CHANGES IN METAL HOMEOSTASIS IN EXPERIMENTALLY INDUCED FATTY LIVER BY THE EFFECT OF SOUR CHERRY CONSUMPTION

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Sour cherry (*Prunus cerasus*) is a widely favored fruit worldwide. Sporadic studies were done to determine the metal ion content of this fruit. Nevertheless its effect on metal ion homeostasis has not been examined so far therefore, experiments on animal (Wistar rats) were carried out to determine the changes of metal homeostasis in liver by the effect of this fruit. Wistar rats were divided into four groups: 1. control animal with normal diet; 2. hyperlipidemic rats were fed with fat-rich diet (chow contained plus 2% cholesterol, 0.5% cholic acid and 20% sunflower oil); 3. rats were fed with normal diet + lyophilized sour cherry (0.75 g daily ad libitum); 4. rats were fed with fat-rich diet and lyophilized sour cherry. The experiment was terminated after 10 days. From the sample handed rat liver homogenate metal ion content was determined by inductively plasma optical emission spectrometry (ICP-OES). Liver fragments were fixed in 4% neutral buffered formalin, embedded in paraffin, and 5 micrometer sections were cut and stained with hematoxylin–eosin. As a result of our experiment the concentration of metal elements were found to decrease significantly in the hyperlipidemic animals fed with sour cherry (Újfehértói fürtös), although there wasn't any significant change in result between the 1. (control animal with normal diet) and the 3. (rats were fed with normal diet + lyophilized sour cherry) group. On the basis of histological study it was established that the treatment with Fanal was the best, although Pipacs and Újfehértói fürtös were also significant in liver regression of hyperlipidemic animals with fatty liver, therefore it is concluded, that sour cherry treatment is beneficial to lower the hyperlipidemy and fatty degeneration.

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Introduction

Nowadays it is common that improper nutrition has a deteriorative effect in the redox- and metal homeostasis of the human organism and that can lead to cardiovascular or gastrointestinal diseases and hyperlipidemia also.

Sour cherry (*Prunus cerasus* L.) is one of the most popular fruits in Europe. It could be used as a "functional food", because of its anti-inflammatory, anti-diabetic and lipid lowering properties[1-4]. The bioactive compounds in sour cherry (*Prunus cerasus* L.) also have beneficial effects on cardiovascular diseases[5] and certain types of cancers. Some studies report that its bioactive agents such as anthocyanins (e.g. cyanidin, peonidin, cyanidin-3-O-rutinoside) and other polyphenols in *Prunus cerasus* L. have protective effects on the neuron cells too[6]. Results of the investigations revealed the strong antioxidant activity of anthocyanins and their possible use as chemotherapeutics[7-8]. Furthermore it attracted much attention that antochyanins possess anti-inflammatory properties as well[9]. Since in previous studies it was justified that the presence of some transition metal (Fe, Ca, Cu, Mn, Zn) elements has a significant effect on the signal transduction process[10-11], our aim was to determine the microelements in three types of sour cherry and their biological effects in hyperlipidemic animals.

Experimental

Materials

Ketamine (Calypsol) were purchased from Richter Gedeon (Budapest, Hungary) and xylazin (Sedaxylan) were from Eurovet Animal Health BV (Bladel, Hollandia). ICP standars originates from Spectro Ltd. (Kleve, Germany).

Fruit samples and extraction

The following three types of sour cherry(*Prunus cerasus* L.) were examined: Pipacs 1 (M1), Fanal (M2) and Újfehértói fürtös (M3).

The cherry trees were cultivated at the Research and Extension Center for Fruit Growing in Újfehértó, Hungary. Fruits were harvested in 2011 in the optimal ripening stage and later halved and pipette out. The fresh fruits were lyophilized (Labconco, USA) and stored at -80°C until experiments. The lyophilized sample (1 g) was delivered

into a 100 mL centrifuge tube, and 50 mL of MeOH:H_2O:HCOOH mixture (60:39:1, v/v) were added. The sample was vortexed and after 1 hour of ultrasonic bath, the suspension was filtered. The extract aliquot solvent (10 mL) was evaporated using a rotary evaporator, under vacuum to dryness at a temperature of 40 °C.

Animal experiments

Young male Wistar albino rats (150-200 g body weight) were weighed and randomly divided into four types of groups with 5 animals in each group.

The rats in group 1 were kept on a normal diet obtained from BIOFARM FARM PROMT Kft. (BFP, Gödöllő, Hungary). The rats of the second group were kept on a fat-rich diet containing cholesterol (1.0%), sunflower oil (11%) and cholic acid (0.3%) added to the control BFP. The third group was fed with the same normal diet completed with lyophilized sour cherry powder mixed into the diet (0.75 g daily ad libitum). The rats in group 4 were kept on fat-rich diet completed with lyophilized sour cherry powder (0.75 g daily ad libitum). The rats were kept on the diets for 10 days. The animals in group 3 and 4 were divided into three-three other groups correspondently the three types of sour cherry: 'Pipacs 1', 'Fanal' and 'Újfehértói fürtös'.

Finally, the rats were anaesthetised with ketamine (75 mg/kg) and xylazin (7.5 mg/kg). After laparotomy, blood was collected from the abdominal vein and the animals were exsanguinated. Liver was removed, washed and homogenized in ice-cold isotonic KCl solution.

Hyperlipidemy was proved by histological study.

Histology

Liver fragments were fixed in 4% neutral buffered formalin, impregnated in paraffin, and 5 mm sections were cut and stained with hematoxylin–eosin.

Protein measurement

The protein content of liver homogenate was set at 10 mg/mL using bovine albumin as a standard, which was measured in accordance with the method of Lowry et al. (1951)[12].

Determination of metal elements

The homogenized liver samples (3 g, 5 parallel for each) were digested with heating in 10 mL HNO_3 (65%) and 2 mL H_2O_2 (30%). After all, the solution was filled up to 10 mL with bidistilled water. The element (Al, B, Ba, Ca, Co, Cu, Fe, Li, Mg, Mn, Ni, P, Pb, Sr, Zn) content was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) with Spectro Genesis (Kleve, Germany) appliance[13].

Statistics

The results are reported in mean values and standard deviation, which were determined by Excel 2010 software programme. Differences between two independent groups of data were analyzed with 2-tailed-t-test. One-way analysis of variance (ANOVA) was used to compare multiple groups. Differences at $P<0.05$ were considered significant. Statistical analysis was carried out by Graphpad software version 1.14. ANOVA was calculated for control, control+M1, fat-rich diet, and fat-rich diet +M1; for control, control+M2, fat-rich diet, and fat-rich diet +M2; as well as for control, control+M3, fat-rich diet and fat-rich diet +M3.

Results

Histology

According to the histological studies treatment with Fanal was the most beneficial to lower the hyperlipidemy in animals. Pipacs1 and Újfehértói fürtös were also effective against tissue necrosis but the treatment with Fanal resulted lower quantity of lipid droplets in hepatic cells (Figures 1 and 2).

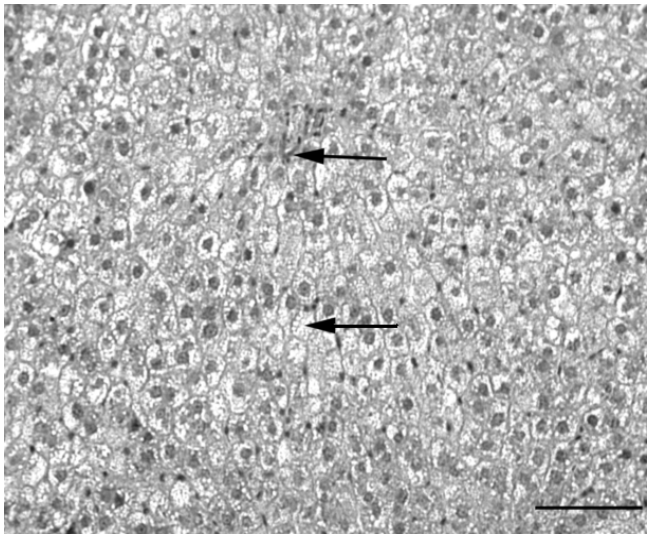

Figure 1. Fatty liver (Arrows show the diffuse hepatocellular degeneration with balloon cell-like hepatocytes in the liver of animals fed with fat-rich diet. Bar scale: 100 μm)

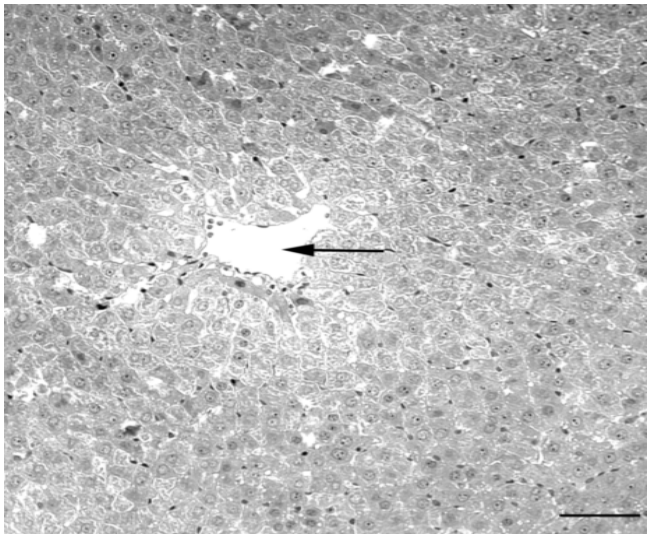

Figure 2. Hepatic lobule shows the beneficial effect of Újfehértói fürtös sour cherry treatment in hyperlipidemic animals (Arrow shows the vena centralis. Bar scale: 100 μm)

Table 1. Element concentrations (μg/g) in rat liver of control groups (n=5)

	Control		Control+M1		Control+M2		Control+M3	
	Main	**Standard deviation**	**Main**	**Standard deviation**	**Main**	**Standard deviation**	**Main**	**Standard deviation**
Al	0.388	0.330	1.006	1.629	0.117	0.082	0.479	0.382
B	1.62	1.426	1.511	0.279	0.635	0.416	1.587	0.945
Ba*	0.0351	0.014	0.028	0.003	0.0195	0.006	0.0318	0.013
Ca	2.00	0.85	3.15	1.29	2.03	0.84	1.77	0.66
Co	0.0051	00.001	0.004	0.0001	<dl	-	<dl	-
Cu	0.186	0.019	0.180	0.027	0.175	0.036	0.252	0.101
Fe	6.78	1.37	7.79	1.67	6.26	1.69	8.83	5.83
Li	0.0043	0.0030	0.0023	0.0010	0.0019	0.0010	0.0029	0.0010
Mg	8.52	0.944	8.23	0.954	8.08	1.41	9.37	1.41
Mn	0.102	0.025	0.107	0.023	0.0861	0.017	0.099	0.016
Ni	0.0112	0.006	0.0080	0.0030	0.0073	0.0060	0.0210	0.0190
P	187.9	18.9	179.4	17.9	169.3	31.3	195.8	28.7
Pb	0.0370	0.0300	0.0258	0.020	0.0282	0.0001	0.0104	0.0001
Sr	0.0053	0.0040	0.0031	0.0010	0.0024	0.0020	0.0025	0.0020
Zn	1.34	0.17	1.44	0.25	1.29	0.28	1.48	0.28

*significant difference between control and control+M2, M1: Pipacs 1 type sour cherry, M2: Fanal type sour cherry, M3: Újfehértói fürtös type sour cherry,<dl under detection limit

It can be stated that sour cherry consumption indicates protective effect on fatty liver, because lipid droplets appear in less quantity than in the necrotic liver. Supposing the bioactive content is similar in every types of sour cherry; however its quantity is different. It is also considerable that the environmental factors may have some influence on the content quality.

Metal elements

The results of control groups are shown in Table 1, while the results of fat-rich diet groups are in Table 2.

According to the results in Table 1, the consumption of Pipacs1 (M1) causes raising tendency in the concentration of Al, Ca, Fe, Mn and Zn in the control group. The ascent of the metal elements with Fanal (M2) was different, because only Ca, Fe and Ni concentrations altered considerably. The most essential changes of metal element concentration occurred by Újfehértói fürtös (M3). Considerable increase was observed in Al, Cu, Fe, Mg, Ni, P and Zn concentrations. The control group shows only one significant difference in the Ba concentration, which is caused by Fanal (M2).

In Table 2 the concentrations of most metal elements, such as Al, B, Cu, Mg, Mn, Ni, P and Zn are raised by the effect of sour cherry Pipacs1 (M1) consumption. Three metal elements (Cu, Mg, Mn) show raising tendency in the group with Fanal (M2), and only two (Al, B) in the group with Újfehértói fürtös. Significant decrease can be apparent in the concentration of Ba, Fe, Sr in fatty liver, which is brought about by Pipacs1 (M1). Beside that Fanal (M2) results significant reduction in the concentration of B, Ca, and Sr. The concentrations of Al, Ba, Ca, Fe, Sr are significantly decreased by the effect of Újfehértói fürtös compared to the atherogen group. The results of ANOVA show that the metal element content in the liver is mostly influenced by Újfehértói fürtös (M3).

On average the changes of the metal element concentration between the control and fat-rich diet fed groups, were different by the effect of each of the sour cherry consumption. Compared to the fat-rich diet fed groups, the changes of the metal element concentrations were almost the opposite, except the results of Fanal (M2), which shows almost the same decreasing tendency in the fat-rich diet fed and control groups as well.

Discussion

On the basis of histological studies it was established that treatment with Fanal was the best, although Pipacs and Újfehértói fürtös were also significant in liver regression of hyperlipidemic animals with fatty liver.

The results also showed that none of the three types of sour cherry change the metal-homeostasis in the control groups. Therefore, it may be considered that, sour cherry don't have a negative effect on the healthy metal-homeostasis.

In conclusion to the result of our experiment all the three types of sour cherry have a positive effect on the metal-homeostasis and its treatment is also beneficial to lower the hyperlipidemy and fatty degeneration.

Table 2. Element concentrations (μg/g) in rat liver of atherogen groups (n=5)

	Atherogen		Atherogen+M1		Atherogen+M2		Atherogen+M3		ANOVA between C, C+MX, A and A+MX
	Main	Standard deviation	Main	Standard deviation	Main	Standard deviation	Main	Standard deviation	
Al ****	0.268	0.221	0.437	0.466	0.141	0.0795	0.913	0.395	M3
B ***, ****	0.769	0.266	1.922	1.223	0.358	0.1079	1.3007	0.593	-
Ba*,**,****	0.0371	0.003	0.0278	0.0028	0.0293	0.0211	0.0265	0.007	-
Ca ***,****	2.75	0.799	2.56	1.288	1.49	0.3990	1.66	0.754	-
Co	0.003		<dl		<dl		0.002		-
Cu	0.155	0.029	0.176	0.0250	0.175	0.0289	0.135	0.030	M3
Fe **,****	7.14	1.232	4.51	0.484	5.61	2.26	4.01	1.63	M1
Li	0.0041	0.0040	0.0034	0.0016	0.0031	0.0022	0.0023	0.001	-
Mg	7.92	0.708	8.10	0.569	8.01	0.884	7.0014	1.23	M3
Mn	0.0692	0.0050	0.0714	0.0077	0.0794	0.0167	0.0609	0.014	M1, M3
Ni	0.0069	0.0040	0.0289	0.0257	0.0067	0.0058	<dl	<dl	-
P	162.2	11.89	167.5	16.71	161.1	17.62	140.4	31.36	M3
Pb	0.0978	0.156	0.0522	0.0025	<dl		<dl		-
Sr **,***,****	0.0063	0.002	0.0034	0.0019	0.0021	0.0019	0.0023	0.001	M3
Zn	1.344	0.326	1.409	0.1809	1.3004	0.1703	1.13	0.277	-

significant difference between A and A+M1, * significant difference between A and A+M2, **** significant difference between A and A+M3; M1: Pipacs 1 type sour cherry, M2: Fanal type sour cherry,M3: Újfehértói fürtös type sour cherry,; <dl under detection limit

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