An Investigation into Hypolipidemic, Anti-inflammatory and Antioxidant Effects of the Inclusion of the Dietary Preventive Product “Garmonicum Oil with Crude Turpentine” in the Diet of Patients with Arterial Hypertension and Ischemic Heart Disease

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Abstract: The study investigates the effects of the vegetable oil mixture “Garmonicum oil with crude turpentine” on blood lipid parameters, particular inflammation markers, the levels of oxidant-modified proteins and products reacting with thiobarbituric acid (TBA-products) in blood plasma in patients with cardiovascular pathology. The double-blind study examined 75 patients with cardiovascular pathology. On the background of standard therapy, Product #.1 (vegetable oil) and Product #.2 (the “Garmonicum oil with crude turpentine” vegetable oil mixture) had been added in the patients’ diet over a period of 8 weeks. The results revealed that the “Garmonicum oil with crude turpentine” vegetable oil mixture (produced in Perm Krai) is capable of increasing the levels of high-density lipoprotein cholesterol (HDLC) in the blood as well as producing an anti-inflammatory, antioxidant and hypo-coagulation effect, while being harmless to the liver and kidneys. The “Garmonicum oil with crude turpentine” vegetable oil mixture can be recommended as a dietary preventive product that is an additional source of polyunsaturated fatty acids, natural antioxidants and microelements, which are intended for the correction of metabolism and prevention of atherosclerosis.

Key words: Atherosclerosis • Arterial hypertension • Ischemic heart disease • Polyunsaturated fatty acids

INTRODUCTION

Cardiovascular diseases (CVD’s) continue to remain the most topical issue in the health care agendas of most countries, despite considerable progress over the last decades in diagnosing and treating cardiovascular pathology. Experts at the World Health Organization (WHO) are predicting further future growth in cardiovascular morbidity and mortality rates in both developed and developing countries, including Russia [1, 2, 3]. CVD’s are a leading cause of mortality in Russia (accounting for 57% of all mortality) [1].

In the majority of cases, the pathomorphological basis of CVD’s is atherosclerosis. Note that the biggest effect on mortality and population invalidization comes from atherosclerosis of coronary and cerebral vessels. At present, the incidence of ischemic heart disease (IHD) in Russia’s total population is 13,5±0,1%, in men - 14,3±0,3% and in women- 13,0±0,2%. That is 3 times the same indicators in the US where the incidence of IHD, according to data by the American Heart Association, was just 4,9% in 2004 [1,2].

Of late, special significance has been attached to the inflammatory theory of atherogenesis [4-7]. The latest clinical studies have revealed that high levels of proinflammatory cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), etc.), acute-phase proteins (fibrinogen, S-reactive protein (SRP), etc.), as well as an increase in the total number of leukocytes and several substances (homocysteine, lipoprotein (a) (LP(a), apolipoprotein B, oxidized lipids, nitric oxide, etc.), indicate a higher risk and an unfavorable forecast for CVD’s. Apparently, inflammation is that non-specific but stereotypical and universal reaction of the endothelium, smooth muscle cells and leukocytes to a lesion caused by various factors. This theory is
developed by data on certain especially atherogenic fractions of low-density cholesterol – peroxide-modified low-density lipoproteins (LDL’s) [4]. Incompletely oxidized LDL fractions stimulate expression of adhesion molecules on endothelial cells. Besides, LDL’s stimulate the formation of free oxygen radicals, causing the production of proinflammatory cytokines by endothelial cells. There is data that indicates that oxygen radicals reduce the quantity of endothelium-derived relaxing factor (nitric oxide (NO)) and stimulate the migration of smooth-muscle cells [4,6]. The impact of the products of lipid peroxide oxidation on the development of inflammation is apparently also effected through the participation of cytokines and adhesion molecules released by the endothelium [4,6].

Arterial hypertension (AH), which is oftentimes concomitant to IHD, should be regarded as a factor catalyzing the tempo of atherosclerosis progressing almost 3 times. Apparently, when there is AH, one of the major factors causing the impairment of endothelial function with subsequent involvement of inflammatory cells in the process, is a hydraulic trauma – shear stress, in particular [8].

We are currently observing a paradoxical situation with regard to the prevention and treatment of CVD’s in the majority of developed countries of Europe and North America. On one hand, the last two decades have been distinguished by unprecedented achievements in the area of cardiological pharmacology, as a result of which within a relatively short period of time there appeared a number of principally new classes of cardiological medications with the broadest range of therapeutic capabilities. However, 70-75% of morbidity and mortality from cardiovascular pathology is attributable to risk factors (RF’s) not relating to pharmacotherapy [5]. These are, most importantly, faulty nutrition, low physical activity, smoking and alcohol abuse. Without correcting these RF’s, we cannot expect high rates of success with regard to treating and preventing CVD’s even if there are the most state-of-the-art medicamental means on hand. Of the RF’s cited, faulty nutrition can be well regarded as one of the leading factors. Note that here we have to understand that faulty nutrition implies not just increases in the amounts of cholesterol, saturated fats, simple carbohydrates, animal proteins and the general caloricity of one’s diet, but, most importantly, increases in the amount of macronutrients on the background of a progressing decrease in the amount of variety of micronutrients. Micronutrients and biologically active nutritional supplements containing them can be considered as the most crucial element in treating and preventing cardiovascular pathology.

One of the sources of necessary micronutrients is vegetable oil, which is diverse in nature. Each oil is rich in its specific set of dietarily significant nutritious and biologically active substances. The most valuable vegetable oils are flax-seed, camelina, cedar, mustard, sunflower, olive, pumpkin-seed, grape-seed, watermelon-seed, sesame and soybean oils, which are rich in essential polyunsaturated fatty acids and characterized by consisting of a broad spectrum of biologically active compounds and having good taste properties. It should be noted that the fatty-acid make-up of the majority of vegetable oils is distinguished by the prevalence of some fatty acids and low amounts or the absence of others.

MATERIALS AND METHODS

The Study Consisted of Two Stages: The first stage involved a comparative study of inflammation markers and the cytokine status in patients with isolated AH (20 individuals, average age 55±7,79) and a combination of IHD and AH (20 individuals, average age 58,9±7,52). The comparison group was composed of practically healthy individuals (20 individuals, average age 57±6,63).
The objective of the study’s first stage was to substantiate the use of “Garmonicum oil with crude turpentine” as not only an expected hypolipidemic but anti-inflammatory remedy for patients with atherosclerosis.

The second stage involved a double-blind study that featured 75 patients (61 females and 14 males). The criteria for inclusion in the study: IHD (angina pectoris of tension, functional classes 1-3 (by the Canadian Cardiovascular Society (CCS) Angina Grading Scale, 1979) and/or post-infarction cardio-sclerosis) in combination with Stage 3 AH, Risk Group 4 (BHOK (the All-Russian Scientific Society of Cardiologists), 2010) and atherogenic dyslipidemia (types 2-a, 2-b, 4 by D. Fredrickson, WHO, 1970) after keeping to a hypolipidemic diet and taking standard IHD therapy medication (beta-adrenoreceptor antagonists, angiotensin-converting-enzyme (ACE) inhibitors, thiazide-type diuretics, aspirin, short-acting nitrovasodilators on demand) no less than 8 weeks before being included in the study. The study was conducted in compliance with the standards of Good Clinical Practice and principles of the Declaration of Helsinki. The study’s protocol was approved by the Ethical Committees of all the participating clinical centers. The criteria for exclusion from the study: acute coronary syndrome, life-threatening heart rhythm disturbances, stagnant heart failure, primary hyperlipidemias, chronic liver failure, liver disorders, disorders of the pancreas in the decompensation phase, diabetes mellitus (DM), disorders of the thyroid gland with a disturbance in the gland’s functional activity, chronic alcoholism, ages older than 75, TG levels higher than 4.5 mmol/l (400 mg/dl). The patients were divided into 3 groups comparable by gender, age and concomitant pathology: Group 1 (a comparison group) featured 25 patients in middle age, age 59.3±7.0, receiving standard IHD and AH therapy, taking in no vegetable oils; Group 2 (a comparison group) was composed of 25 patients in middle age, age 57.3±6.0, receiving standard IHD and AH therapy in combination with Product #1 (sunflower vegetable oil); Group 3 (the experimental group) featured 25 patients in middle age, age 62.3±12.0, who along with standard therapy received Product #2 (“Garmonicum oil with crude turpentine”).

The type of intervention was oil (Products #1 and #2) used as a supplement to food in the amount of 15 drops three times per day. The observation period: baseline and 8 weeks later.

Methods of the Study: The programme for patient examination in the 1st stage envisioned the studying of the anamnesis and physical examination in combination with additional laboratory methods (determination of the quantity of S-reactive protein (SRP) serum concentrations, tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8)). The study programme in the 2nd stage included exploring the lipid spectrum (total cholesterol (TC), low-density lipoproteins (LDL), triglycerides (TG), very-low-density lipoproteins (VLDL) and high-density lipoproteins (HDL); calculating the atherogenicity index (AI); determining the level of glycemia on an empty stomach; studying the therapy safety indicators (the activity of serum aminotransferases (AST, ALT), alkaline phosphatases (ALP), total bilirubin, total creatine kinase (CK) and the concentration of creatinine); determining inflammation markers (concentrations of SRP, fibrinogen and interleukin-1 (IL-1); assessing the levels of oxidant-modified proteins in the blood serum [9]; determining the concentration of products reacting with TBA [10]. The statistical processing of data was conducted by means of the STATISTICA 6.0 applied software system, as well as a program engaged in nested-package statistical data processing. The results are presented in the form M + σ, where M is the sample mean deviation and σ is the sample standard deviation. In analyzing all the forms, the differences were considered as statistically significant at p < 0.05.

Results of the study. The study’s first stage revealed that the levels of IL-6 and TNF-α in the blood serum of the patients with isolated AH did not exceed the normal indicators according to the recommendations of the test-kit used and did not verifiably differ from those of the healthy individuals. The results obtained for the cytokine spectrum in the patients with isolated AH revealed that the normative concentration of IL-8 and SRP in the serum of the patients with isolated AH (Table 1) was exceeded, with the differences from the practically healthy patient group being statistically significant (p < 0.05).

The study of proinflammatory cytokines and biomarkers in the combined AH and IHD group revealed a verifiable increase in the levels of all the indicators studied compared with the group of the patients with isolated AH and practically healthy individuals (Table 2).

Thus, the patients with AH and IHD combined (Group 2) demonstrated higher indicators of proinflammatory serum biomarkers than those with isolated AH.
Table 1: The levels of IL-8 (pg/ml) and SRP (ng/ml) in the blood serum of patients with isolated AH

<table>
<thead>
<tr>
<th>Indicator</th>
<th>N</th>
<th>Median</th>
<th>Std.Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8, pg/ml</td>
<td>20</td>
<td>8,30</td>
<td>9,39</td>
</tr>
<tr>
<td>SRP, µg/ml</td>
<td>20</td>
<td>6,54</td>
<td>3,98</td>
</tr>
</tbody>
</table>

Table 2: The levels of proinflammatory cytokines in the blood serum of the patients with isolated AH and AH and IHD combined

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group 1</th>
<th>Group 1</th>
<th>p level</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/ml</td>
<td>Median</td>
<td>3,55</td>
<td>4,3</td>
</tr>
<tr>
<td></td>
<td>Std.Dev.</td>
<td>2,9</td>
<td>8,07</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>Median</td>
<td>0,71</td>
<td>2,6</td>
</tr>
<tr>
<td></td>
<td>Std.Dev.</td>
<td>2,58</td>
<td>5,71</td>
</tr>
<tr>
<td>IL-8, pg/ml</td>
<td>Median</td>
<td>8,3</td>
<td>22,8</td>
</tr>
<tr>
<td></td>
<td>Std.Dev.</td>
<td>9,39</td>
<td>49,90</td>
</tr>
<tr>
<td>SRP µg/ml</td>
<td>Median</td>
<td>6,54</td>
<td>7,05</td>
</tr>
<tr>
<td></td>
<td>Std.Dev.</td>
<td>3,98</td>
<td>3,44</td>
</tr>
</tbody>
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Let us take a look at the results of the study’s second stage. In Group 1, the average concentration of total cholesterol (TC) was 5,7+1,1 mmol/l. No verifiable changes in the levels of TC were detected over the course of the 8 weeks of observation. The HDL concentration in Group 1 was 1,5+0,9 mmol/l; note that no verifiable changes in HDL levels were detected over the course of observation. No changes in the concentration of LDL and TG in the blood were detected over the course of observation either. No significant changes in the AI were detected over the course of the 8 weeks of observation. The level of glycemia in this group was within the normal limits at all the stages of the study. The state of liver function was assessed based on the activity of AST, ALT, the level of total bilirubin and ALP. No changes in these parameters were detected over the course of the 8 weeks of observation. All the patients were investigated for the quantity of total creatine kinase (CK) and the concentration of creatinine in the blood. No verifiable changes were detected. The study detected no verifiable changes in the concentrations of CRP in the blood at baseline and after 8 weeks (0,7+0,2 and consequently 0,6+0,3 mg/l, p > 0,05) and interleukin-1 (2,1+0,9 and consequently 2,0+0,8 pg/ml, p = 0,08). In assessing the activity of the oxidation of lipids based on the concentration of products reacting with thiobarbituric acid (TBA-products) in blood serum and proteins based on the concentration of oxidant-modified in blood serum, it was established that these values were initially within the reference limits and after 8 weeks no significant changes were detected.

We will now examine the results of examining the patients in Group 2. The group consisted of 23 females and 2 males. The therapeutic intervention investigated included standard therapy and the addition of Product #.1 to the diet (vegetable sunflower oil) to be taken in the amount of 15 drops 3 times per day during meals. On average, the TC level was 5,9+1,2 mmol/l. Over the course of the 8 weeks of observation, no verifiable changes in TC concentrations were detected. At baseline, the HDL concentration in the blood was 1,3+0,4 mmol/l and after 8 weeks the level had a trend towards increase - 1,4+0,4 mmol/l. No changes in the concentrations of LDL, VLDL and TG were detected over the period of observation. The study detected a trend towards decrease in AI over the course of the 8 weeks of observation: 3,7+1,2 units at baseline and 3,2+1,0 over time (p = 0,06). The level of glycemia in the Group 2 patients was within the normative limits at baseline and over time after 8 weeks. No changes in the activity of AST, ALT, the level of total bilirubin, ALP, creatinine and the quantity of total CK were detected over the course of the 8 weeks of observation. In assessing the activity of the oxidation of lipids based on the concentration of products reacting with thiobarbituric acid (TBA-products) and proteins based on the concentration of oxidant-modified proteins in blood serum, it was established that these values were within the reference limits at baseline and after 8 weeks no verifiable changes were detected. By the 8th week of taking Product #.1, a statistically significant decrease in the interleukin-1 concentration in the blood was detected.

In the Group 3 patients (18 females, 7 males), who received standard therapy and Product #.2 (“Garmonicum oil with crude turpentine”) added to the diet, the average level of TC was 5,9+1,0 mmol/l. Over the course of the 8 weeks of observation, no verifiable changes in TC levels were detected. At baseline, the concentration of HDL in the blood was 1,3+0,8 mmol/l and after 8 weeks it increased up to 1,6+0,2 mmol/l (p = 0,01). No changes in the concentration of LDL, VLDL and TG were detected over the period of observation. The study detected a verifiable decrease in the AI over the course of the 8 weeks of observation: 3,6+0,9 units at baseline and 3,2+1,0 (p = 0,01) after 8 weeks. In the Group 3 patients, the level of glycemia was within the normal limits at all the stages of the study. No significant changes in the concentrations of AST, ALT, ALP, total CK and creatinine were detected over the course of the 8 weeks of observation. In assessing the activity of the oxidation of lipids based on the concentration of products reacting with thiobarbituric acid (TBA-products) in blood serum and proteins based on the concentration of oxidant-modified in blood serum, it was established that these values were initially within the reference limits and after 8 weeks no significant changes were detected.
time) (p = 0.002). In assessing the activity of the oxidation of lipids by the concentration of products reacting with thiobarbituric acid (TBA-products) and proteins by the concentration of oxidant-modified proteins in blood serum, it was established that these values were within the reference limits; after 8 weeks the study detected a number of verifiable changes: the level of TBA-products at baseline was 4.0±2.9 µmol/l and after 8 weeks - 2.2±0.7 µmol/l (p = 0.005); a decrease in the level of oxidant-modified proteins at the wave length of 370 nm from 1.9±0.8 at baseline down to 1.2±0.5 opt. den. units/ml after the 8 weeks of observation (p = 0.005).

**DISCUSSION**

The first stage of the study showed that in the patients with isolated AH, compared with the practically healthy individuals, there were detected symptoms of the systemic inflammatory reaction and, most importantly, an increase in the levels of IL-8. It is known that it is IL-8 that activates neutrophiles and to a lesser degree other granular leukocytes and causes their chemotaxis to the site of inflammation. An increased level of IL-8 is associated with chronic and acute inflammatory states and correlates with tissue infiltration of neutrophiles. The question remains: What initiates an increase in IL-8 levels in patients with AH? A possible cause for this cytokinemia is the cascade of pathogenetic events associated with endothelial dysfunction, which is characteristic of even the initial stages of the development of AH. The results of exploring cytokines and inflammation biomarkers in the patients with a combination of IHD and AH demonstrated that the significance of the systemic inflammatory reaction was growing as the atherosclerotic damaging of vessels progressed. The results of studying the clinical effects of “Garmonicum oil with crude turpentine” lead us to admit there are number beneficial effects, which are examined below.

**The Lipid-Corrigent Effect:** On the background of standard therapy, the study did not establish any effect on the patient’s lipid blood parameters. The Group 3 patients demonstrated a verifiable increase in the concentration of HDL in the blood. The origin of this effect should be investigated in the future.

**The Anti-Inflammatory Effect:** The most marked anti-inflammatory effect in relation to SRP was detected in applying Product #.2. The result obtained is in line with what literature says: derivatives of polyunsaturated fatty acids (PUFA) compete with arachidonic acid for the formation of prostaglandines and leukotrienes. As a result, the equilibrium shifts towards PUFA derivatives. In applying standard therapy and Product #.1, such changes are not detected. These facts indicate there being an own anti-inflammatory effect of Product #.2. In applying Product #.1 and Product #.2 on the background of standard therapy, the study detected a decrease in the levels of interleukin-1 in the blood. No verifiable differences between the values of this parameter were detected in the groups. It is known that a number of research works have showed that enriching the diet of healthy volunteers with PUFA leads to a decrease in the production of interleukins-1, -6 and -2 and TNF by mononuclear blood cells [4]. This effect is attributable to the competition between arachidonic acid and PUFA at the cyclooxygenase-lipoxygenase level.

**The Antioxidant Effect:** The study investigated the influence of the type of therapy on processes of the oxidation of lipids and proteins. Thus, standard therapy and applying Product #.1 do not have a substantial effect on the levels of oxidant-modified proteins and TBA-products in blood serum. In the group of patients who received Product #.2 on the background of standard therapy, the study verifiably detected a decrease in the levels of TBA-products and oxidant-modified proteins in the blood at the wave length of 370 nm – ketondinitrophenylhydrazone (KPH), which is a late marker of the oxidative destruction of protein (Table 2). The decrease in the concentration of KPH in blood serum indicates a decrease in the extent of the oxidative destruction of the protein molecule or an increase in the tempo of the utilization of the modified protein (the secondary antioxidant effect). This effect of Product #.2 is construed as the product’s own antioxidant effect.

Besides, we can talk about the safety of the therapy investigated. The safety criteria were there being no increase in the activity of hepatic aminotransferases, ALP, total bilirubin, total CPK (creatine phosphokinase) fraction and creatinine. These results indicate the safety of applying Products #.1 and #.2 on the background of standard therapy.

**CONCLUSION**

The comparative blind study assessed the expediency of including the “Garmonicum oil with crude turpentine” vegetable oil mixture (Product #.2) in the diet of patients with cardiovascular pathology. It was established that the “Garmonicum oil with crude
“Garmonicum oil with crude turpentine” vegetable oil mixture produced by OOO Bolshoye Zagarye (Perm Krai) is capable of increasing the levels of HDL in the blood and producing anti-inflammatory and antioxidant effects. These effects are actualized 8 weeks after taking in the product. Note that “Garmonicum oil with crude turpentine” is a safe remedy in relation to the function of the liver and kidneys. The “Garmonicum oil with crude turpentine” vegetable oil mixture produced under the “Zolotaya Formula” trademark by OOO Bolshoye Zagarye (Perm Krai) is recommended as an additional source of polyunsaturated fatty acids, natural antioxidants, microelements and is intended for the correction of metabolism and prevention of atherosclerosis.

REFERENCES