Trans ferulic acid	4.179	616	4 133.16
Ethyl gallate	4.178	56	2.144
Phlorizin	4.224	12 364	925.11
Oleuropein	4.249	1 377	223.25
Myricetin	4.284	2 383	863.46
Aloin a	4.055	2 023	40.80
Quercetin	4.386	617 128	84 108.31
Luteolin	4.428	5 418	523.84
Abscisic acid	4.422	2 756	1 693.34
Naringenin	4.488	811	378.11
Kaempferol	4.496	3 331	8 504.05
Isorhamnetin	4.511	161 288	7 233.13

The effects of aronia on body weight (g), body weight growth rate (g/w)

Effects of aronia on body weight (g) and body weight growth rate (g/w) are presented in Figure 1 *a* and *b*. The study used periodic measurements that evaluated the rats' baseline and final weight changes. The rats' initial body weights were homogeneously given out, and rats weighing between

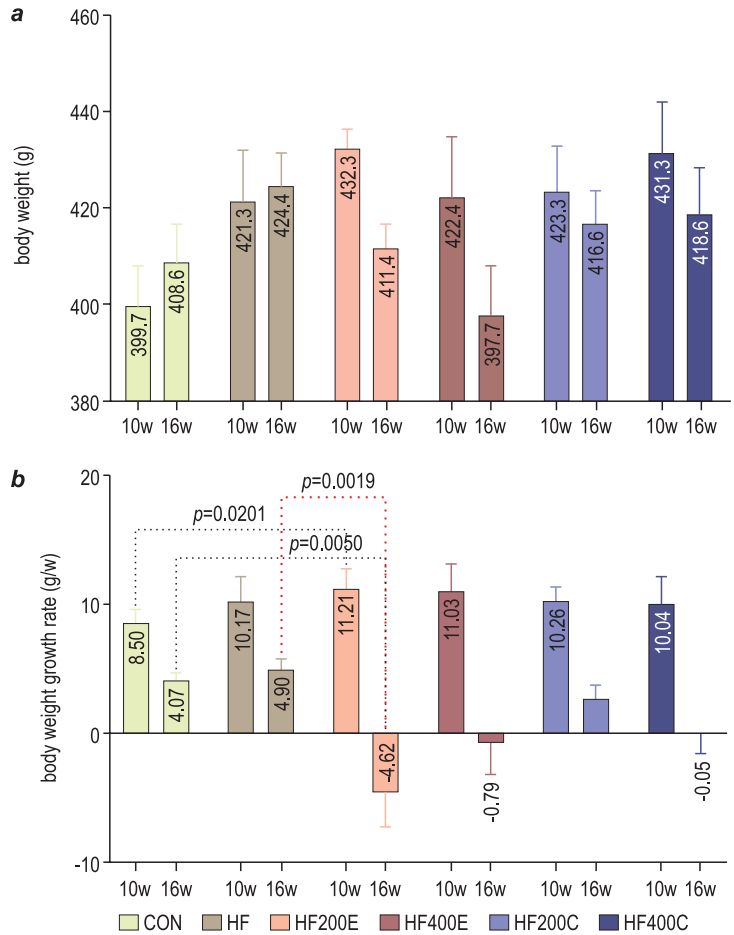

Fig. 1. Body weight (g) changes – *a*, body weight growth rate changes (g/w) – *b*

271-283 g were chosen. The average body weights of rats before and after aronia use ranged from 399.71-408.57 g in the CON group; 421.29-424.43 g in the HF group; 423.29-416.57 g in the HF200C group; 432.29-411.43 g in HF200E group; 431.29-418.57 g in HF400C group; 422.43-397.71 g in HF400E group. There was no significant difference between the diet groups according to BW (g) changes (Figure 1*a*).

The average body weight gain rate of rats in groups was determined before and after aronia application to the rats' diets, grams/week (g/w). The rats in the HF200E group gained body weight (BW) at a slower and more significant rate than the rats in both the CON (P=0.0050) or HF (P=0.0019) groups (Figure 1*b*).

The mean body mass index (BMI kg m^{-2}) of the rats at the end of the 10^{th} week was calculated as 0.20±0.004 in the CON, 0.21±0.010 in the HF,

0.20±0.005 in the HF200C, 0.22±0.010 in the HF200E, 0.21±0.005 in the HF400C and 0.21±0.01 in the HF400E groups. BMI 1 of the rats in the HF200E group was calculated to be higher than the rats in the CON group (P=0.0394; Figure 2*a*). BMI 2 averages of rats after aronia application were not significantly different (Figure 2*b*).

Figure 2*c-d* is shows a comparison of CON, HF and experiment groups regarding the Lee index, with no significant difference between the groups. After the aronia application, the lowest Lee index between the groups was determined in HF400E (Figure 2*d*).

Effects of aronia on serum lipid profiles

Table 3 shows the effects of aronia on the levels of serum TC, TG, LDL, HDL, VLDL and LDL/HDL in all experimental groups.

Table 3

Effects of aronia on the levels of serum TC, TG, LDL, HDL, VLDL and LDL/HDL in all experimental groups

Parameters	Groups (n=7)					
	CON	HF	HF200E	HF400E	HF200C	HF400C
TC	158.9±2.05	159.7±2.13	160.3±2.48	167.7±0.57$^{*,\#}$	160.0±1.63	159.0±1.51
TG	141.1±8.29	172.1±23.27	115.1±6.03	93.0±1.67$^{\#}$	140.9±20.20	174.3±26.72
LDL	67.54±2.17	64.21±3.51	83.37±5.42$^{\#}$	71.96±2.65	70.83±5.70	63.90±5.50
HDL	63.09±2.34	61.08±0.97	53.89±3.21*	77.15±2.93$^{***,\#\#\#}$	61.00±2.06	60.24±1.60
VLDL	28.23±1.66	34.43±4.65	23.03±1.21	18.60±0.33$^{\#}$	28.17±4.04	34.86±5.35
LDL/HDL	1.08±0.07	1.06±0.07	1.62±0.21$^{*,\#}$	0.95±0.07	1.19±0.13	1.07±0.10

CON – normal diet, HF – high-fat diet, HF200E – high fat diet + 200 mg kg^{-1} aronia extract, HF400E – high fat diet + 400 mg kg^{-1} aronia extract, HF200C – high fat diet + 200 mg kg^{-1} encapsuled aronia, HF400C – high fat diet + 400 mg kg^{-1} encapsuled aronia, TC – total cholesterol, TG – triglyceride, LDL – low-density lipoprotein-cholesterol, HDL – high-density lipoprotein-cholesterol, VLDL – very low-density lipoprotein-cholesterol. Values are means ± SEM (n=7). * P<0.01, ** P<0.001, *** P<0.0001 compared with CON; $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 compared with HF diet

Serum TC levels in the HF400E group were significantly (P<0.05) higher than the CON (P=0.0070) and HF (P=0.0166) groups.

Serum TG levels in the HF400E (93.00±1.67 mg dl^{-1}) group were found to be lower than those in HF (P=0.0107) groups, while the opposite was observed for serum LDL levels. The decrease in TG levels in the blood of rats in the HF200E, HF400E and HF200C groups compared to those in the HF group was due to the positive effect of aronia supplemented into the rats' diets.

Rats of the HF400E group had higher HDL levels (77.15 mg dl^{-1}) compared to the CON (63.09±2.34 mg dl^{-1}, P=0.0006) and HF (61.08±0.97 mg dl^{-1}, P=0.0001). The higher HDL level in the HF400E group may be attributed to the positive effect of aronia extract.

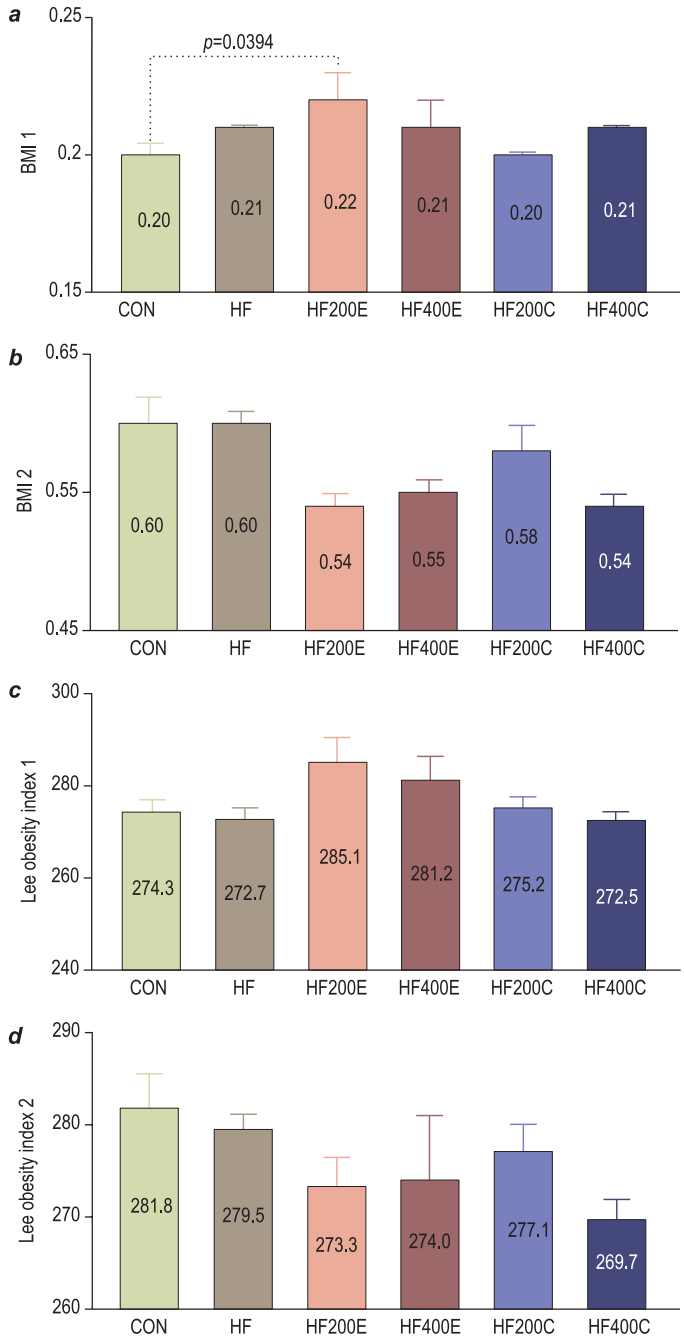

Fig. 2. BMI 1 of rats before aronia application – *a*, BMI 2 of rats after aronia application – *b*, Lee obesity index 1 of rats before aronia application – *c*, Lee obesity index 2 of rats after aronia application – *d*

LDL levels in the HF200E rats (83.37±5.42 mg dl^{-1}) were found to be higher than in rats from HF group (64.21±3.51 mg dl^{-1}). Serum HDL levels in the HF400E group were significantly (P<0.05) higher than the CON (P=0.0006) and HF (P=0.001) groups, and were significantly lower in HF200E group than in the CON (P=0.0327). Serum VLDL levels in the HF group were significantly higher than in the HF400E group (P=0.0107).

Serum LDL/HDL levels in the HF200E group were significantly higher than in the CON (P=0.0134) and HF (P=0.0094) groups.

Effects of aronia on liver

Effects of aronia on liver weight, liver weight/body weight ratio, liver damage score (LDS), ALT, AST and MDA values are presented in Tables 4 and 5. The LWs (g) of the rats in the HF400E group were found to be lower

Table 4

Effects of aronia on liver weight, liver weight/body weight ratio and liver damage score (LDS)

Groups (n=7)	LW mean ± SEM (g)	LW/BW ratio mean ± SEM (%)	LDS mean ± SEM
CON	17.4±0.79	4.37±0.21	0.65±0.01$^{\#\#\#}$
HF	18.03±0.81	4.34±0.16	5.38±0.02***
HF200E	14.30±0.55**,$^{\#\#\#}$	3.30±0.13***,$^{\#\#\#}$	3.81±0.01***,$^{\#\#\#}$
HF400E	12.80±0.24***,$^{\#\#\#}$	3.20±0.18***,$^{\#\#\#}$	2.00±0.01***,$^{\#\#\#}$
HF200C	15.2±0.71$^{\#\#}$	3.60±0.17**,$^{\#}$	5.51±0.01
HF400C	14.60±0.51*,$^{\#\#}$	3.39±0.13***,$^{\#\#}$	5.20±0.06

CON – normal diet, HF – high-fat diet, HF200E – high fat diet + 200 mg kg^{-1} aronia extract, HF400E – high fat diet + 400 mg kg^{-1} aronia extract, HF200C – high fat diet + 200 mg kg^{-1} encapsuled aronia, HF400C – high fat diet + 400 mg kg^{-1} encapsuled aronia, LW – liver weight, LW/BW ratio – liver weight/body weight ratio, LDS – liver damage score. Values are means ± SEM (n=7). * P<0.01, ** P<0.001, *** P<0.0001 compared with CON, $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 compared with HF diet

Table 5

Effects of aronia on liver tissue ALT, AST and MDA

Groups (n=7)	ALT mean ± SEM (U L^{-1})	AST mean ± SEM (U L^{-1})	MDA mean ± SEM (nmol ml^{-1})
CON	44.15±5.61	64.54±6.50	2.77±026
HF	54.13±3.30	75.37±4.32	2.80±0.33
HF200E	42.49±4.52	40.45±3.82*,$^{\#\#\#}$	2.64±0.18
HF400E	35.10±1.98	26.68±2.58***,$^{\#\#\#}$	2.38±0.28
HF200C	38.91±3.61	62.11±8.23	2.46±0.37
HF400C	38.91±5.48	52.13±5.50$^{\#}$	2.36±0.17

CON – normal diet, HF – high-fat diet, HF200E – high fat diet + 200 mg kg^{-1} aronia extract, HF400E – high fat diet + 400 mg kg^{-1} aronia extract, HF200C – high fat diet + 200 mg kg^{-1} encapsuled aronia, HF400C – high fat diet + 400 mg kg^{-1} encapsuled aronia, ALT – alanine amino transferase, AST – aspartate aminotransferase. Values are means ± SEM (n=7). * P<0.01, *** P<0.001 compared with CON, $^{\#}$ P<0.05, $^{\#\#\#}$ P<0.001 compared with HF diet

than that of the rats in the CON (P=0.0001) and HF(P=0.0001) groups. Aronia was found to have a reducing effect on liver weights in the HF200E, HF400E and HF400C groups compared to the HF group. This situation was evaluated as a positive effect of aronia given in the diet of rats, which reduces liver steatosis.

The LW/BW ratio (%) of the rats in the aronia treatment groups were found to be lower than that of the rats in the CON (P=0.0001) and HF(P=0.0001) groups. In the HF400E group, the lowest liver-to-body weight ratio was found to be 3.20±0.18%. Compared with the HF and CON groups, a significant reducing effect of aronia on the LW/BW ratio was also found in the HF200E, HF400E and HF400C groups.

Changes in the liver tissue levels of ALT, AST and MDA of each group are presented in Table 5. ALT and AST values in all treatment groups were determined within the normal standard intervals. Although ALT and MDA levels in the groups HF400E, HF400C and HF200C tended to be lower than in the CON and HF groups, the differences were not significant. Rats in both the CON group (P=0.0001) and HF group (P=0.0001) had significantly higher AST values than rats in the HF200E and HF400E group.

Histopathological evaluation of liver sections

Effects of aronia on liver histopathological findings (Figure 3) and liver damage score (LDS) are presented in Table 6.

Table 6

Liver morphology damage assessment criteria
(Brunt and Tiniakos 2010, Brown and Kleiner 2016)

Steatosis (S)	Lobular Inflammation (L)	Ballooning of Hepatocyte (B)
0: < 5% 1: 5% - 33% 2: 34% - 66% 3: > 66%	0: none 1: < 2 2: 2-4 3: > 4	0: none 1: a small amount 2: large amount

Evaluation was performed at 200X magnification. The score obtained according to the criteria is S+L+B (0-8)

Liver sections of rats in the CON group showed normal hepatic cells, sinusoidal spaces, and a central vein (Figure 3F). There was nearly minimal inflammation found in the portal section of the rats' liver tissues that belonged to the HF group. However, the liver's response to HF was found to manifest as notable microsteatosis and moderate macrosteatosis (Figure 3E). This group had a greater liver damage score than the CON group (P<0.001). Rat liver findings for the groups HF200C and HF400C were comparable, and the HF group's results were similar. Compared the liver scores of each of these groups to the HF group, Figures 3C and 3D demonstrate that there was no significant statistical difference. The liver scores of the HF200E

and HF400E groups were statistically lower than the one for the HF group (Figure 3E, 4F, $P<0.001$). However only the HF400E-treated group had the same histology as the CON group (Figure 3F). Although the HF200E group showed a decline in liver score, the level of micro- and macrosteatosis remains relatively unaltered (Figure 3E). When the HF group is compared with the HF400E group, it can be said that aronia has a significant reducing effect on LSD.

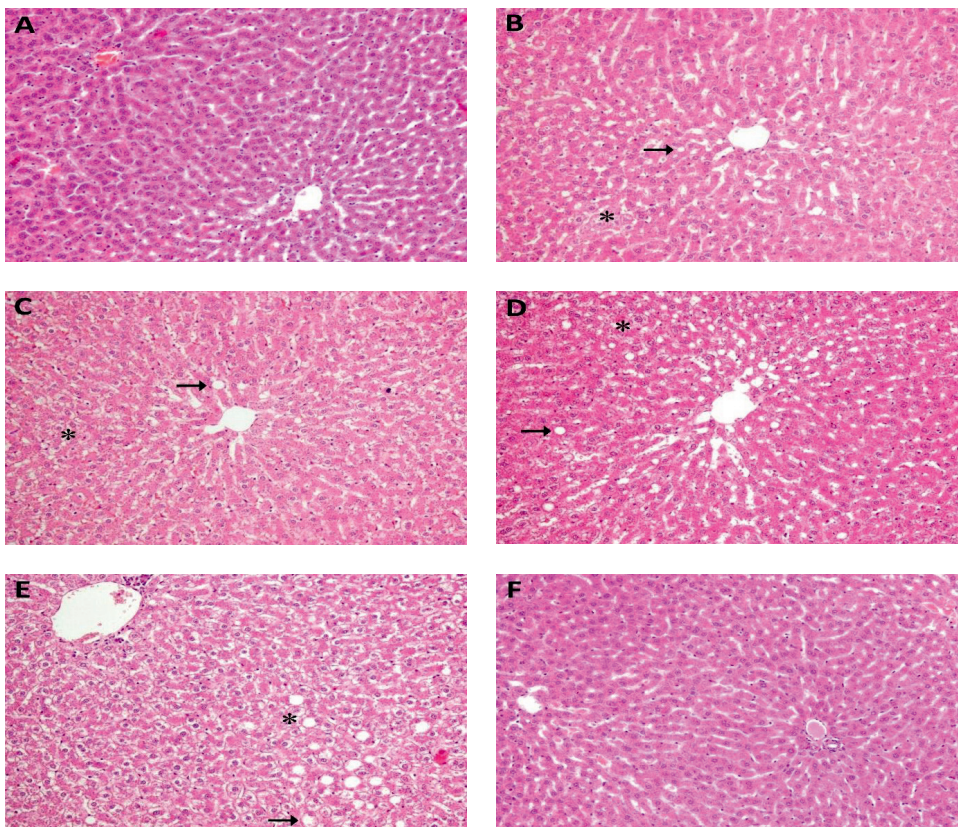

Fig. 3. Liver H-E stained micrographs: A – HF400E, B – HF200E, C – HF400C, D – HF200C, E – HF, F – CON (*) microsteatosis findings, (→) – macrosteatosis findings x 200 magnification

DISCUSSION

Aronia melanocarp (Michx.) or Elliot (chokeberry) has numerous potential health benefits owing to its high levels of anthocyanins and other functional compounds. Epidemiological and randomized controlled intervention trials have highlighted the potential of polyphenols in aronia, such as anthocyanins, proanthocyanidins, and phenolic acids, to prevent or attenuate

chronic diseases associated with oxidative stress, such as cardiovascular diseases, insulin resistance, and neurological conditions (Jurikova et al. 2017, Hein et al. 2019, Tena et al. 2020, Alam et al. 2021, Godos et al. 2021, Sandoval-Ramírez et al. 2021, Sun et al. 2023).

The objective of the present study was to determine the biofunctional effects of extract aronia and its microencapsulated form on lipid metabolism and liver tissue in high-fat-diet-fed rats. After 42 days of aronia treatment in rats fed a high-fat diet, there were significant changes in body weight, liver weight, and blood lipid levels between the six groups.

These results have been frequently observed in many other studies using similar experimental animal models. Our study demonstrates that aronia extract reduces body weight and food intake of HF-fed rats. It also decreases liver weight and improves the liver lipid profile. The underlying mechanisms of action of aronia-induced weight loss are likely due to the combined effects of several phytochemicals found in aronia extract, such as anthocyanins proanthocyanidins, and phenolic acids (Hein et al. 2019, Tena et al. 2020, Sandoval-Ramírez et al. 2021). Anthocyanins, a subfamily of flavonoids, act by modulating glucose, lipid, and amino acid metabolic pathways in various targets such as liver, skeletal muscle, adipose tissues, and pancreas, leading to the increase in fatty acid oxidation, the improvement of insulin sensitivity and glucose uptake, the reduction of oxidative stress, inflammation, and fatty acids, and the inhibition of cholesterol biosynthesis, resulting in a decrease in body weight and fat accumulation (Azzini et al. 2017). Not only the phenolic compounds, but also the polysaccharides isolated from aronia fruits have been shown to stimulate lipolysis and inhibit pre-adipocyte proliferation, thus reducing fat cell numbers and adipose mass (Chen et al. 2017). Aronia displays the highest polyphenol content among when berries, such as blueberries, blackcurrants, grapes, lingonberries, cranberries, raspberries and strawberries (Denev et al. 2018, Sidor et al. 2019), and has a higher antioxidative potential based on *in vitro* radical-scavenging assays, such as DPPH, Ferric reducing antioxidant capacity (FRAP), or oxygen radical antioxidant capacity – ORAC (Forbes-Hernande et al. 2016, Denev et al. 2018, Sidor et al. 2019). In our study, total phenolic compounds composed 3.42% of aronia extract and its antioxidant capacity was 51.46 ppm in terms of IC50. Inhibition concentration 50 (IC50) means the minimal concentration of the extract that can neutralize at least 50% of free radicals of 1, 1-diphenyl--2-picrylhydrazyl (DPPH). IC50 of less than 50 ppm is considered very strong antioxidant capacity, while between 50 to 100 ppm is strong.

Studies in animals and humans have shown that aronia effectively modulates lipid metabolism (Valcheva-Kuzmanova et al. 2007, Kulling, Rawel 2008, Kim et al. 2013, Daskalova et al. 2015, Lipińska, Jóźwik 2018). In other experiments involving spontaneous or induced hyperlipidaemias, the lipid-lowering effects of black chokeberry are similar to those detected in our experiment. Kulling and Rawel (2008) indicated that Aronia extract

reduces high levels of total cholesterol (TC), triglycerides (TG) and LDL in hypertensive rats and also improves the level of HDL lipoprotein that of Daskalova et al. (2015) demonstrated that the juice changes only the LDL concentration. Moreover, Lipińska and Jóźwik (2018) in feeding merino lambs with chokeberry pomace (300 g of pomace kg^{-1} of feed) found that the feed increases the HDL and decreases the TG level; however, the TC and LDL concentrations remain unchanged. Other authors (Kim et al. 2013) conducted research on inactivated apolipoprotein E (ApoE) in mice which were supplemented with chokeberry extract. They observed that after four weeks of feeding the mice, their plasma TC levels decreased, but no differences in plasma TG levels were found. Valcheva-Kuzmanova et al. (2007) have reported that aronia juice lowers the diet-induced increase in serum TC, LDL-C and TG, an effect resulting from the large amount of polyphenols contained in the juice. The possible underlying mechanisms of the lipid--lowering properties of flavonoids are as follows: cholesterol uptake suppression (by silymarin and tea catechins); improved lipoprotein catabolism (by cyanides); increased bile efflux, elimination of bile cholesterol and bile acids (proven for narginin). Quercetin, a flavonoid contained in black chokeberry, has an inhibiting effect on the enzymes that take part in the process of cholesterol synthesis and esterification (Ren et al. 2022).

The histopathological analysis showed that the livers of rats fed with HF diet developed signs of hepatosteatosis, indicating that HF400E may reduce hepatocellular damage and play a protective role against liver damage caused by high-fat diet. Therefore, administration of aronia extract may reduce hepatic lipid accumulation to prevent the occurrence of fatty liver. In general, hypertrophy and lipid exchange in hepatocytes caused by high-fat diet are accompanied by increased AST and ALT activities (Karakçı et al. 2023). Since these enzymes are found intracellularly in the liver, an increase in liver tissue damage entails increased levels of these enzymes in the blood. Our study did not reveal a statistically significant result in ALT levels. However, the administration of aronia caused a decrease in AST levels and liver weight compared to the HF group; this suggests that aronia shows hepatoprotective effects. Increased MDA concentrations reflect the level of lipid peroxidation in tissues and are considered a marker of tissue damage. At the same time, this increase may be associated with decreased antioxidant levels and increased ROS. In our study, an increase in MDA levels was observed in liver tissues, consistent with the studies of Ara et al. (2005) and Campbell et al. (2019) in HF-induced obese mice and rats. This result suggests that hepatic MDA levels may be related to hypercholesterolaemia. In our study, the hepatic MDA concentration decreased numerically in aronia applications.

In conclusion, high-fat diet significantly increased serum ALT, AST, TC, TG and LDL-C and hepatic MDA levels, whereas administration of HF400E to hyperlipidaemic rats caused a significant decrease in the levels of these

parameters. Weight gain was largely inhibited. Furthermore, histopathological analysis of liver sections showed that aronia treatment also protected against liver damage. These results indicated that HF400E administration improved lipid profiles, inhibited lipid peroxidation and played a protective role against liver injury in hyperlipidaemic rats. The lack of a significant effect in the encapsulation application of aronia may be due to the short duration of treatment. In future studies, it should be taken into consideration that encapsulation application studies should be conducted for longer periods (≥6 weeks) and at higher doses.

CONCLUSIONS

In conclusion, high-fat diet significantly increased serum ALT, AST, TC, TG and LDL-C and hepatic MDA levels, whereas administration of HF400E to hyperlipidaemic rats caused a significant decrease in the levels of these parameters. Weight gain was largely inhibited. Furthermore, the histopathological analysis of liver sections showed that aronia treatment also protected against liver damage. These results indicated that HF400E administration improved lipid profiles, inhibited lipid peroxidation and played a protective role against liver injury in hyperlipidaemic rats. The lack of a significant effect in the encapsulation application of aronia may be due to the short duration of treatment. In future studies, it should be taken into consideration that encapsulation application studies should be conducted for longer periods (≥6 weeks) and at higher doses.

Author contributions

STK – formal analysis, validation, investigation, data curation, methodology, data curation, visualization, writing original draft, writing review & editing, SK – writing review, conceptualization, supervision, OE – histopathological evaluation, SA – HDL ELISA analysis.

Conflicts of interest

The authors declare no competing interests.

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