INFLUENCE OF OXIDATED VEGETABLE OIL AND GARLIC EXTRACT UPON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

JOLANTA ZALEJSKA–FIOLKA¹, SŁAWOMIR KASPERCZYK¹, ALEKSANDRA KASPERCZYK¹, EWA BIRKNER¹, EWA GRUCKA-MAMCZAR¹, BARBARA STAWIARSKA-PIĘTA² AND ANNA SCHNEIDER¹

¹Department of Biochemistry in Zabrze, ²Department of Pathology in Sosnowiec, Medical University of Silesia, 40-006 Katowice, Poland
e-mail: jfiolka@slam.katowice.pl

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Abstract

The aim of the study was to find out if the consumption of oxidised oils aggravates the undesired changes in the concentration of lipids and homocystein in blood and in the aorta endothelium region and whether the addition of garlic to diet results in anti-atherosclerotic action. The experiments were carried out on mixed breed rabbits divided into 3 groups. The body weight of the rabbits was checked once a week and every six weeks blood samples were taken. The concentration of homocystein, total cholesterol, HDL cholesterol, and triacylglyceroles were determined in blood. After the experiment was completed, aortas were dissected for histological examinations. It was found that oxidised rapeseed oil, administered to animals, caused the development of atherosclerotic alterations in the aorta wall region and increased the homocystein content in blood serum. The administration of garlic in diet inhibited atherosclerotic changes in the aorta wall and this seems to be related to the decreasing concentration of triacylglyceroles in blood serum.

Key words: rabbits, garlic, vegetable oil, oxidation, atherosclerosis.

Diseases of the cardiovascular system still take a leading world-wide position, as regards incidence and mortality (1). Prevention and treatment of ischaemic heart disease is connected with a recommendation to consume vegetable fats and to reduce animal fat content in the diet (2). Research proved, though, that such prophylaxis is not risk-free (3). Vegetable fats contain polyunsaturated fatty acids, which are prone to oxidation. Frying in the same vegetable oil and increasing its temperature to 180°C-190°C several times results in a chemical reaction which is similar to that taking place during vegetable fat hardening in industrial manufacturing. A substantial administration of oxidised vegetable oils in diet may lead to aggravation of the free radical processes. It is believed that such a diet, similar to excessive consumption of products rich in cholesterol, results in the development of vascular lesions, leading to atherosclerosis and then to diseases of the cardiovascular system (4, 5).

Research revealed also that high consumption of antioxidant substances reduces the risk of developing circulatory system diseases (6, 7). It would seem crucial to find out whether additions of antioxidant substances to food results in reducing the disadvantageous changes caused by the consumption of oxidised vegetable oils. One of such natural substances having an anti-oxidative character is garlic extract, the protective role of which in the oxidation of edible oils we have proven in our earlier studies (8, 9). Garlic extract also appears to have a multidimensional influence upon the organism. Among others, it causes a reduction in the concentration of lipids, which have been recognised as one of the risk factors of developing cardiovascular system diseases (10, 11). Numerous communications indicate homocystein to be the new, independent factor testifying to the development of atherosclerosis in early stages of the disease (1, 12, 13).

Having the above in mind, we aimed of our studies at the following questions:

1. Does the oxidation of edible oil in 120°C cause changes in the content percentage of fatty acids?
2. Will the consumption of oils oxidised in 120°C aggravate the undesired changes in the concentration of lipids and homocystein in blood and cause sclerotic lesions in the aorta endothelium region?
3. Does the addition of garlic to diet result in anti-atherosclerotic action?

Material and Methods

Preparation of oil: „Kujawski” rapeseed oil (ZT „Kruszwica” S.A., Poland) was used in the study. The oil was oxidised for 7 d at 120°C. The oxidation
process was controlled by determining the peroxide value, in accordance with the Norm of the Polish Standardisation Committee (PN – ISO 3960) (14), and the content of fatty acids by means of gas-chromatography (15).

**Animals.** The experiments were carried out on mixed breed rabbits, with the initial body weight of 2500g ± 50g. The animals after initial selection (lipid parameters) were divided into 3 groups, six rabbits in each.

**Experimental design.** The rabbits of group I were fed fresh rapeseed oil, group II – oxidised rapeseed oil and group III – oxidised rapeseed oil and garlic (*Allium sativum* L.). Garlic was chopped and homogenized, then 1 g of the homogenized garlic was added to the feed. The detailed experimental design and fodder content were presented in Table 1. The experiment lasted 24 weeks.

The body weight gain in the rabbits was checked once a week and every six weeks blood samples were taken from the central auricular vein, in the amount of 5 ml each time. After the experiment was completed, the animals were anaesthetised and blood was taken from their hearts for biochemical examination. After lethal anaesthesia, their aortas were dissected for histological examinations.

**Biochemical and histological examinations.** Serum concentration of homocystein was determined with the liquid chromatography (HPLC) method by Kuo (16). The contents of total cholesterol, HDL cholesterol, and triacylglyceroles were determined by enzymatic methods, using tests by Alpha Diagnostics (Germany). Histopathological examination of the aortas was performed in the Department of Pathology of the Medical University of Silesia in Sosnowiec. The aortas was fixed in 10% formalin and paraffin sections stained with haematoxylin and eosin (H-E) and frozen sections stained with Sudan III for neutral fats (17) were prepared. Colour microphotographs were taken with the Docuval microscope equipped with the photo device (Carl Zeiss Jena).

**Statistical analysis.** Software Statistica PL was used for statistical analysis. For comparison of changes between particular groups of animals the test ANOVA by Kruskal-Wallis and test U by Mann-Whitney were used.

**Results**

The results of the investigations are presented in Tables 2 and 3, as well as in Figs 1 through 8. As can be seen from Table 1, the rapeseed oxidized for 7 d at 120°C showed an increased content of palmitic acid by 27% and oleic acid by 21% and a decreased content of linolic acid by 63% and linolenic acid by 92%.

At the same time, the peroxide value (PN) increased 39 times (Table 3).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>before oxidation</th>
<th>after oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>16.0</td>
<td>5.6±0.06</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18.1</td>
<td>71.4±0.10</td>
</tr>
<tr>
<td>Linolic acid</td>
<td>18.2</td>
<td>15.5±0.06</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>18.3</td>
<td>7.4±0.21</td>
</tr>
</tbody>
</table>

* standard deviation (SD); all results are averages of 4 analyses

### Table 1

<table>
<thead>
<tr>
<th>Fodder composition</th>
<th>Group I – control (C)</th>
<th>Group II – experimental (O)</th>
<th>Group III – experimental (OG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>21%</td>
<td>21%</td>
<td>21%</td>
</tr>
<tr>
<td>Fats</td>
<td>34%</td>
<td>34%</td>
<td>34%</td>
</tr>
<tr>
<td>Saccharide</td>
<td>45%</td>
<td>45%</td>
<td>45%</td>
</tr>
<tr>
<td>Fresh rapeseed oil</td>
<td>(12 g/200 g of fodder, accounted for in total fat content)</td>
<td>Oxidised rapeseed oil (12 g/200 g of fodder, accounted for in total fat content)</td>
<td>Oxidised rapeseed oil (12 g/200 g of fodder, accounted for in total fat content) and 1 g of garlic</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Content of fatty acids (%) in rapeseed oil before and after its oxidation for 7 d at 120°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>before oxidation 5.6±0.06 after oxidation 7.1±0.06</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>before oxidation 71.4±0.10 after oxidation 86.5±0.10</td>
</tr>
<tr>
<td>Linolic acid</td>
<td>before oxidation 15.5±0.06 after oxidation 5.8±0.10</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>before oxidation 7.4±0.21 after oxidation 0.6±0.08</td>
</tr>
</tbody>
</table>
Table 3
Peroxide value (PV) in rapeseed oil before and after oxidation

<table>
<thead>
<tr>
<th>Rapeseed oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before oxidation</td>
</tr>
<tr>
<td>After oxidation</td>
</tr>
<tr>
<td>4.5±0.25</td>
</tr>
<tr>
<td>181.24±0.10</td>
</tr>
</tbody>
</table>

± standard deviation (SD); all results are averages of 4 measurements

The concentration of homocysteine in blood serum increased temporarily (the 12th week of the experiment) by 20-25% in groups receiving oxidised oil and oxidised oil with garlic, and at the end of the experiment no changes in the concentration of that parameter were observed in comparison with control (Fig.1). In the OG group, in week 24 of the experiment, a statistically significant (P<0.010) drop in triacylglycerols concentration was noted (by 35%) in comparison with the group administered oxidised oil only (group O). At the same time no difference in the concentration of that parameter was noted in comparison with control (Fig.2). The concentration of total cholesterol and HDL cholesterol revealed no statistically significant differences throughout the experiment (Figs 3 and 4). The body weight of animals in the experimental groups did not differ from that of animals in control during the experiment (Fig.5).

Histological examinations of aorta specimens revealed that the presence of fresh rapeseed oil in diet caused no histopathological changes in the aorta membrane region (Fig. 6), whereas the presence of oxidised rapeseed oil in diet caused focal thickening of the wall, with proliferation of myocytes in the aortic media in all animals examined (Fig. 7). In rabbits receiving oil diet supplemented with garlic, focal thickening of the internal layer of the aorta was noted only in 2 animals (Fig. 8).

Histochemical examination of aorta specimens stained with Sudan III revealed that the presence of fresh rapeseed oil and garlic with the oxidised oil in diet caused no changes in neutral fat content in the aorta membrane region (Fig. 10).

![Graph](image)

**Fig. 1.** The concentration of homocysteine in blood serum at the start of the experiment, at its 12th week, and at the end (average ± SEM).
**Fig. 2.** The concentration of triacylglyceroles in blood serum at the start of the experiment, at its 12th week, and at the end (average ± SEM).

**Fig. 3.** The concentration of total cholesterol in blood serum at the start of the experiment, at its 12th week, and at the end (average ± SEM).
Fig. 4. The concentration of HDL-cholesterol in blood serum at the start of the experiment, at its 12th week, and at the end (average ± SEM).

Fig. 5. Body weight of animals at the start of the experiment, at its 12th week, and at the end (average ± SEM).
Fig. 6. Control group I. Rabbit aorta after 3-month administration of fresh rapeseed oil. H-E. x 150.

Fig. 7. Group II after 3-month administration of oxidised rapeseed oil. H-E. x 150.

Fig. 8. Group III after 3-month administration of oxidised rapeseed oil and garlic. H-E. x 150.

Fig. 9. Control group I. Rabbit aorta after 3-month administration of fresh rapeseed oil. Sudan III. x 150.

Fig. 10. Group II after 3-month administration of oxidised rapeseed oil. Sudan III. x 150.
Discussion

It was noted that the oxidation of rapeseed oil caused a significant increase in the peroxide value (PN), as well as changes in the percentage content of fatty acids in the oil, which indicate the disadvantageous process taking place during the oxidation (Tables 2 and 3). We obtained similar results in our earlier in vitro studies (8). The changes in the content of fatty acids prove that the oxidation of rapeseed oil at 120°C lead to the saturation of polyunsaturated fatty acids. Due to the fact that numerous studies point to the diets rich in saturated fatty acids as diets inducing atherosclerotic lesions (4, 18, 19), it could then be assumed that diet enriched with rapeseed oil oxidised in such a way would cause the development of atherosclerotic lesions in the experimental model assumed.

The results of the research revealed that the supplementation of diet with oxidised rapeseed oil and oxidised rapeseed oil with garlic did not cause changes in the level of total cholesterol or HDL cholesterol in comparison with control. After 24 weeks of the experiment it was found that garlic added to oxidised rapeseed oil as part of fodder caused a decrease in the level of triacylglyceroles (TG) in comparison with the group receiving only oxidised rapeseed oil. It follows that addition of garlic reduces the level of TG compared to the group receiving fresh oil. It could be assumed then that garlic acted effectively in the initial phase of lipid accumulation.

No relative changes were observed throughout the experiment in animal body weight, in all experimental groups. Still, increased condition of the hair and claws was noted in the group receiving garlic, which may testify to its multi-directional influences.

Both the histopathological and histochemical examinations demonstrated that fresh rapeseed oil did not cause lesions in the aortas of experimental animals. The lesions were noted in animals given the oxidised rapeseed oil. The beneficial influence of garlic upon the animal aortas was confirmed. The results are in line with the reports of other authors (10, 11) that indicate the protective role of garlic in hypercholesterolemia.

Our study revealed a temporary increase in homocysteine concentration in the groups of animals receiving oxidised oil and oxidised oil with garlic. Perhaps longer oxidation of oil in higher temperatures would result in a more substantial increase in homocysteine concentration and more profound progression of atherosclerotic lesions.

Acknowledgments: The work has been reviewed by the Ethic Committees and obtained the consent of the Academic Animal Ethic Committees in Katowice.

References