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Antihyperlipidemic, antioxidant and weight-lowering effects of “Vitalplant”

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Abstract: BACKGROUND: “VITALPLANT” medicinal plant mixture, aimed at body weight regulation and metabolism enhancement was formulated in order to be incorporated as an ingredient in functional food formulations on the basis of known biological activity of the alder buckthorn bark, peppermint, caraway and parsley. METHODOLOGY: The body weight-lowering potential, hypolipidemic effects and antioxidant activity of “Vitalplant” mixture were examined on five groups of male Wistar rats on different food regimes for 14 weeks. RESULTS: “Vitalplant” mixture supplementation caused an increase in food intake, but induced a decrease in body weight gain. The lipid status and atherogenic index (AI) were significantly improved in “Vitalplant” mixture supplemented hyperlipidemic (HF) groups in comparison to HF group. “Vitalplant” mixture was able to influence the activity of antioxidant enzymes and to protect erythrocytes of the rats fed the HF diet from oxidative damage, which was indicated by significantly lower malondialdehyde (MDA) levels in “Vitalplant”- HF groups in comparison to the HF group. CONCLUSION:

“Vitalplant” mixture seemed to be beneficial for the reduction of body weight and improvement of antioxidant status of the erythrocytes, and its antihyperlipidemic property was highly active for enhancing the profile of plasma lipids in rats.

Keywords: Antihyperlipidemic, Antioxidant, Peppermint, Caraway, Parsley, Alder buckthorn, Rats, In vivo

1 Introduction

Obesity is a disarray of energy balance and primarily considered as a disorder of lipid metabolism [1]. It is one of the major public health problems in the world because of its association with an increased risk of various chronic diseases, including cardiovascular diseases, type 2 diabetes, hypertension, dyslipidemia and cancer [2].

Hyperlipidaemia is mainly characterised by increased levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) cholesterol along with a decrease in high-density lipoprotein (HDL) cholesterol. This condition is an indicator of both coronary artery disease and atherosclerosis and is the main cause of cardiovascular disease worldwide [3]. Treatment of hyperlipidemia involves diet control, exercise and pharmaceutical therapy.

Polyphenolic plant compounds (especially flavonoids) have been shown to offer protection against atherosclerosis and metabolic disorder (hyperlipidemia, hyperglycaemia, hypercholesterolemia) related processes [4]. These compounds provide protection via a range of mechanisms including lowering the concentration of plasma non-HDL cholesterol, reducing serum lipid oxidation, lowering vascular resistance [5] and altering cellular inflammatory signalling pathways [6].

Medicinal plants have long been known as sources of biologically active substances. Because of their long traditional use, they appeal to the consumers who question the safety of synthetic food additives. Essential

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oil component apiole - a proven kidney stimulant and parsley (*Petroselinum crispum* (Mill.) A.W. Nym. ex Hill) are known for their diuretic action. Peppermint (*Mentha x piperita* L.) and caraway (*Carum Carvi* L.) essential oils are frequently used in herbal drugs for treatment of abdominal discomfort and pain, while the alder buckthorn bark (*Rhamnus Frangula* L.) contains anthraquinone glycosides with purgative effects [7].

By using the above-mentioned medicinal plants, a mixture – “VITALPLANT” aimed at body weight regulation and metabolism enhancement was formulated. Selected medicinal plants and the mixture were already shown to be a rich source of plant phenolics [8] and to possess antioxidant activity by *in vitro* tests [7]. A relatively high *in vitro* antioxidant activity of “Vitalplant” mixture was explained by synergistic effects of its components [7]. In addition, regardless of the way it is incorporated into cookies, either in the form of alcoholic extracts or as a powdered mixture, “Vitalplant” addition was shown to be efficient in improving antioxidant activity and oxidative stability of the cookies in a dose-dependent manner [7-9].

Referring to the known pharmacological action of “Vitalplant” biologically active substances and proven *in vitro* antioxidant activity, the aim of this work was to test weight-lowering, antihyperlipidemic and antioxidant properties of “Vitalplant” mixture in an animal model.

2 Experimental Procedures

2.1 Preparation of experimental diets

“Vitalplant” mixture (*Rhamnus Frangula* L. bark (35%), *Mentha x piperita* L. leaves (20%), *Carum Carvi* L. fruit (20%) and *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill fruit (25%)) in dried powder form was obtained from the Institute for Medicinal Plants Research “Dr Josif Pančić”, Belgrade, Serbia, where the plants were identified and the voucher specimens were deposited (*Rhamnus Frangula* L. No. 13510412, originated from Bosnia and Herzegovina; *Mentha x piperita* L. No. 36681113, originated from Serbia; *Carum Carvi* L. No. 05560213, originated from Lithuania; *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill No. 15190513, originated from Serbia). A commercial complete mixture for laboratory rats, containing 20% proteins in a powder form was obtained from the Veterinary Institute JSC, Subotica, Serbia. The mixture was processed at a pilot plant to obtain the experimental diets. The control diet was obtained by steam conditioning of the commercial

powder at 80°C in a batch type steam conditioner (Muyang SLHSJ0.2A, China). Afterwards, steam conditioned mixture was extruded in a single screw extruder (OEE 8, Amandus Kahl, Germany) at 103°C, to obtain granules with diameter of 11.5 mm. “Vitalplant” supplemented control (VP-5%) was prepared the same way, after supplementing the commercially available powder with 5% “Vitalplant” mixture. Hyperlipidemic diets, high fat diet (HF) and “Vitalplant” supplemented high fat diet (HFVP-5%), were obtained after 2.5% cholesterol, 20% sunflower oil and 0.5% sodium cholate were added to the control and VP-5% granules by vacuum coating technology.

2.2 Chemical analysis of experimental diets

Standard methods of analysis [10] were used to determine crude protein, fat, reducing sugar, crude cellulose, starch and water content in experimental diets. For protein determination, a nitrogen-to-protein conversion factor of 6.25 was used. Gross energy content of feed was measured by oxygen calorimetric bomb (model AC 500, Leco, USA) calibrated using benzoic acid.

Direct saponification of the sample (0.4 M potassium hydroxide in ethanol and heating at 60°C for 1 h) was applied for the determination of total cholesterol. Unsaponifiable matter was extracted using hexane and was washed with water until the extract was free of alkalis. After evaporation to dryness, the extract was dissolved in HPLC grade ethanol and filtered through 0.45 µm pore size PTFE filters (Rotilabo-Spritzenfilter 13 mm, Roth, Karlsruhe, Germany) before injection into the HPLC system [11]. HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD) and an evaporative light-scattering detector – ELSD (Agilent G4218A LT-ELSD) on an Agilent, Eclipse XDB-C18, 1.8 µm, 4.6 × 50 mm column, at a flow-rate of 1.000 mL min⁻¹. Methanol was used as a mobile phase. Separated compounds were detected using DAD at 212 nm with reference wavelength set at 550/100 nm and the spectra were acquired in the range 210–400 nm. Run time was 10 min, and the post time 5 min.

Experimental diets were extracted with ethanol for the determination of Na-cholate [12]. Mobile phase consisting of the mixture of methanol and 1% (v/v) HCOOH in water (75/25) was injected into the same apparatus and column, as used for the determination of cholesterol. Na-cholate was detected using ELSD detector set up as follows: nitrogen carrier gas pressure 3.5 bar, temperature 40°C, gain 1.

2.3 Experimental animals' treatment

Experiments were carried out on forty male Wistar rats, (four months age, b.w. 295-388 g), obtained from the vivarium (Galenika a.d., Belgrade, Serbia). All experiments and protocols employed in the study were reviewed and approved by the Institutional Animal Care and Use Committee [No. III-2011-01]. Experimental animals were housed in groups of two or three per standard cage, in a room with a 12 h light-dark cycle and an ambient temperature of 24°C. All rats were fed a pelletized commercial chow diet for 2 weeks after arrival. They were then randomly divided into five groups. The animals from group I (control) were fed normal chow (n = 11). The rats of the group II were fed with VP-5% (n = 6). The group III (n = 11) was fed with the HF diet and the group IV (n = 6) was fed with the “Vitalplant” supplemented lipogenic diet HFVP-5%. This regime was maintained for 14 weeks. The animals of the group V were maintained on the same food regime as the animals in the group III (n = 6). After 6 weeks, animals were reported to have doubled the plasma cholesterol concentration and they were maintained on the same food regime as the animals in the group IV for 8 weeks. The composition of the experimental diet is shown in Table 1. Calculated energy and gross energy values of the high-fat diets (HF and HFVP) were significantly higher compared to other diets due to differences in dietary fat content. The animals were given food and tap water *ad libitum* during the experimental period. Food consumption was measured daily. Weight gain and faecal mass were measured weekly.

At the beginning (0 day), and after 6 and 14 weeks of experiment, the animals were fasted overnight and then sacrificed under ether anaesthesia. Blood samples (5-6 ml) were collected from the inferior vena cava in tubes containing heparin as anticoagulant, and plasma was separated for biochemical analysis.

2.4 Biochemical analysis

2.4.1 Plasma lipid levels and transaminase activities

The levels of plasma total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), alanine transaminase (ALT) and aspartate transaminase (AST) were measured using the automated enzymatic methods (Roshe Diagnostics GmbH, Mannheim, Germany) by Cobas c111 analyzer.

2.4.2 Oxidative stress and antioxidant protection capacity

Blood samples were centrifuged at 15,000 g for 15 minutes at +4°C where the erythrocytes were separated and the plasma samples were then frozen at -20°C until the analyses. After two washes with saline solution (0.9% NaCl), an equal volume of water was added to the erythrocytes and they were centrifuged at 15, 000 g for 15 minutes. The resulting supernatant represented the hemolysate, which was aliquoted and stored at -20°C until

Table 1: Composition of the control and the experimental diets (VP-5% control supplemented with 5% “Vitalplant “ mixture; HF-high fat; HFVP-5% high-fat supplemented with 5% “Vitalplant” mixture).

	Control	VP	HF	HFVP	Control powder
Protein (%)	20.44 ± 0.09 ^d	19.76 ± 0.10 ^c	15.72 ± 0.04 ^b	15.57 ± 0.01 ^a	20.92 ± 0.03 ^e
Moisture (%)	9.40 ± 0.01 ^a	9.46 ± 0.05 ^a	6.87 ± 0.07 ^b	7.31 ± 0.03 ^c	8.22 ± 0.02 ^d
Ash (%)	6.19 ± 0.02 ^a	6.61 ± 0.07 ^d	4.80 ± 0.01 ^b	5.22 ± 0.08 ^c	6.13 ± 0.04 ^a
Cellulose (%)	4.82 ± 0.02 ^c	6.28 ± 0.05 ^e	2.16 ± 0.01 ^a	4.23 ± 0.02 ^b	4.98 ± 0.07 ^d
Fat (%)	1.51 ± 0.01 ^a	2.03 ± 0.01 ^b	20.91 ± 0.01 ^d	23.05 ± 0.01 ^e	2.41 ± 0.01 ^c
Reducing sugars (%)	2.90 ± 0.04 ^c	1.31 ± 0.01 ^b	1.16 ± 0.04 ^a	1.13 ± 0.01 ^a	5.18 ± 0.04 ^d
Starch (%)	36.68 ± 0.01 ^a	36.46 ± 0.20 ^a	30.79 ± 0.15 ^c	29.93 ± 0.15 ^b	38.23 ± 0.14 ^d
Cholesterol (mg/g)	0.80 ± 0.02 ^a	0.82 ± 0.12 ^a	12.37 ± 0.98 ^b	11.52 ± 1.82 ^b	0.22 ± 0.06 ^a
Na-cholate (mg/g)	-	-	5.95 ± 0.15	4.75 ± 0.30	-
Calculated energy value (kJ/100 g)*	1558	1518	1937	1985	1544
Gross energy value (kJ/100 g)	1621 ± 3.50 ^b	1643 ± 6.42 ^c	2086 ± 25.3 ^a	2091 ± 18.5 ^a	1610 ± 2.48 ^b

Values are expressed as mean ± SD

* calculated from the chemical composition by the calculator [13]

^{a,b,c,d,e} Means in the same row not sharing a common superscript are significantly different (p < 0.05) between groups

the analysis. The catalase (CAT), glutathione S-transferase (GST), the superoxide dismutase (SOD), the glutathione peroxidase (GPx) and other parameters of oxidative stress were measured in the hemolysate. The hemoglobin level was measured with Drabkin's Reagent [14]. The activities of all determined enzymes in the hemolysate samples were expressed in the units per mg hemoglobin (U/mg Hb), while the malondialdehyde levels (MDA) in nmol per mg hemoglobin.

- CAT activity was determined according to Aebi [15]. The GST activity was measured according to the Habig *et al.* [16] method. The amount of the reduced glutathione (GSH) was measured using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid DTNB in phosphate buffer), based on the amount of thiols, i.e. non-protein sulfhydryl residues [17]. The data are expressed as nmol of GSH per mg Hb. Before the determination of SOD activity, the hemolysates were treated with ethanol/chloroform mixture (hemolysate:ethanol:chloroform = 1:1:0.6) to remove hemoglobin from the samples, and then were centrifuged at 15,000 g for 3 min. The supernatants were used for SOD activity determinations. SOD activity was measured by Misra and Fridovich [18] method in the sample free of hemoglobin in system cytochrom c (Fe^{3+})/xantine/xantine oxidase, while the GSH-Px activity was determined using method of Chin *et al.* [19]. The MDA levels in both plasma and hemolysate samples were estimated by TBARS Assay Kit (Cayman Chemicals, USA). The antioxidant activity was determined measuring total antioxidant capacity (TAC) using a commercially available kit, *OxiSelect™ TAC Assay Kit* (purchased from *Cell Biolabs*, USA).

The spectrophotometric assays were performed with a Nicolet Evolution 100 UV/VIS scanning spectrophotometer equipped with a cell positioned or with a multi plate reader Thermo LabSystem Multiskan EX.

2.5 Statistical analysis

The results were expressed as mean values \pm standard deviation (SD). The statistical significance was determined using one-way analysis of variance (ANOVA). The differences between control and experimental diets were determined by the Turkey's test. Values of $p < 0.05$ were considered significant.

3 Results

3.1 Food intake, weight gain, and organ weights

All high-fat diet groups III, IV and V exhibited significantly lower food intake ($p < 0.05$) when compared to the normal diet groups, without (group I) or with (group II) "Vitalplant" mixture (Table 2). Interestingly, the food intake within the HF groups was significantly higher ($p < 0.05$) in groups IV and V than in the group III.

After 14 weeks of the experiment, body weight gain and the food efficiency ratio (FER) was significantly higher ($p < 0.05$) in the group III compared to other groups (Table 2). The average weight gains and FER of groups IV and V were not significantly different from that of groups I and II ($p > 0.05$). The supplementation of "Vitalplant" mixture contributed to an increased food intake, but decreased

Table 2: Food intake, weight gain, and weight of organs in the control (I) and the experimental groups of rats (II-control supplemented with 5% "Vitalplant" fed group; III-high-fat fed group; IV-high-fat supplemented with 5% "Vitalplant" fed group; V-high-fat fed for 6 weeks and high-fat supplemented with 5% "Vitalplant" for 8 weeks fed group) after 14 weeks of the experiment.

Group	I	II	III	IV	V
Food intake (g/day)	23.18 \pm 0.95 ^a	25.60 \pm 0.67 ^b	17.73 \pm 0.71 ^c	19.32 \pm 0.73 ^d	19.06 \pm 0.88 ^d
Weight gain (g/day)	9.51 \pm 0.62 ^a	8.57 \pm 0.72 ^a	13.65 \pm 2.12 ^b	9.14 \pm 0.83 ^a	10.68 \pm 1.65 ^a
FER*	0.40 \pm 0.08 ^a	0.33 \pm 0.07 ^a	0.73 \pm 0.11 ^b	0.44 \pm 0.04 ^a	0.51 \pm 0.07 ^a
Feces (g/day)	11.14 \pm 1.45 ^a	17.52 \pm 1.80 ^b	6.35 \pm 0.99 ^c	7.77 \pm 1.18 ^d	8.67 \pm 1.63 ^e
Organ weights (mg/g b.w.)					
Heart	2.90 \pm 0.19 ^a	2.85 \pm 0.16 ^a	2.97 \pm 0.14 ^a	3.07 \pm 0.16 ^a	3.18 \pm 0.18 ^a
Liver	26.13 \pm 2.23 ^a	31.17 \pm 1.74 ^b	41.49 \pm 1.41 ^c	41.41 \pm 2.58 ^c	46.67 \pm 1.67 ^d
Kidney	3.93 \pm 0.21 ^a	3.85 \pm 0.23 ^a	3.89 \pm 0.29 ^a	3.91 \pm 0.28 ^a	3.92 \pm 0.15 ^a
Brain	4.14 \pm 0.73 ^a	4.30 \pm 0.65 ^a	4.00 \pm 0.61 ^a	3.42 \pm 0.66 ^a	4.49 \pm 0.47 ^a

Values are expressed as mean \pm SD

* Food efficiency ratio: weight gain/food intake

^{a,b,c,d,e} Means in the same row not sharing a common superscript are significantly different ($p < 0.05$) between groups

body weight gain when compared with HF groups, which is in agreement with the significantly higher ($p < 0.05$) faecal mass in groups IV and V. The highest faecal mass was measured in the group II compared to other groups.

Organ weights were expressed as their relative weight per body weight (Table 2). The relative weights of liver were significantly higher in HF groups than in groups I and II ($p < 0.05$). Increase in the liver weight in the HF groups was attributed to the high-fat diet. However, the heart, kidney and brain weights were not significantly different between the groups ($p < 0.05$).

3.2 Biochemical analysis

3.2.1 Plasma lipid levels and transaminase activities

Concentrations of plasma lipids and transaminase activities after 0 day, 6 and 14 weeks of the experiment are shown in Table 3. After 6 weeks of the experiment, a significant difference was observed in plasma triglycerides, total cholesterol, low-density lipoprotein cholesterol levels and atherogenic index (AI) between groups I and III. An increase in total cholesterol level and atherogenic index by 101.5% and 284.0%, respectively was observed in group III (with induced hyperlipidemia). After that period, feeding regime was changed to the group V of animals from a lipogenic diet to a lipogenic diet with "Vitalplant".

In the next 8 weeks the supplementation of "Vitalplant" to the group V animals significantly lowered TC by 29.6%, LDL by 32.1%, AI by 56.2%, ALT by 13.1%, and significantly increased HDL by 11.6%, compared to the group III animals ($p < 0.05$). Also, after 14 weeks the supplementation of "Vitalplant" to the group IV

significantly lowered TC by 18.2%, LDL by 18.8%, AI by 43.3%, ALT by 18.0%, and significantly increased HDL by 11.6%, compared to the group III ($p < 0.05$), which indicated that "Vitalplant" mixture had anti-hyperlipidemic effect. TG concentrations and AST activities were not significantly different ($p > 0.05$) between groups III, IV and V.

After 14 weeks of the experiment there were no significant differences in HDL and transaminase activities, while significant differences were observed in TG, TC, LDL and AI when comparing groups I and II with groups IV and V ($p < 0.05$). Compared with groups I and II, there were significant differences in all analyzed parameters, except HDL and AST in the group III with induced hyperlipidemia ($p < 0.05$).

In our study, as well as in previous feeding experiments with rats [20], it was observed that addition of cholesterol, fat component and cholic acid to normal diet increase plasma cholesterol concentration, i.e. enhance the hyperlipidemic effect.

TC, LDL, AI and ALT were significantly lower, and HDL significantly higher in rats fed a high-fat diet supplemented with "Vitalplant" mixture but the supplement had no effect in cholesterol-free diet group (group II). It has been reported that the hypocholesterolemic effect of dietary fiber and polyphenolic compounds were more effective in hypercholesterolemic animals and humans than in normocholesterolemic ones [21].

3.2.2 Oxidative stress and antioxidant capacity

The activity of enzymes, such as CAT, SOD, GSH-Px, GST, were measured in the homogenate samples to determine the antioxidant activity of the erythrocytes *in vivo*.

Table 3 Biochemical parameters in plasma of the control (I) and the experimental groups of rats (II-control supplemented with 5% "Vitalplant" fed group; III-high-fat fed group; IV-high-fat supplemented with 5% "Vitalplant" fed group; V-high-fat fed for 6 weeks and high-fat supplemented with 5% "Vitalplant" for 8 weeks fed group) after 14 weeks of the experiment.

	Group	TG (mmol/L)	TC (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	AI*	AST (IU/L)	ALT (IU/L)
0 day	I	0.43 ± 0.08	1.02 ± 0.16	0.82 ± 0.12	0.17 ± 0.06	0.25 ± 0.06	137 ± 19	45 ± 7
6 week	I	1.20 ± 0.12 ^a	1.37 ± 0.15 ^a	0.91 ± 0.10 ^a	0.22 ± 0.02 ^a	0.50 ± 0.03 ^a	157 ± 32 ^a	52 ± 3 ^a
	III	0.40 ± 0.09 ^b	2.76 ± 0.32 ^b	0.91 ± 0.05 ^a	2.04 ± 0.29 ^b	1.92 ± 0.28 ^b	138 ± 27 ^a	54 ± 10 ^a
14 week	I	0.90 ± 0.30 ^a	1.47 ± 0.08 ^a	1.25 ± 0.06 ^{ac}	0.22 ± 0.05 ^a	0.28 ± 0.01 ^a	113 ± 12 ^a	53 ± 3 ^a
	II	1.05 ± 0.16 ^a	1.28 ± 0.15 ^a	1.02 ± 0.09 ^a	0.19 ± 0.03 ^a	0.40 ± 0.08 ^a	117 ± 15 ^a	47 ± 2 ^a
	III	0.43 ± 0.07 ^b	3.68 ± 0.31 ^b	1.21 ± 0.03 ^a	3.15 ± 0.42 ^b	2.03 ± 0.26 ^b	120 ± 27 ^a	61 ± 8 ^b
	IV	0.41 ± 0.03 ^b	3.01 ± 0.27 ^c	1.35 ± 0.10 ^c	2.55 ± 0.30 ^c	1.15 ± 0.12 ^c	110 ± 25 ^a	50 ± 2 ^a
	V	0.41 ± 0.06 ^b	2.59 ± 0.26 ^d	1.35 ± 0.22 ^c	2.14 ± 0.23 ^d	0.89 ± 0.11 ^d	112 ± 24 ^a	53 ± 2 ^a

Values are expressed as mean ± SD

TG - triglycerides; TC - total cholesterol; HDL-c - high-density lipoprotein cholesterol; LDL-c - low-density lipoprotein cholesterol; AST - aspartate transaminase; ALT - alanine transaminase

*Atherogenic index: (TC-HDL-c)/HDL-c

^{a,b,c,d} Means in the same column and time period not sharing a common superscript are significantly different ($p < 0.05$) between groups

Our results indicate that CAT, GSH-Px and SOD activities were significantly increased ($p < 0.05$) in the homogenate samples of the group III in comparison with the other groups (Table 4). High activity of SOD in the group III may be a result of an adaptive cell mechanism, which was a result of the oxidative stress induced by the high-fat diet. It has been reported that oxidative stress increased SOD levels [22].

MDA levels were significantly increased ($p < 0.05$) in group III in comparison to the other groups (Table 4), which was most probably the result of oxidative damage to the lipids. These results were consistent with many studies pointing out that hyperlipidemia is closely associated with an increased lipid peroxidation [23].

As for the other examined parameters in the erythrocyte samples, such as GST and GSH, only mild increase in GST level was recorded in the group III during the experiment, but this increase was not statistically significant (Table 4). CAT, GSH-Px and SOD seem to provide the most important antioxidant enzyme protection in hemolysate samples of the hyperlipidemic rats.

“Vitalplant” herbal mixture supplementation of the high-fat diet caused a significant decrease ($p < 0.05$) in CAT, GSH-Px and SOD enzyme activities in groups IV and V in comparison to group III. The activities of these enzymes were similar to the activities in the control group. Furthermore, MDA content of groups IV and V was lower than of the group III. The difference between group III and group V was significant ($p < 0.05$) (Table 4), which confirms the results of a good antioxidant capacity of “Vitalplant” herbal mixture obtained by *in vitro* tests [7].

Significantly increased TAC level ($p < 0.05$) in group III is probably in a connection with the increased level of uric acid, which has been shown to be an indicator of the obesity [24].

Increased TAC level most likely showed the body response against the present oxidative stress in the group fed only with fatty chow. Decreased levels of TAC in group IV, but statistically significant ($p < 0.05$) only in the V group, indicated a fall in the concentration of uric acid, and clearly showed the protective effect of “Vitalplant” herbal mixture.

4 Discussion

This study showed that the supplementation of “Vitalplant” herbal mixture significantly increased food intake, but decreased body weight gain, plasma lipid concentrations (TC, LDL) as well as ALT and AI activities in rats that were fed a high-fat diet.

The body-weight lowering activity of “Vitalplant” mixture can be explained by the already known biological activity of its constituents: diuretic action of parsley and laxative activity of the alder buckthorn.

Parsley has been used in folk medicine as a remedy for many different ailments. In addition to its recognized role as a stomachic, carminative, emmenagogue and abortifacient, the herb has also gained a widespread reputation as a powerful diuretic. In addition to that, Leung [25] ascribes hypotensive properties to the plant, which could be considered a consequence of its diuretic effect. Rats offered an aqueous parsley seed extract to drink, eliminated a significantly larger volume of urine per 24 h as compared to when they were drinking water. The mechanism of action of parsley seems to be mediated through an inhibition of the $\text{Na}^+ - \text{K}^+$ pump that would lead to a reduction in Na^+ and K^+ reabsorption leading thus to an osmotic water flow into the lumen, and diuresis [26].

Table 4 Erythrocyte antioxidant status and oxidative stress in the control (I) and the experimental groups of rats (II-control supplemented with 5% “Vitalplant” mixture fed group; III-high-fat fed group; IV-high-fat supplemented with 5% “Vitalplant” mixture fed group; V-high-fat fed for 6 weeks and high-fat supplemented with 5% “Vitalplant” mixture for 8 weeks fed group) after 14 weeks of experiment.

	I	II	III	IV	V
CAT (U/mg Hb)	6564 ± 1160 ^b	6664 ± 1023 ^b	9336 ± 1228 ^a	5819 ± 1308 ^b	6372 ± 957.2 ^b
SOD (U/mg Hb)	44.22 ± 5.45 ^b	45.22 ± 4.24 ^b	65.68 ± 13.32 ^a	48.08 ± 4.97 ^b	45.33 ± 6.16 ^b
GPx (U/mg Hb)	1.84 ± 0.27 ^b	1.92 ± 0.22 ^b	2.67 ± 0.50 ^a	2.16 ± 0.46 ^{ab}	1.46 ± 0.32 ^{bc}
GST (mU/mg Hb)	14.15 ± 1.42 ^a	14.52 ± 1.80 ^a	15.24 ± 2.85 ^a	14.85 ± 1.28 ^a	14.39 ± 1.06 ^a
GSH (nmol/mg Hb)	7.44 ± 0.80 ^a	7.32 ± 0.45 ^a	7.65 ± 1.66 ^a	9.16 ± 1.00 ^a	9.63 ± 2.40 ^a
MDA (nmol/mg Hb)	2.13 ± 0.28 ^b	2.19 ± 1.74 ^b	3.47 ± 1.02 ^a	2.89 ± 0.55 ^{ab}	1.92 ± 0.43 ^b
TAC (nmol UAE/mg Hb)	60.53 ± 8.09 ^a	62.02 ± 8.21 ^a	85.85 ± 12.46 ^b	79.25 ± 17.04 ^a	68.94 ± 11.39 ^a

Values are expressed as mean ± SD.

CAT - catalase; SOD - superoxide dismutase; GPx - glutathione peroxidase; GST - glutathione S-transferase; GSH - reduced glutathione; MDA - malondialdehyde; TAC - total antioxidant capacity

^{a,b,c} Means in the same row not sharing a common superscript are significantly different ($p < 0.05$) between groups

The alder buckthorn (*Rhamnus Frangula* L.) bark, belongs to the stimulant laxatives. Emodin-9-anthrone is the most important metabolite, which is produced by the bacteria of the large intestine. The mode of action is based on two mechanisms. Firstly, colonic motility is increased leading to a reduced transit time. Secondly, an influence on secretion processes by two concomitant mechanisms, namely inhibition of absorption of water and electrolytes (Na⁺, Cl⁻) into the colonic epithelial cells (antiabsorptive effect) and an increase in the leakiness of the tight junctions and stimulation of secretion of water and electrolytes into the lumen of the colon (secretagogue effect), results in enhanced concentrations of fluid and electrolytes in the lumen of the colon [27].

Combined action of parsley and the alder buckthorn bark probably resulted in the enhanced metabolic activity, which prevented excessive weight gain in the rats of the groups IV and V in our experiment. Apart from that, according to Williams et al. [28] polyphenols are a class of phytochemicals that are likely candidates as anti-obesity agents due to their ability to modulate the adipocyte lifecycle as shown in several studies. The strongest evidence for this effect comes from: phenolic acid derivatives such as chlorogenic; flavonols e.g. quercetin; and flavones such as luteolin.

In the previous study, analysis of phenolic composition of “Vitalplant” mixture showed the presence of the compounds originating from the herbs of which it was composed. “Vitalplant” mixture contains a wide range of phenolic compounds, free phenolic acids and phenolic acids derivatives. Flavonoids are mostly presented in glycosidic form [8].

Anti-obesity mechanisms of phytochemicals appear to involve mediation of complex and interconnected cell signalling pathways, therefore the combination of multiple phytochemicals may give rise to synergistic and enhanced anti-obesity effects [28].

Caraway fruit (*Carum carvi* L.) has traditionally been used to treat diabetes, cardiovascular diseases and hypertension. Lemhadri et al. [29] demonstrated significant hypocholesterolemic effects of caraway aqueous extracts in both normal and severe hyperglycemic rats after repeated oral administration, but he could not elucidate the mechanism of action.

Badal et al. [30] found that an oral administration of aqueous leaves' extract of *Mentha piperita* decreased the plasma glucose concentration of fructose-fed rats in a dose-dependent manner, showing the beneficial action of *Mentha piperita* in rats with insulin resistance. In their experiment, treatment with the aqueous extract of *Mentha piperita* leaves for 21 days significantly reduced serum total cholesterol,

triglycerides and LDL-C associated with concomitant significant increase in HDL-C levels and decrease in atherogenic index in hyperlipidemic rats indicating its potent antihyperlipidemic and antiatherogenic activity. The glucose lowering action of the extract was explained by the improved lipid metabolism, apart from the direct interaction with glucose homeostasis. The triglyceride lowering properties (activity) of extract can indirectly contribute to the overall antihyperglycemic activity through a mechanism called glucose-fatty acid cycle [30].

The erythrocyte is pivotal to the overall antioxidant status of the blood, as the red cell functions to maintain plasma antioxidants in the reduced state, and eliminates superoxide and hydrogen peroxide derived from the plasma. Furthermore, the blood is a tissue that comes into direct contact with the endothelium and previous *in vitro* studies have shown that intact erythrocytes can protect endothelial cells against oxidant-induced damage [31].

In our experiments, “Vitalplant” mixture was shown to be able to protect erythrocytes of the rats fed the high-fat diet from oxidative damage, which was indicated by significantly lower MDA levels in groups IV and V. Although some studies suggested that feeding rats with plant materials rich in plant phenolics increased the activity of antioxidant enzymes [32]. The results of the present study show a decrease in the antioxidant enzymes, possibly due to the capacity of dietary antioxidants, phenolic compounds from “Vitalplant” mixture, to scavenge oxygen radicals and consequently reduce the need for enzymatic endogenous antioxidants. Our results are consistent with the results of da Silva Pereira et al. [33].

Finally, “Vitalplant” mixture consisting of *Rhamnus Frangula* L. bark, *Mentha x piperita* L. leaves, *Carum carvi* L. fruit and *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill fruit in dried and powdered form was shown to be efficient in decreasing body weight gain, plasma lipid concentrations (TC, LDL) and ALT and AI activities in rats fed a high-fat diet, indicating its potential as an ingredient for functional food formulations. Previously estimated by *in vitro* tests, antioxidant activity of “Vitalplant” mixture was confirmed by *in vivo* tests.

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Conflict of interest: Dr. Popović, dr. Đurendić-Brenesel, dr. Pilića, dr. M. Milić, dr. Mišan and dr. Arsić report to have a patent at national level issued. Dr. Kojić and dr. N. Milić have nothing to disclose.

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