ANTI-ATHEROGENIC PROPERTIES OF A FUNCTIONAL HERBAL MIXTURE

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In this work, anti-atherogenic and anti-hyperlipidemic effects of a herbal mixture, rich in polyphenols, and composed of 35% of buckthorn bark (Frangulae cortex), 20% of mint leaves (Menthae piperita folium), 20% of caraway fruit (Carvi fructus) and 25% of parsley fruit (Petroselinum fructus) were studied by monitoring biochemical parameters in experimental animals. Experimental animals (Wistar rats) were subjected to five different feeding regimes. Plasma levels of total cholesterol, triglycerides, cholesterol bound to high-density lipoproteins (HDL) and cholesterol linked to low-density lipoproteins (LDL) were monitored in different time periods. The feces of experimental animals was analysed for seven bile acids, as well as for total cholesterol. Simultaneously, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured. The introduction of herbal mixture into fatty food did not have significant influence on enzyme activities; however, the effect on induced hyperlipidemia was significant. Total atherogenic index was reduced by 43.3%, whereas total cholesterol and cholesterol bound to low density lipoproteins were reduced by 18.2% and 18.8%, respectively. Total bile acids concentrations dropped by 13.2%, whereas cholesterol was reduced by 33%.

KEY WORDS: herbal mixture, atherosclerosis, bile acids, anti-hyperlipidemic

INTRODUCTION

Atherosclerosis is a disease that causes more deaths and disabilities than any other disorder in the industrialized world, and more than all forms of cancer together (1). Compromised brain arteries due to atherosclerosis are the most common cause of stroke, while heart diseases are the result of coronary artery atherosclerosis. This health condition is caused by accumulation of cholesterol transported by lipoproteins in arteries,

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leading to tissue damage, inflammation and the appearance of fibroproliferative scarring. Tissue damage of the inner arterial walls leads to the formation of thrombi that can cause heart attack or stroke (1).

Intensive medical research opened the possibilities for the prevention and treatment of atherosclerosis and currently the most commonly applied medical treatments include catheterization, stenting and bypass surgery (2). Nevertheless, it is necessary to stress the importance of the prevention and treatment of atherosclerosis through appropriate diet and lifestyle, as well as the role of drugs that affect balance of cholesterol transporting lipoproteins.

The link between the development of coronary heart diseases, atherosclerosis and antioxidants is known for a long time. The formation and development of atherosclerotic lesions are affected both by fat in blood and antioxidants (3). Oxidation of low density lipoproteins (LDL) and consequent formation and growth of foam cells are responsible for the process of endothelial injury and arterial plaque deposition (4). Previous studies related to the influence of antioxidants focused mainly on examining the effects of dietary tocopherols (5,6) and carotenoids (7). Other studies also dealt with antioxidant effects of certain vitamins. The influence of vitamin E has been particularly emphasized (6). It was also proven that the levels of vitamin D were about 50% lower in the blood of people who suffered from atherosclerosis compared to healthy individuals (8).

Polyphenols are large group of naturally occurring compounds which have long been known to exhibit antioxidant properties (9). The effects of intake of foods rich in polyphenols on the development of atherosclerosis were studied in mice and hamsters with hereditary deficiency of apolipoprotein E (apoE - / -). Anti-atherogenic effects were observed for orally taken extracts of green and black tea (10, 11), pomegranate extract (12), grape extract (13) and red wine (14), thus previous studies mainly focused on catechin, anthocyanins and tannins. In this paper, anti-atherogenic effects of diet enriched with herbal mixture of parsley (Petroselinum crispum), peppermint (Mentha piperita), caraway (Carum carvi) and buckthorn (Rhamnus frangula) were studied by monitoring plasma levels of total cholesterol, cholesterol bound to low and high-density lipoproteins, atherogenic index and activities of aspartate aminotransferase and alanine aminotransferase in experimental animals.

**EXPERIMENTAL**

**Chemicals and reagents**

HPLC grade hexane, ethanol, methanol, formic and benzoic acid were obtained from Merck. Chemical sets for the determination of cholesterol, triglycerides and activities of alanine aminotransferase and aspartate aminotransferase were purchased from Sigma-Aldrich. Potassium hydroxide, magnesium chloride, sodium chloride and sodium carbonate were purchased from Centrohem (Stara Pazova). Bile acid and cholesterol standards were a kind gift of Prof. Mihalj Poša, from the Faculty of Medicine, Department of Pharmacy in Novi Sad.
Instruments

The levels of total cholesterol (TC), triglycerides (TG), cholesterol bound to high-density lipoproteins (HDL-c), alanine transaminase activity (ALT) and aspartate transaminase activity (AST) were determined using automated enzymatic method (ELITECH Diagnostic, Sees, France). HPLC analysis was performed by using a liquid chromatograph, 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 x 50 mm column, at a flow-rate of 1.000 mL min⁻¹.

Materials and methods

**Preparation of functional mixture.** The herbal mixture was prepared by mixing 35% of buckthorn 35% of buckthorn bark (Frangulae cortex), 20% of mint leaves (Menthae folium), 20% of caraway fruit (Carvi fructus), and 25% of parsley fruit (Petroselinum fructus). Control of individual plant sources included the determination of water content according to SRPS EN ISO 6540:2012, ash content according to SRPS EN ISO 2171:2012, and essential oil content according to SRPS E.38.018:1994 (Table 1).

**Table 1.** Characteristics of raw plant materials used for the preparation of functional mixture

<table>
<thead>
<tr>
<th>Plant</th>
<th>Origin of plant</th>
<th>Part used</th>
<th>Moisture content (%)</th>
<th>Ash content (%)</th>
<th>Essential oil content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mint (Mentha piperita)</td>
<td>Serbia</td>
<td>Leave</td>
<td>9.30</td>
<td>5.31</td>
<td>2.90</td>
</tr>
<tr>
<td>Parsley (Petroselinum crispum)</td>
<td>Serbia</td>
<td>Fruit</td>
<td>12.03</td>
<td>10.87</td>
<td>2.26</td>
</tr>
<tr>
<td>Caraway (Carum carvi)</td>
<td>Lithuania</td>
<td>Fruit</td>
<td>5.73</td>
<td>4.95</td>
<td>2.35</td>
</tr>
<tr>
<td>Buckthorn (Rhamnus frangula)</td>
<td>Bosnia and Herzegovina</td>
<td>Bark</td>
<td>3.38</td>
<td>9.86</td>
<td>1.90</td>
</tr>
</tbody>
</table>

In the prepared herbal mixture, the moisture content was 7.91%, ash content 6.71% and granulation 0.75 mm. The microbiological quality, pesticide residues, mycotoxins content and radioactivity corresponded to the European Pharmacopoeia requirements (15).

**Animal experiments.** Standard feed for experimental animals (mixture of 20% protein powder) was acquired from the Veterinary Institute in Subotica. All experimental meals were prepared by extruding the feed mixture powder on the single screw extruder, at a temperature of 103°C, and the resulting granules had a diameter of 11.5 mm. Cholesterol (Sigma Grade, ≥99%; Sigma-Aldrich), and Na-cholate (98%, Sigma-Aldrich) were suspended in sunflower oil (Vital, Vrbas) and subsequently added to the granules by using vacuum coating technology. Animals had access to food and water ad libitum.

Experiment was conducted on 40 male Wistar rats 4 months old, weighing 295 to 388 g, in accordance with Ethics Commission standards. During the experiment, animals were kept in standard cages (two or three animals per cage) at a temperature of 24°C and twelve hours light/dark regime. During adaptation period, which lasted two weeks, all animals were fed with standard diet for laboratory rats. After this period the animals were...
randomly divided into five groups. First group included eleven animals that had standard diet through the entire experiment period of 14 weeks. Second group consisted of six animals fed with standard diet supplemented with the herbal mixture in an amount of 5%. Third group consisted of eleven animals fed with high-fat content diet prepared by addition of sodium cholate, cholesterol and sunflower oil to a standard diet in amounts of 0.5%, 2% and 20% respectively. Fourth group of six rats was fed with the feed prepared in the same manner as for the third group, but with the addition of herbal mixture in an amount of 5%. Fifth group consisted of six animals which were on the same diet regime as the third group for the first six weeks, following eight weeks at the same diet regime as the fourth group.

Blood samples were collected at the beginning of the experiment, at 6 weeks, and at the end of the experiment (16). Faeces samples were collected at the end of the experiment (Table 2).

Table 2. Dynamics and number of samplings in animal groups

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Group</th>
<th>Number of blood samples</th>
<th>Number of faecal samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>1st</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>After 6 weeks</td>
<td>1st</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>After 14 weeks</td>
<td>1st</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5th</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

A number of animals were sacrificed at the beginning of the experiment after starvation during the night and a number after 6 and 14 weeks. The sacrifices were carried out with prior ether anesthesia. On this occasion, blood samples were collected in amounts from 5 to 6 ml of inferior vena cava in heparinized tubes, after which the blood plasma was separated by centrifugation for biochemical analysis (16).

Determination of cholesterol blood concentration. Levels of cholesterol bound to high-density lipoproteins were determined by precipitation with phosphotungstic acid and magnesium chloride (17). The method is based on the treatment of the serum with phosphotungstic acid in the presence of magnesium ions. Low-density lipoproteins, very-low-density lipoprotein (VLDL) and chylomicrons were precipitated from the serum. Remaining HDL cholesterol was further dissolved in the supernatant. The analysis of HDL cholesterol was carried out from the supernatant by enzymatic method.

Total cholesterol was determined by an enzymatic colorimetric method, hydrolyzing cholesterol esters to cholesterol with cholesterol esterase from Pseudomonas sp., purchased from Sigma-Aldrich (21). Briefly, free cholesterol in the reaction with cholesterol oxidase forms 4-cholesten-3-one and hydrogen peroxide. Hydrogen peroxide was treated with peroxidase in the presence of phenol and 4-aminoantipyrine, forming the red com-
pound chinonimine. Serum cholesterol level was determined by measuring absorbance of the formed product at 500 nm. Total cholesterol was calculated as the ratio of sample absorbance \((As)\) and standard absorbance \((Ast)\) multiplied with 2.29 \(((As/Ast) \times 2.29)\). (17). Atherogenic index \((AI)\) was calculated as \((TC - HDL-c)/HDL-c\). Cholesterol linked to low-density lipoproteins was calculated by using Friedewald's formula (16).

**Determination of triglycerides.** Due to the fact that triglycerides, like cholesterol, do not circulate freely in the blood, methods for their determination are based on previous alkaline or enzymatic hydrolysis to glycerol and free fatty acids, catalysed by lipase. The released glycerol was measured by enzymatic method (19).

**Determination of aspartate aminotransferase activity.** The assay is based on the ability of aspartate aminotransferase to catalyse reaction of aspartate and \(\alpha\)-ketoglutarate to give oxaloacetate and glutamate. Oxaloacetate is converted to malate by malate dehydrogenase and \(NAD^+\). The conversion of NADH to \(NAD^+\) (measured at 450 nm) is proportional to the level of AST enzyme in the sample (20).

**Determination of alanine aminotransferase activity.** The method is based on the quantification of pyruvate produced by alanine aminotransferase. Pyruvate and nicotinamide adenine dinucleotide (NADH) are converted to lactate and NAD\(^+\) respectively, with a lactate dehydrogenase (LDH). The decrease in absorbance of NADH at 340 nm is proportional to the activity of alanine transaminase (20).

**Determination of cholesterol and bile acids in faeces.** Faecal cholesterol was determined after sample saponification and the extraction of non-saponificated residue with hexane. Saponification was carried out by heating 500 mg of sample in 100 ml of 0.4 M solution of potassium hydroxide in methanol at 60°C for one hour. Non-saponificated matter was extracted with 30 ml of n-hexane in order to separate the residual base. After evaporation, the dry residue was dissolved in ethanol and passed through a Teflon filter (0.45 µm pore size) prior to HPLC analysis. For the determination of bile acid, solid faecal samples (100 mg) were immersed in 1 ml of methanol, extracted in an ultrasonic bath for 5 minutes, and heated in an oven for half an hour at 80°C. The supernatant was separated and the process was repeated two more times. The extracts were combined, evaporated to dryness and dissolved in 400 ml of methanol. To the prepared sample, 3.6 ml of 1% formic acid was added. Purification was carried out on Agilent SampliQ OPT Polymer columns with a loading of 60 mg.

Cholesterol and bile acids in faeces were determined by liquid chromatography using different detectors. The chromatographic analysis was performed on an Agilent 1200 Series chromatograph equipped with a Diode Array detector (DAD) and Evaporative Light Scattering Detector (ELSD). The separation was performed on a 4.6x50 mm Agilent Eclipse XDB-C18 column at a rate of 1000 ml/min, using methanol as the mobile phase. Cholesterol was determined with DAD at 212 nm with a reference wavelength set at 550 nm and the spectra were recorded in the range from 210 to 400 nm. The analysis time was 10 min with the post-time of 5 minutes.

For the bile acid determination a mixture of methanol and 1% (v/v) formic acid in water (75/25) was used as a mobile phase (21). The apparatus and the columns were the same as in the determination of cholesterol. Bile acids were determined using an ELS de-
tector adjusted to the nitrogen carrier gas pressure of 3.5 bars and the temperature of 40°C.

**Statistical analysis.** The results were expressed as mean values ± 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 Tukey’s test. Values of $p < 0.05$ were considered significant.

**RESULTS AND DISCUSSION**

**Anti-atherogenic parameters in plasma**

The first (control) and the second group of animals expressed an increase in the plasma triglycerides by 109% and 144%, respectively, throughout the experiment. The levels of triglycerides in the third, fourth and fifth group after 14 weeks were not significantly different from the values at the beginning of the experiment (Fig. 1).

After 6 weeks of the experiment, a significant difference was observed in TG, TC and LDL-c levels, and AI between the first and third group of animals (16).

The level of total plasma cholesterol in the first group increased by 34% after 14 weeks. In the second group, the total cholesterol increased by 25% at the end of the experiment. In the third group, which was fed with fatty food during entire experiment, the increase in cholesterol level was 101% after 6 weeks and 260% after 14 weeks, indicating induced hyperlipidemia. Fifth group of animals was on the same diet regime as the third group during the first six weeks. After that period, the diet of the fifth group was changed by adding the herbal mixture in an amount of 5%. During the last eight weeks of the experiment, the diets of the fourth and fifth groups of animals were the same.

The values for cholesterol linked to high density lipoproteins (HDL-c) show that in the first, second and third groups there were no significant changes after 14 weeks of the experiment. However, in the case of LDL-c the value for the third group of animals was significantly greater after 14 weeks (1753%) (Fig. 1) (16).

Addition of herbal mixture to the diet of the fifth group of animals, which was fed exclusively with fatty food during the first 6 weeks, reduced TC by 41%, LDL-c by 75%, and AI by 64%. Compared to the third group of animals, the increase of HDL-c was 33% ($p<0.05$). Also, it can be concluded that the addition of herbal mixture to the fatty food given to the fourth group of animals led to a decrease in TC by 25%, LDL-c by 64%, and in AI by 49%, and to an increase in the HDL-c by 33% in comparison to the third group ($p<0.05$) (Fig. 1).
For all tested animal groups there were no significant changes in the activity of aspartate aminotransferase, while alanine aminotransferase activity was significantly higher only for the third group (Fig. 2) (16).

**Figure 1.** Changes of biochemical parameters evaluated in plasma in different animal groups after 14 weeks

**Figure 2.** Alanine transaminase (ALT) and aspartate transaminase (AST) activity per groups after 14 weeks
Faeces analysis

The data for the faeces content, gross energy value of diet and faeces, net caloric intake, cholesterol content of faeces, cholesterol excretion, protein and fat content of faeces, protein and fat excretion, apparent fat and protein absorption are presented in Table 3 (16).

Table 3. Faeces content, gross energy value of diet and faeces, net caloric intake, cholesterol content of faeces, cholesterol excretion, protein and fat content of faeces, protein and fat excretion, apparent fat and protein absorption in the control (I) and the experimental groups of rats: second (II), third (III), fourth (IV) and fifth (V), after 14 weeks of experiment

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces (g/day)</td>
<td>11.14 ± 1.45</td>
<td>17.52±1.80</td>
<td>6.35±0.99</td>
<td>7.77 ± 1.18</td>
<td>8.67±1.63</td>
</tr>
<tr>
<td>Gross energy value of faeces (kJ/day)</td>
<td>158 ± 1.09</td>
<td>246±8.67</td>
<td>116 ± 0.22</td>
<td>142 ± 1.47</td>
<td>161 ± 1.13</td>
</tr>
<tr>
<td>Gross energy value of diet (kJ/day)</td>
<td>409 ± 2.65</td>
<td>458 ± 0.566</td>
<td>397 ± 1.83</td>
<td>401 ± 0.30</td>
<td>401 ± 0.30</td>
</tr>
<tr>
<td>Net caloric intake (kJ/day)</td>
<td>251</td>
<td>212</td>
<td>281</td>
<td>359</td>
<td>240</td>
</tr>
<tr>
<td>Cholesterol content of faeces (mg/g)</td>
<td>0.817 ± 0.075</td>
<td>0.869 ± 0.044</td>
<td>19.14 ± 0.525</td>
<td>17.03 ± 0.590</td>
<td>13.07 ± 0.392</td>
</tr>
<tr>
<td>Cholesterol excretion (g/day)</td>
<td>9.10 ± 0.588</td>
<td>15.23 ± 0.044</td>
<td>119 ± 0.087</td>
<td>140 ± 0.555</td>
<td>120 ± 0.500</td>
</tr>
<tr>
<td>Protein content of faeces (%)</td>
<td>20.8 ± 0.26</td>
<td>20.6 ± 0.24</td>
<td>18.3 ± 0.41</td>
<td>16.5 ± 0.44</td>
<td>18.1 ± 0.54</td>
</tr>
<tr>
<td>Protein excretion (g/day)</td>
<td>2.32 ± 0.03</td>
<td>3.62 ± 0.04</td>
<td>1.16 ± 0.03</td>
<td>1.28 ± 0.02</td>
<td>1.60 ± 0.02</td>
</tr>
<tr>
<td>Apparent protein absorption (%)</td>
<td>104</td>
<td>39.9</td>
<td>140</td>
<td>134</td>
<td>89</td>
</tr>
<tr>
<td>Fat content of faeces (%)</td>
<td>2.81 ± 0.26</td>
<td>1.91 ± 0.19</td>
<td>11.2 ± 0.42</td>
<td>8.7 ± 0.31</td>
<td>11.4 ± 0.28</td>
</tr>
<tr>
<td>Fat excretion (g/day)</td>
<td>0.231 ± 0.028</td>
<td>0.336 ± 0.032</td>
<td>0.740 ± 0.027</td>
<td>0.679 ± 0.024</td>
<td>0.989 ± 0.024</td>
</tr>
<tr>
<td>Apparent fat absorption (%)</td>
<td>84</td>
<td>83</td>
<td>96</td>
<td>97</td>
<td>96</td>
</tr>
</tbody>
</table>

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

The faeces content is expressed in grams, as an averaged value per animal per group per day. It is obvious that the herbal mixture supplementation stimulated digestion of both the low-fat and high-fat diets, as significant differences in faces content are observed between the herbal mixture containing and non-containing groups (p<0.05). The gross energy value of diet refers to the averaged food energy intake per animal per day. Referring to the results, the herbal mixture supplementation increased food intake as significant differences appeared between the herbal mixture containing and non-containing low-fat and high-fat group counterparts (p<0.05). Contrary to this, the gross energy value of faeces was significantly increased by the herbal mixture presence in the diet. The net caloric intake presents the difference of the energy consumed and excreted. Although not statistically interpreted, the presented values clearly point out that the herbal mixture supplementation reduced the net caloric intake in both high and low fat fed groups. In our experiment, the cholesterol diet supplementation was used to induce hyperlipidemia in high fat fed groups (III, IV and V), and that fact explains a huge difference in the faecal cholesterol content between low and high fat fed groups. The results of cholesterol determination in faeces show that the contents were the highest in the third group that was fed with fatty food without addition of the herbal mixture. The values for the first and the second animal groups did not differ significantly, while those of the other groups was signi-
significantly higher (p<0.05). The values for the fifth group of the experimental animals were significantly lower than the values for the fourth group.

Based on the previously presented results on plasma cholesterol levels it is obvious that there was a strong correlation between blood and faeces cholesterol levels in all tested animal groups.

Regarding the cholesterol content, it could be concluded that the herbal mixture addition stimulated cholesterol absorption from high fat diet. However, if the mass of faeces is taken into account and cholesterol content of faeces is expressed as cholesterol excretion in g/day, it is clear that the herbal mixture addition stimulates cholesterol excretion regardless of the fat content in the chow. Regarding protein content of faeces, significant differences existed between the low-fat (I and II) and high-fat groups (III, IV and V). Referring to these results, as well as to those of protein excretion and apparent protein absorption, the herbal mixture supplementation seems to reduce protein absorption from the feed. A negative protein balance was noticed in the groups I, III and IV, meaning that the protein content of faeces was higher than the protein content of the control feed. According to Mahipala et al. (22), a large proportion of faecal protein is of bacterial and metabolic origin. The same authors concluded that faecal protein content does not accurately provide reliable quantitative prediction of the differences of the digestibility of dietary crude proteins. Regarding fat content of faeces, significant differences existed between the low- and high-fat fed groups. Fat excretion of the group II was increased in comparison with the control group I, indicating that herbal mixture stimulated fat excretion in low-fat fed animals. However, in high-fat fed groups, the increase existed in the group V, but a decrease in the group IV, in comparison with the group III. Unlike the apparent protein absorption, apparent fat absorption does not seem to be much influenced by the herbal mixture addition.

Digestion stimulating activity of the herbal mixture can be explained by the presence of *Rhamnus Frangula* L. bark in its composition. 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, the influence on the 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 (23).

In order to examine if the herbal mixture influenced the enterohepatic circulation of bile acids, bile acid composition in the faeces of the control and the experimental groups of rats was performed and the results are presented in Table 4.

As expected, total bile acids showed no significant differences between the first and second group (Table 4). Looking at the values of individual bile acids it could be clearly seen that the contents were higher in experimental animals that were fed with food enriched with fats, except in the case of β-muricholic, hyocholic and hyodeoxycholic acids.
Table 4. The content of bile acids in faeces

<table>
<thead>
<tr>
<th>Bile acid</th>
<th>first group</th>
<th>second group</th>
<th>third group</th>
<th>fourth group</th>
<th>fifth group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content (μmol/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ω-Muricholic</td>
<td>0.90±0.19</td>
<td>0.98±0.17</td>
<td>16.08±0.09*</td>
<td>10.37±0.08*</td>
<td>16.23±0.90*</td>
</tr>
<tr>
<td>β-Muricholic</td>
<td>1.49±0.31</td>
<td>1.95±0.10</td>
<td>1.82±0.85</td>
<td>9.75±0.10*</td>
<td>3.63±0.18*</td>
</tr>
<tr>
<td>Hyocholic</td>
<td>1.21±0.22</td>
<td>0.94±0.20</td>
<td>0.86±0.05</td>
<td>4.95±0.04*</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hyodeoxycholic**</td>
<td>2.18±0.24</td>
<td>1.60±0.34</td>
<td>6.49±0.19*</td>
<td>13.63±1.47*</td>
<td>9.03±0.99*</td>
</tr>
<tr>
<td>Cholic</td>
<td>n.d.</td>
<td>n.d.</td>
<td>2.32±0.02*</td>
<td>1.38±0.01*</td>
<td>5.38±0.38*</td>
</tr>
<tr>
<td>Deoxycholic**</td>
<td>0.75±0.00</td>
<td>0.72±0.11</td>
<td>25.99±0.83*</td>
<td>7.61±0.46*</td>
<td>13.91±1.39*</td>
</tr>
<tr>
<td>Lithocholic**</td>
<td>n.d.</td>
<td>n.d.</td>
<td>4.52±0.88*</td>
<td>3.58±0.34*</td>
<td>2.23±0.14*</td>
</tr>
<tr>
<td>Total</td>
<td>6.54±0.97</td>
<td>6.19±0.86</td>
<td>58.08±3.42*</td>
<td>51.27±1.92*</td>
<td>50.41±1.01*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± relative standard deviation with a confidence interval of 95%
* Values for the same parameter is statistically significantly different (p <0.05)
** Secondary bile acids

As could also expected, the values for the third animal group differed significantly from the values for the first and second or the fourth and fifth groups. In contrast to cholesterol levels, the concentration of total bile acids in the faeces of the fourth and fifth groups did not differ significantly, indicating that the addition of the herbal mixture had the same effects independently on timing of the mixture introduction into the diet (Figure 3).

Figure 3. Levels of individual bile acids in the faeces samples after 14 weeks
In the faeces of experimental animals that were on a combined diet regime (fatty food with the addition of herbal mixture) the levels of β-muricholic, hyocholic (γ-muricholic) and hyodeoxycholic acids were higher than in the third group, which was fed only with fatty food. Comparing these values with total cholesterol and LDL-c levels in blood it could be concluded that in the case of the fourth group, the addition of herbal mixture to fatty foods led to lowering of blood cholesterol and increase of faecal bile acids; β-muricholic by 436% hyocholic by 475% and hyodeoxycholic by 110%. In the animals that were fed first six weeks with fatty food exclusively and last eight weeks with the fatty food enriched with herbal mixture (fifth group), there was an increase in fecal β-muricholic and hyodeoxycholic acid by 99% and 39%, respectively.

CONCLUSION

The analysis of blood and faeces biochemical parameters of experimental animals showed anti-atherogenic and antihyperlipidemic effects of the herbal mixture rich in natural phenolic compounds. The evaluated biochemical parameters showed that the introduction of herbal mixture into a high-fat content food had positive effects in induced hyperlipidemia, and the concentration of high density lipoproteins. Namely, high-fat content food enriched with 5% of herbal mixture lowered the total plasma cholesterol (41%) and low density lipoproteins (75%). Under the influence of tested herbal mixture, the atherogenic index dropped by 64%. Activities of aspartate transaminase and alanine transaminase showed that the diet did not have significant effects on liver functions. The introduction of the herbal mixture into the diet of experimental animals resulted in a decrease of cholesterol and bile acids concentrations in the faeces with the exception of β-muricholic, hyocholic (γ-muricholic) and hyodeoxycholic acids, whose concentrations increased with the introduction of the herbal mixture. In this respect, the diet had a more pronounced effect on cholesterol if administered after 6 weeks than when the herbal mixture was added to fatty foods from the start. On the other hand, in the case of biliary acids the timing of the diet introduction (at the start or after 6 weeks) had no effect.

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АНТИАТЕРОГЕНЕ ОСОБИНЕ ФУНКЦИОНАЛНЕ БИЉНЕ МЕШАВИНЕ

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Кардиоваскуларна обољења су најбројнији узрок смрти и инвалидитета у индустријализованом свету. Најчешћи тип кардиоваскуларног обољења је атеросклероза која настаје због нагомилавања холестерола на одређеним местима унутар артерије. С обзиром на то да поред лекова на баланс холестерола велики утицај има и начин исхране, циљ овог истраживања је био да се утврди ефекат биљне мешавине на излучивање фекалних жучних киселина и апсорпцију холестерола. In vivo експеримент
је спроведен на пет група мушких Вистар пацова који су били на различитим режимима хране током 14 недеља. За одређивање сирових протеина и масти су коришћене стандардне методе анализе, док је за сепарацију и одређивање фекалног холестерола и жучних киселина примењена течна хроматографија. Припрема фецеса код одређивања холестерола подразумевала је сапонификацију узора и екстракцију несапонификоване материје хексаном а жучне киселине екстраховане су етанолом. Овако припремљени узорци су испитивани HPLC/DAD и HPLC/ELSD техником.

Кључне речи: биљна мешавина, атеросклероза, жучне киселине, антихиперлипидемијски

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