

## Dose-Dependent Influence of Commercial Garlic (*Allium sativum*) on Rats Fed Cholesterol-Containing Diet

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The aim of this investigation was to evaluate the dose-dependent influence of commercial garlic on rats fed cholesterol-containing diets. It was found that commercial garlic contains high concentrations of dietary fibers, microelements, and total polyphenols, and its total antioxidant capacity as determined by two independent assays [1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)] was similar to that of the original garlic samples. Wistar rats (35) were randomly divided into five diet groups, named control, Chol, Garlic500, Garlic750, and Garlic1000. Control rats were fed basal diet (BD), which included wheat starch, casein, soybean oil, and vitamin and mineral mixtures. To the BD of the Chol group was added 1% of cholesterol. To the BD of the other three groups (Garlic500, Garlic750, and Garlic1000) were added 1% of cholesterol and commercial garlic equal to 500, 750, and 1000 mg of raw garlic per kilogram of animal weight. After 4 weeks of the experiment only in rats from the Garlic500 group were a significant hindering in the rise in plasma lipids and also a significant hindering in a decrease of plasma antioxidant activity registered. A significant decrease in plasma circulating fibrinogen and an increase in the clotting time were found in the same group of rats ( $P < 0.05$  in both cases). The fibrinolytic effect of garlic diets was visualized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In the fibrinogen fraction of Garlic500 the 66, 24, and 14 kDa protein bands were detected with weaker protein intensity than in the corresponding ones in the Garlic750 and Garlic1000 diet groups. In conclusion, the positive influences of commercial garlic on plasma lipids, proteins, antioxidant activity, and some indices of blood coagulation are dose-dependent. Therefore, commercial garlic (Elena, Żelazków, Poland) could be a valuable component of atherosclerosis-preventing diets only in optimal doses.

**KEYWORDS:** Commercial garlic; bioactive compounds; antioxidant potential; rats; plasma lipids; proteins; antioxidant activity; blood coagulation

### INTRODUCTION

Scientific investigations mainly on laboratory animals showed that garlic (*Allium sativum* L.), on the basis of its synergism

(1), positively affects a wide range of diseases including cancer (2). It was shown that garlic contains a wide spectrum of bioactive compounds: dietary fibers, microelements, especially Se, and phenolics (3). Studies on animals and humans show the beneficial effects of garlic and other dietary plants rich in total antioxidants (4–6). It was reviewed that aged garlic extract contains antioxidant compounds and increases nitric oxide production and decreases the output of inflammatory cytokines from cultured cells. These data suggest that garlic may improve impaired endothelial function in men with coronary disease treated with aspirin and statin (6). No doubt, the successful use

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of garlic in folk medicine for treatments of different diseases is connected to bioactive compounds (1, 2).

However, its main effect is cardioprotective (5–7). In recent years there has been renewed interest in garlic research (4–8). Investigators continue studies concerning mostly the cardioprotective properties of this vegetable (9–11). It is known that the main cause of mortality from dangerous coronary atherosclerosis is thrombosis of the coronary arteries, which leads to myocardial infarctions that in many cases can be fatal (12). Despite this fact the influence of garlic on the anticoagulant system is less studied (13). Fibrinogen is one of the plasma-circulating proteins. This protein is synthesized in liver and circulates in the plasma of Wistar rats at a concentration of 2–3 g/L. Fibrinogen plays a central role in blood clotting, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, and neoplasia. This is regulated to a large extent by fibrin formation itself and by complementary interactions between specific binding sites on fibrinogen and extrinsic molecules including proenzymes, clotting factors, enzyme inhibitors, and cell receptors. Therefore, it was decided to study the influence of garlic consumption on plasma-circulating fibrinogen—one of the important elements of the blood coagulation system and also coagulation time in an experiment *in vivo*.

Nowadays the public prefers to use commercial garlic (14). Therefore, in this experiment the influence of the commercial garlic (Elena, Żelazków, Poland) on rats fed cholesterol-containing diets was studied. It was discovered (15) that it is a dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. Therefore, different doses of commercial garlic in the experiment *in vivo* were used to check if the influence of garlic on other indices is also dose-dependent.

As far as we know, there are no such combined investigations *in vitro* and *in vivo*.

## MATERIALS AND METHODS

**Chemicals.** 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), Folin–Ciocalteu reagent, and nonoxidized cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO).

**Samples and Preparation.** The samples of commercial garlic were obtained from the producer (Elena) by one of the investigators. The samples were prepared by the producer in the following way: (1) The frozen raw garlic samples were lyophilized, and the producer received from 1 kg of raw garlic 250 g of lyophilized garlic. (2) The lyophilized garlic was transformed by the producer into powder, and this form was used in the present investigation. Commercial and original garlic samples were treated with solvents of different lipophilicities to obtain fractions for testing the antioxidant activity (16). The following extracts (EXT) were obtained: to 10–100 mg of garlic powder, 1 mL of either water (EXTA), methanol/water (70:30 v/v) (EXTB), or ethanol (EXTC) was added. A highly lipophilic fraction (EXTD) was prepared from 1 g of garlic powder in 50 mL of acetone/water (75:25 v/v).

**Determination of Major Bioactive Compounds.** The contents of dietary fibers and microelements were determined as previously described (3).

**Determination of Total Polyphenols and Antioxidant Potentials.** Total polyphenols were determined according to the Folin–Ciocalteu method and measured at 765 nm. The results are given in milligrams of gallic acid (17) equivalent per gram of dry weight (DW). Some antioxidant assays give different antioxidant activity trends (18). To receive reliable data concerning the total antioxidant potential of the studied commercial garlic, two independent complementary assays were used.

*Determination of Total Antioxidant Potential by 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) Diammonium Salt (ABTS<sup>+</sup>) and*

*K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>.* The ABTS<sup>+</sup> radical cation was generated by the interaction of ABTS<sup>+</sup> (250 μM) and K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (40 μM). After the addition of 990 μL of ABTS<sup>+</sup> solution to 10 μL of Trolox standards (final concentration = 0–20 μM) in phosphate-buffered saline (PBS), the absorbance was monitored at exactly 1, 6, and 9 min. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the sample's concentration and of Trolox for the standard reference data. The ABTS<sup>+</sup> solution was diluted with ethanol when the samples were dissolved in ethanol, hexane, or dichloromethane. For samples dissolved in water, acetone, or methanol/water (70:30 v/v), dilution was done with water. Standards such as BHA were dissolved in dichloromethane, and Trolox was prepared in both water and ethanol (16, 19).

*Determination of Total Antioxidant Potential by Radical Scavenging Activity Using DPPH.* Garlic extracts were taken in different test tubes. The volume was adjusted to 100 μL by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5 μL) to these tubes. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity = (control OD – sample OD/control OD) × 100, where OD is optical density. Changes in the sample's absorbance were measured at 517 nm. BHA was used for comparison (18).

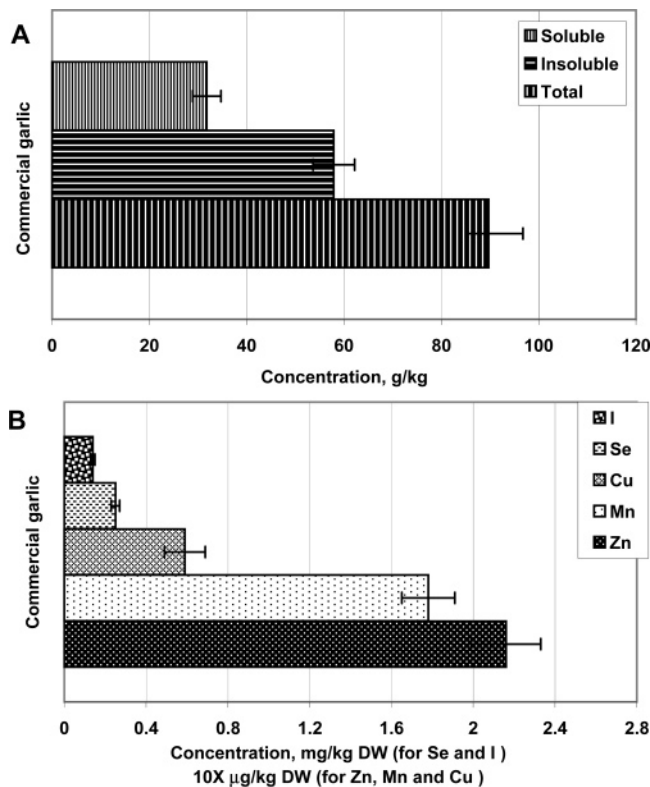
**Rats and Diets.** The Animal Care Committee of the Warsaw Agricultural University approved this study protocol, which was successfully used in our previous investigation (11). Wistar male rats (*n* = 35) with mean weight 150 g at the beginning of the experiment were divided into five diet groups, each of seven. The groups were named control, Chol, Garlic500, Garlic750, and Garlic1000. The rats were housed in individual plastic cages in an air-conditioned room (temperature = 21–22 °C and humidity = 55–65%). During 4 weeks of the experiment the rats of all five groups were fed basal diet (BD), which included wheat starch, casein, soybean oil, and vitamin and mineral mixtures. The rats of the control group were fed BD only. To the BD of the Chol group was added 1% of cholesterol. The BD of the other three groups (Garlic500, Garlic750, and Garlic1000) was supplemented with 1% of cholesterol and commercial garlic in doses equal to 500, 750, and 1000 mg of raw garlic per kilogram of animal weight, respectively. During the experiment the amount of the supplemented garlic was adjusted to the increasing weights of the animals. The daily dose of garlic for every rat was prepared just before the feeding. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. The diets contained as percentage of energy 65.5% of carbohydrates, 25.5% of protein, and 9% of fat. The calculated energy was from 395.9 to 400.1 kcal/100 g, and the differences were not significant.

As in our previous experiment (11), all rats were fed once a day at 10:00 a.m. *ad libitum*. The rats had unrestricted access to drinking water. The feed intake and body gains were monitored daily. Blood samples were taken from the left atrium of the heart before and at the end of the experiment. Plasma was prepared and used for laboratory tests. Total plasma cholesterol (TC) and TC in liver (TCL) were determined with Randox kit reagents, catalog no. CH 280, application no. 7; low-density lipoprotein cholesterol (LDL-C) was determined using the Friedewald method; and triglycerides (TG) were determined with Randox kit reagents catalog no. 1697, application no. 8 (11).

**Determination of Fibrinogen, Blood-Clotting Time, and Antioxidant Activity.** The content of blood-circulating fibrinogen (grams per liter) and closely related blood-clotting time (minutes) were determined according to the classical method of Roche (Roche Diagnostics, Warszawa, Poland). Serum fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 h, and lyophilized.

The ABTS<sup>+</sup>/K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> test was adopted for the determination of the plasma antioxidant (millimoles of TE per liter) capacity (19). The antioxidant capacity was determined in the full plasma (S), as well as in two fractions: (E), ethanol-soluble (ethanol/plasma), and (A), acetone-soluble (acetone/plasma).

**Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE).** Plasma fibrinogen samples were dissolved in sample buffer: 2% SDS, 10% glycerol, 2% mercaptoethanol, 0.002% bro-



**Figure 1.** (A) Fiber content (g/kg) and (B) microelements (mg/kg and  $\mu\text{g/kg}$ ) in commercial garlic. Means  $\pm$  SD of three measurements (vertical lines).

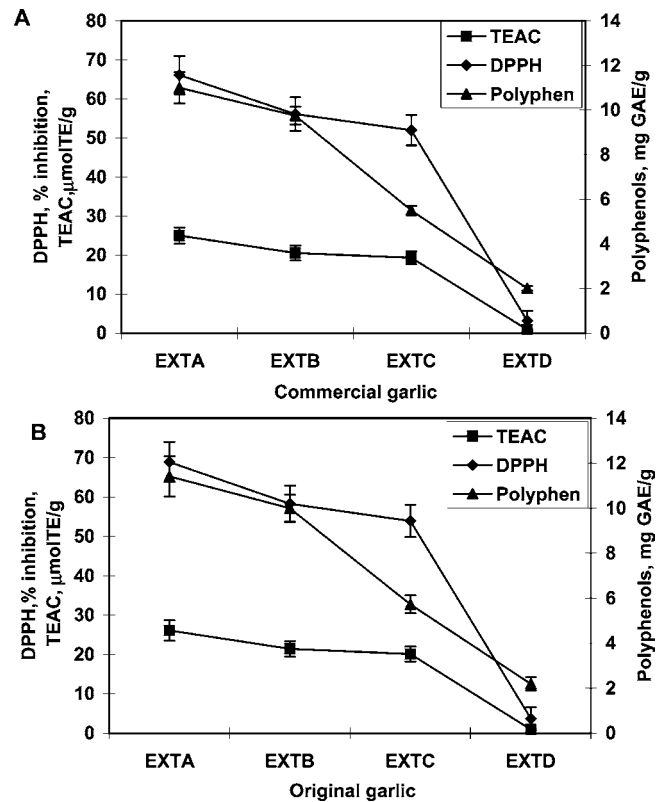
mophenol blue, and 0.62 M Tris HCl, pH 6.8. Electrophoresis was performed with the Hoefer SE 600 vertical unit (Hoefer Pharmacia Biotech Inc., San Francisco, CA) according to the Laemmli method (20), using polyacrylamide gels (resolving gel T = 13.7%, C = 1.7%, stacking gel T = 3.8%, C = 1.8%) with gel size of 180  $\times$  160  $\times$  1.5 mm. Sample size was 5  $\mu\text{L}$ . The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water, and scanned in transmission light with an Agfa SNAPSCAN 1236 (Agfa-Gevaert NV, Belgium, Agfa SnapScan 1236 s color image scanner).

**Statistics.** The results of this investigation in vitro are means  $\pm$  standard deviation (SD) of three measurements. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, the Spearman correlation coefficient (*R*) was used. Linear regressions were also calculated. *P* values of <0.05 were considered to be significant.

## RESULTS

**In Vitro. Major Bioactive Compounds.** Total, insoluble, and soluble dietary fibers (Figure 1A) and microelements (zinc, manganese, copper, selenium, and iodine) (Figure 1B) in commercial garlic were similar to those in the original Polish garlic (3).

**Total Polyphenols and Antioxidant Potentials.** Total polyphenols (milligrams of GAE per gram) in the extracted fractions of commercial garlic were as follows: EXTA, 11.0  $\pm$  0.7; EXTB, 9.75  $\pm$  0.4; EXTC, 5.5  $\pm$  0.2; and EXTD, 2.0  $\pm$  0.1. Accordingly, the antioxidant potentials in two scavenging assays were as follows: TEAC, EXTA, 25.0  $\pm$  2.1  $\mu\text{mol}$  of TE/g; EXTB, 20.5  $\pm$  1.9  $\mu\text{mol}$  of TE/g; EXTC, 19.0  $\pm$  1.7  $\mu\text{mol}$  of TE/g; EXTD, 0.9  $\pm$  0.04  $\mu\text{mol}$  of TE/g; and DPPH, EXTA, 66.1  $\pm$  4.9% of inhibition; EXTB, 56.1  $\pm$  4.3% of inhibition; EXTC, 52.0  $\pm$  3.9% of inhibition; and EXTD, 3.11  $\pm$  0.6% of inhibition (Figure 2A). All of these studied indices were



**Figure 2.** Polyphenols (mg of GAE/g) and antioxidant potentials: TEAC ( $\mu\text{mol}$  of TE/g) and DPPH (% of inhibition) in (A) commercial garlic and (B) original garlic extracts (EXT). GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl. Garlic extracts (EXT): EXTA, water; EXTB, methanol/water; EXTC, ethanol; EXTD, acetone/water extract.

**Table 1.** Weight Gains, Feed Consumption, and Feed Efficiency Ratio in Groups of Rats

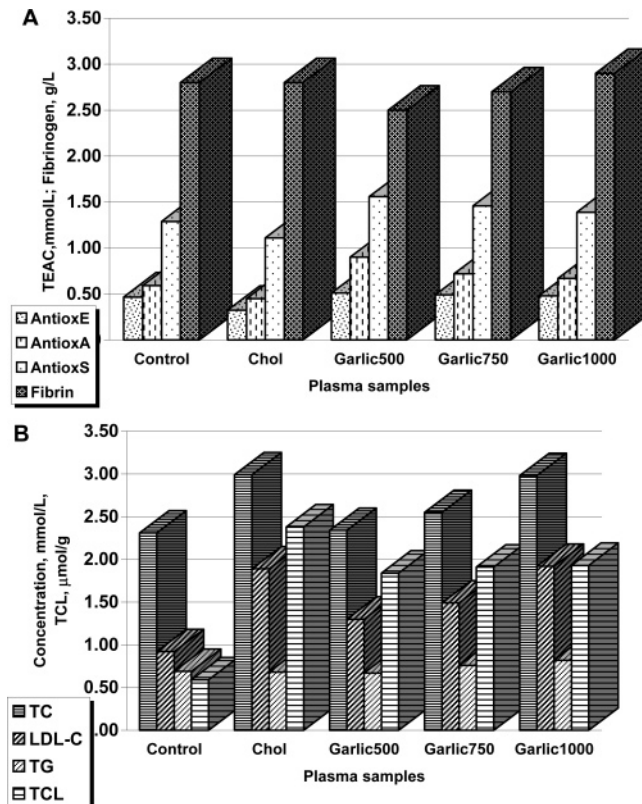
diet group	wt gain (g/day)	feed consumption (g/day)	feed efficiency ratio
control	3.8 $\pm$ 0.4a	13.9 $\pm$ 0.11a	0.273 $\pm$ 0.03a
Chol	3.4 $\pm$ 0.1a	14.4 $\pm$ 1.81a	0.236 $\pm$ 0.03a
Garlic500	4.6 $\pm$ 0.9b	16.1 $\pm$ 2.49b	0.285 $\pm$ 0.02a
Garlic750	3.9 $\pm$ 0.3a	14.1 $\pm$ 1.05a	0.276 $\pm$ 0.03a
Garlic1000	3.3 $\pm$ 0.3a	14.5 $\pm$ 2.18a	0.227 $\pm$ 0.02b

<sup>a</sup> Values are means  $\pm$  SD (*n* = 7). Means in columns without letters in common differ significantly (*P* < 0.05).

compared with the same ones in original garlic samples, and the differences were not significant (Figure 2B). In vitro results showed that commercial and original garlic samples were similar in their bioactive substances and antioxidant activities; therefore, only commercial garlic was used in in vivo experiments.

**In Vivo.** Weight gains, feed consumption, and feed efficiency ratio in all five diet groups after the trial were summarized (Table 1). All three indices varied, but the best results were registered in the Garlic500 group of rats (*P* > 0.05).

After the trial, a decrease in the plasma antioxidant activities in all groups of rats fed added cholesterol was found (Figure 3A). The decrease in the antioxidant activity only in rats of the Garlic500 group (1.11  $\pm$  0.06 mmol/L) in comparison with the control group (1.29  $\pm$  0.05 mmol/L) was not significant (*P* > 0.05). An increase in antioxidant capacity in full plasma AntioxS (1.56  $\pm$  0.06 mmol/L) and in its two subfractions [AntioxE (0.51  $\pm$  0.08 mmol/L) and AntioxA (0.9  $\pm$  0.04) mmol/L] was indicated.



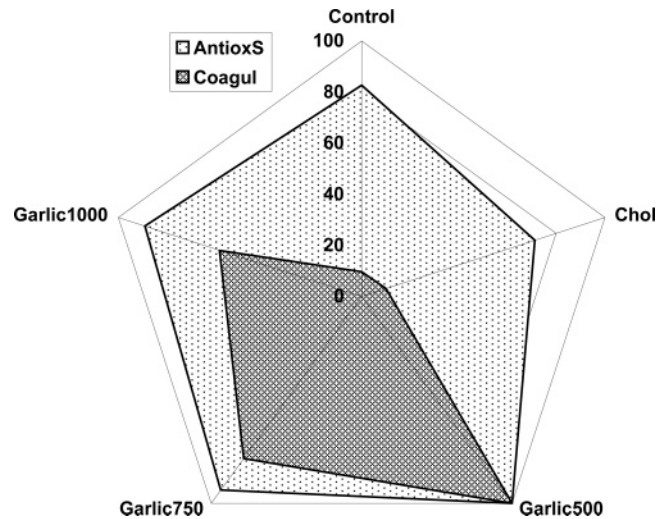
**Figure 3.** Plasma samples after different diets supplemented with garlic and cholesterol: (A) total equivalent antioxidant capacity (mmol of TE/L) in total plasma (AntioxS), ethanol-soluble (AntioxE), and acetone-soluble (AntioxA) fractions, and fibrinogen content (g/L); (B) lipid profile (mmol/L) as TC, total cholesterol (TC), low-density lipid cholesterol (LDL-C), triglycerides (TG), and total cholesterol (TCL) in liver ( $\mu\text{mol/g}$ ).

In rats of the Garlic500 diet group a significant decrease in the content of the blood-circulating fibrinogen ( $2.5 \pm 0.18$  mmol/L) was found (Figure 3A). Only commercial garlic in a dose of 500 mg significantly hindered the rise in plasma lipids (Figure 3B) in all fractions: TC,  $2.34 \pm 0.15$  mmol/L; LDL-C,  $1.3 \pm 0.12$  mmol/L; TG,  $0.67 \pm 0.06$  mmol/L; and TCL,  $18.4 \pm 1.24$   $\mu\text{mol/g}$ , which was connected with the cholesterol feeding ( $P < 0.05$ ).

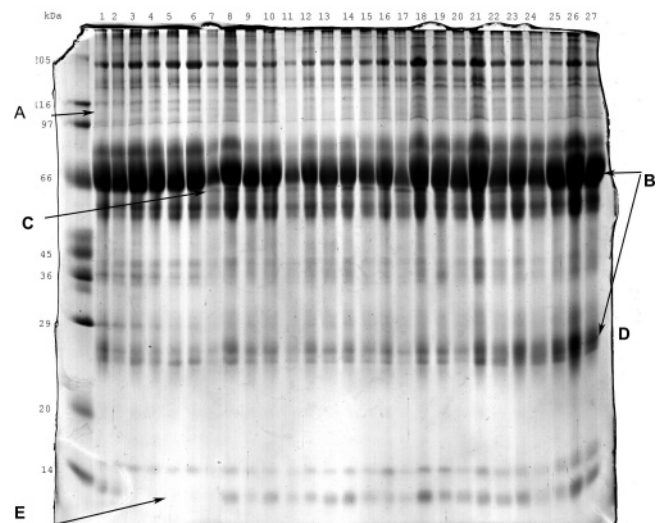
In rats of the Garlic500 group a significant increase in blood-clotting time and the highest total antioxidant capacity (Figure 4) were registered ( $P < 0.05$  in both cases). The protein profile of plasma standard samples (Figure 5, lanes 1–2) showed that in the fibrinogen fraction was less protein than in the experimental groups (arrow A). Garlic750 (lanes 14–20) and Garlic1000 (lanes 21–27) diet groups had more protein at 66 kDa than Garlic500 (Figure 5, lanes 7–13, arrows B and C, respectively). At 24 kDa in Garlic1000 was observed more protein than in Garlic750 and Garlic500 diet groups (arrow D). There was indicated also a lack of 14 kDa protein in the control and Chol groups and very weak 14 kDa protein in Garlic500 (arrow E) in comparison with the Garlic750 and Garlic1000. The main patterns were located in the range of 50–90 kDa (Figure 5).

## DISCUSSION

Garlic has been used as an herbal medicine for thousands of years, and it contains several medically active substances that possess many favorable effects, such as decrease in LDL-C, antioxidation, antithrombosis, and suppression of platelet ag-



**Figure 4.** Plasma samples after different diets supplemented with garlic and cholesterol: AntioxS, total antioxidant capacity (mmol of TE/L); Coagul, blood clotting time (min).



**Figure 5.** Comparison of band intensity of serum proteins (methanol extraction) after different diets dissolved with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. Molecular markers (kDa): 205, myosin; 116,  $\beta$ -galactosidase; 97, phosphorylase *b*; 66, bovine albumin; 45, ovalbumin; 36, glyceraldehyde-3-phosphate dehydrogenase; 29, carbonic anhydrase; 24, trypsinogen, PMSF treated; 20, trypsin inhibitor; 14,  $\alpha$ -lactalbumin. Lanes: 1–12, full serum, loading, 2  $\mu\text{L}$ ; 1, 2, standard serum; 3, 4, control; 5, 6, cholesterol; 7–13, Garlic500; 14–20, Garlic 750; 21–27, Garlic1000. Arrow A at 105 kDa protein shows that in the standard group this band was less intense than in all other groups; arrow B indicates that Garlic750 and Garlic1000 diet groups had more protein at 66 kDa than Garlic500 (arrow C); arrow D shows that at 24 kDa in Garlic1000 there was more protein than in Garlic750 and Garlic500. Arrow E shows a lack of 14 kDa protein in control and cholesterol groups and very weak 14 kDa protein in Garlic500 in comparison with the Garlic750 and Garlic1000.

gregation (21). New data have increased the interest in garlic, and its role in normalization and treatment of cardiovascular disease risk factors (22). It has been discussed that garlic positively influences plasma lipid levels and plasma antioxidant activity in experiments on laboratory animals and humans and can normalize plasma lipid levels and control lipid peroxidation (11, 21–23).

Only a few scientists have claimed that garlic consumption shows no significant effect on markers of oxidation or on subfraction composition of low-density lipoproteins (24, 25). The influence of garlic and its preparations on the blood coagulation system was described (26–28); however, only in a few studies was the dose-dependent influence of garlic preparations on the above-mentioned indices determined (15, 29). Therefore, in the present investigation the dose-dependent influence of commercial garlic on plasma lipids, antioxidant activity and the closely related fibrinogen content, and clotting time was studied.

The investigation in vitro of the commercial garlic used has shown that the contents of dietary fibers, microelements, and total polyphenols were similar to the contents in already studied garlic, coefficient ratio of 1:4 (14). These data are in accordance with other studies, showing highest hydrogen-donating and hydrogen peroxide-scavenging activities and reducing power (8, 30).

In vivo there was registered a significant hindering in the rise of the plasma lipids only in rats of the Garlic500 group versus the Chol group. The hindering of the rise in plasma lipids was dose-dependent, and the best results were registered in the group of rats for which the diet was supplemented with 500 mg of commercial garlic per kilogram of animal weight (Garlic500). A decrease in the plasma antioxidant activity was found in all four groups of rats fed cholesterol-containing diets. However, this decrease was less than in the Chol group, for which the diet was not supplemented with garlic. Also, this effect was dose-dependent: the best results were registered in the rats of the Garlic500 group ( $P < 0.05$ ).

We found that Garlic500 was the most effective. Our results are in accordance with other data (15). In our opinion, the higher doses are less effective because of the well-known in medicine “paradoxical effect”.

The present results correspond with other studies based on synergism of extracts and powders. A 33% increase in LDL inhibition was observed when lycopene used together with garlic powder (1, 2, 4, 7). The reported results are as well similar to the conclusion of other papers (10, 23) that plasma total cholesterol (TC), LDL-C, and triglyceride levels were found to be significantly lowered but that the high-density lipoprotein cholesterol (HDL-C) level increased after the garlic diet. The TC/HDL-C ratio was also found to be decreased in Garlic500 ( $1.72 \pm 0.08$ ) in comparison to Garlic750 ( $1.90 \pm 0.09$ ) and Garlic1000 ( $2.22 \pm 0.15$ ). It was observed that cholesterol-fed animals had a significant increase in serum cholesterol compared to the control group of rats fed a normal diet. However, when the rats were fed with a high-cholesterol diet mixed with garlic powder, there was a significant reduction in their serum cholesterol levels compared with the groups that were on a diet containing high cholesterol without garlic powder. Serum triglyceride levels were also significantly lowered by garlic powder when compared to control and high-cholesterol diet group rats. Our results support as well the results of another study (31) that the effect of garlic is most pronounced with relatively high initial cholesterol and triglycerides.

It is very important to include in coronary atherosclerosis preventing diets natural products that are able to exercise an anticoagulant-increasing influence (12, 31).

Therefore, the blood fibrinogen level and the blood-clotting time were determined in vivo. There were found a dose-dependent decrease in the content of the blood-circulating fibrinogen and a significant increase in the blood-clotting time ( $P < 0.05$  in both cases). Fibrinogen decreased by  $\approx 11\%$ , which

is similar to the decrease of fibrinogen in patients with coronary heart disease (31). The data of the present investigation confirm that the studied commercial garlic possesses dose-dependent hypolipidemic, antioxidant, and anticoagulant properties.

Our obtained results can be compared and evaluated only with very limited data connected with other dietary plants that are used in traditional foods and medicines and were applied under different conditions from those shown in the present experiment (in animal and human models with various duration of administration, a wide range of supplementation to the diets, and other variables). Regular consumption of lettuce, for example, should contribute to improve protection against antioxidant stress, showing a beneficial effect on lipid metabolism and tissue oxidation. A lettuce diet of 20% on rat model during 3 weeks markedly decreased liver cholesterol levels by  $\approx 41\%$  and the LDL-C/HDL-C ratio and significantly increased both ascorbic acid and  $\alpha$ -tocopherol plasma levels, which contributed to improved plasma antioxidant capacity (32). In our experiment it was a decrease of liver cholesterol levels of  $\approx 23\%$ . Some extracts of herbs, similarly as in our experiments, have been shown to have inhibitory effects on blood platelet aggregation, thrombin-induced conversion of fibrinogen to fibrin, and the activity of plasminogen or plasmin. The protection of herbal medicines (33) on the ischemic infarction induced artificially might be related to their inhibitory effects on platelet coagulation and thrombotic action, which may be identical to the action of garlic extracts as in our experiment. Similar effects were described with fruit juice concentrate on rats, showing improved blood fluidity and inhibitory effects on collagen and platelet aggregations and on thrombin-induced conversion of fibrinogen to fibrin (34).

The electrophoretic bands of the fibrinogen fraction showed a difference between the garlic diets: in Garlic500, the 66 and 24 kDa electrophoretic bands had less protein than Garlic750 and Garlic1000. No information exists about the changes in the separated proteins after garlic or other plant diets; therefore, it is not possible to compare our data with other studies. Only two papers mostly describe the use of electrophoretic separation for characterization of a profibrinolytic plasminogen-binding protein (35, 36). In the present paper the detection of the differences in the protein composition of rat plasma after garlic diets was found. The reviewed data of the activity of fibrinogen were around the range of 40–54 kDa (35, 36). As can be seen (Figure 5), most of the proteins were located in this region, but the differences were found in low (14 and 24 kDa) and middle (66 kDa) molecular mass proteins in this study.

The above-mentioned data on serum antioxidant activity, proteins, and lipid metabolism could justify the inclusion of commercial garlic in coronary atherosclerosis-preventing diets. However, it is well-known that the results of experiments on laboratory animals cannot be automatically applied to humans. Therefore, further investigations on human volunteers suffering from coronary atherosclerosis and hyperlipidemia are needed.

In conclusion, the positive influences of the studied commercial garlic on plasma lipids, plasma proteins, plasma antioxidant activity, and some indices of blood coagulation are dose-dependent. Therefore, the studied commercial garlic could be a valuable component of atherosclerosis-preventing diets only in optimal doses.

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