

Supplementation of garlic lowers lipids and increases antioxidant capacity in plasma of rats

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Abstract

The bioactivity of raw and boiled garlic (*Allium sativum* L.), comprising contributions from polyphenols, was determined by cupric-reducing antioxidant capacity and trolox equivalent antioxidant capacity assays. Boiling garlic at 100°C for 20 minutes preserves its bioactivity and makes it comparable with the raw sample. Wistar male rats were randomly divided into 10 diet groups with garlic supplementation. The control group was fed basal diet that included wheat starch, casein, soybean oil, cellulose, mineral, and vitamin mixtures. To the basal diet of the other groups, 25 mg of lyophilized garlic equivalent to 500 mg raw garlic/kg body weight (raw) was added. The same quantity of boiled garlic for 20, 40, and 60 minutes (Gar20, Gar40, and Gar60 groups), 1% of cholesterol (Chol) and 25 mg of lyophilized raw garlic (Chol/Raw), 1% of Chol, and the same quantity of boiled garlic for Chol/Gar20, Chol/Gar40, and Chol/Gar60 groups were added, respectively. After the trial in rats of Chol/Raw and Chol/Gar20 diet groups, the added garlic significantly hindered the rise in plasma lipids. A significant increase ($P < .05$) in plasma antioxidant activity was registered in Raw and Gar20 diet groups. In conclusion, raw and boiled garlic at 100°C for 20 minutes improved the plasma lipid levels in rats fed cholesterol-containing diets and increased the plasma antioxidant activity in groups of rats fed cholesterol-free diets. Garlic boiled for a short time can be used as an additive in cooking.

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1. Introduction

Garlic (*Allium sativum* L.) possesses many healthful properties that are related to its bioactive compounds [1-4]. It was reported that consumption of garlic is very helpful in regulating plasma lipid levels [5] as well as plasma anticoagulant activity [6,7] and in prevention of the atherosclerosis process [8] and even cancer [9]. It was

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shown that garlic also provides protection against ethanol-induced gastric injury [10]. The most studied and reported health-promoting effect of garlic is cardioprotection [5,8]. However, a pilot study of garlic consumption shows no significant effect on markers of oxidation or subfraction composition of low-density lipoprotein (LDL) [11]. It was also reported that short-term garlic therapy in adults with mild to moderate hypercholesterolemia does not affect lipid levels [12]. Kerckhoffs et al [13] claim that it is still uncertain whether garlic or garlic preparations can be used as lipid-lowering agents.

There is no doubt that garlic and garlic preparations possess anticoagulant abilities [6,7]. However, there is still a controversy regarding the plasma lipid regulating and antioxidant increasing properties of garlic. Therefore, we decided to study the possible changes in the plasma lipid levels and antioxidant activity through an experiment on rats fed cholesterol-containing and cholesterol-free diets supplemented with raw or boiled garlic. Fruits and vegetables subjected to temperature lose a certain part of their bioactivity [14,15]. Garlic is widely used as an obligatory ingredient in many cooked dishes [1,3,5]. The knowledge about the influence of temperature on the bioactive properties of garlic is very limited; therefore, the optimal cooking regime that preserves the bioactivity of raw garlic was determined.

2. Methods and materials

2.1. Chemicals

6-Hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid (trolox); 2, 2' -azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS); Folin-Ciocalteu reagent; $\text{CuCl}_2 \times 2\text{H}_2\text{O}$; gallic and caffeic acids; catechin; and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma Chemical Co (St. Louis, Mo, USA) and from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade.

Deionized and distilled water were used throughout.

2.2. Samples preparation

Raw Polish garlic was purchased from a local grower in Warsaw from the harvest of 2004. The garlic samples were prepared manually. The edible parts were weighed and divided into 4 parts: 1 is to be used raw, and the other 3 for boiling at 100°C for 20, 40, and 60 minutes, respectively. Water from boiled samples was removed by filtration. The samples were cooled, crushed, and frozen under a temperature of -20°C. After lyophilization, 500 mg of fresh and boiled garlic samples was equal to 25 mg of lyophilized garlic.

2.3. Determination of the bioactive compounds

Dietary fiber, minerals, minor elements, total polyphenols, total tocopherols, and its most abundant and active isomer α -tocopherol were determined as previously de-

scribed [16-18]. The total phenols were extracted with 5 mL of 1.2 mol/L HCl in 50% methanol/water from 50-mg aliquot of deproteinized lyophilized garlic samples. The samples were cooled and diluted to 10 mL with methanol and centrifuged for 5 minutes at $4000 \times g$ with a benchtop centrifuge to remove solids. The phenols were measured at 750 nm after reacting for 10 minutes, using the Folin-Ciocalteu reagent that was diluted 5-fold before use, with gallic acid as standard [16-18].

2.4. Determination of the total antioxidant potentials

There are many methods for total antioxidant determination with specific limitations. Some of the antioxidant assays give different antioxidant activity trends [19]. Therefore, the antioxidant potential of raw and boiled garlic samples was determined by 2 complementary methods using 2,2' -azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diaminium salt (ABTS^{++}) with $\text{K}_2\text{S}_2\text{O}_8$ [19] and copper (II)-neocuproine [Cu (II)-Nc] [20].

ABTS^{++} radical cation was generated by the interaction of ABTS (250 μM) and $\text{K}_2\text{S}_2\text{O}_8$ (40 μM). After addition of 990 μL of ABTS^{++} solution to 10 μL of different garlic extracts (0.2 mg/mL) or trolox standards (final concentration 0-20 μM) in ethanol or phosphate-buffered saline, the absorbance was monitored exactly 1 and 6 minutes at 734 nm after the initial mixing [19].

Cupric-reducing antioxidant capacity (CUPRAC) is based on using the Cu (II)-Nc reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of Cu (II), Nc, and NH_4Ac buffer solution, antioxidant sample (or standard) solution (x mL) and H_2O ($[1.1 - x]$ mL) were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank [20]. The percentage decrease of the absorbance was calculated and plotted as a function of sample concentration and of trolox for the standard reference data [19,20].

2.5. Animals and diets

The Animal Care Committee of Warsaw Agricultural University approved this study. The Institute of Animal Physiology and Nutrition of Polish Academy of Science (Jablonna, Poland) provided male Wistar rats ($N = 70$) with a mean weight of 150 g at the beginning of the experiment. These rats were randomly divided into 10 diet groups with 7 rats each: control, Raw, Gar20, Gar40, Gar60, Chol, Chol/Raw, Chol/Gar20, Chol/Gar40, and Chol/Gar60.

The diets of all rat groups are summarized in Table 1. As can be seen, the control group was fed basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, mineral, and vitamin mixtures. To the BD of the 9 other groups, 25 mg of lyophilized raw garlic (equivalent of 500 mg raw garlic/kg body weight [Raw]), the same quantity of garlic boiled for 20, 40, and 60 minutes for Gar20, Gar40, and Gar60, respectively, 1% of cholesterol (Chol), 1% Chol and 25 mg/kg body weight of lyophilized raw garlic (Chol/Raw), 1% Chol, and the same quantity of

Table 1
Composition (g/kg) of the basal diet (BD)

Ingredients/Diet groups	Control	Raw	Gar20	Gar40	Gar60	Chol	Chol/Raw	Chol/Gar20	Chol/Gar40	Chol/Gar60
Casein	180	180	180	180	180	180	180	180	180	180
Soybean oil	60	60	60	60	60	60	60	60	60	60
Wheat starch	680	680	680	680	680	680	680	680	680	680
Raw garlic	–	+	–	–	–	–	+	–	–	–
Garlic20*	–	–	+	–	–	–	–	+	–	–
Garlic40*	–	–	–	+	–	–	–	–	+	–
Garlic60*	–	–	–	–	+	–	–	–	–	+
Cholesterol	10	10	10	10	10	10	10	10	10	10
Vitamin mixture ¹	10	10	10	10	10	10	10	10	10	10
Mineral mixture ²	60	60	60	60	60	60	60	60	60	60

* To the BD were added 25 mg of lyophilized raw or boiled for 20, 40 and 60 min (Gar) garlic/kg of the animal's body weight.

¹ Vitamins (per kg of diet): thiamin, 20 mg; riboflavin, 15 mg; pyridoxin, 10 mg; nicotinamide, 100 mg; calcium pantothenate, 70 mg; folic acid, 5 mg; biotin, 0.3 mg; cyanocobalamin, 0.05 mg; retinyl palmitate, 1.5 mg; dl- α -tocopheryl acetate, 125 mg; cholecalciferol, 0.15 mg; menadione, 1.5 mg; ascorbic acid, 50 mg; myo-inositol, 100 mg; carrier wheat starch, 1.36 g.

² Minerals (per kg of diet): CaHPO₄, 15 g; K₂HPO₄, 2.5 g; KCl, 5 g; NaCl, 5 g; MgCl₂, 2.5 g; Fe₂O₃, 2.5 mg; MnSO₄, 125 mg; CuSO₄ × 7H₂O, 0.2 mg; ZnSO₄ × 7H₂O, 100 mg; KIO₃, 0.4 mg.

garlic boiled for 20, 40, and 60 minutes for Chol/Gar20, Chol/Gar40, and Chol/Gar60 were added, respectively. The dose of 500 mg (25 mg of lyophilized garlic/kg body weight) was chosen as the most effective [21].

During the experiment, the dose of the used garlic was adjusted to the growing weight of every animal. The dietary cholesterol was checked by high-performance liquid chromatography and was found not to contain cholesterol oxides. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. These diets contained as percentages of energy 66% carbohydrates, 25% protein, and 9% fat. The calculated energy of the used diets was from 1658.8 to 1675.1 kJ/100 g, and the difference was not significant. All rats were fed once a day at 10:00 AM ad libitum with unrestricted access to drinking water. Garlic was added to the diets before feeding.

It is generally accepted that the most reliable data of the blood lipid metabolism can be obtained from fasting animals, 14 to 16 hours after the last feeding. Therefore, the feed was removed from the cages at 6:00 PM the day before. The samples were collected at 9:00 AM the next day. The plasma was prepared and used for laboratory tests.

Two time points were used in this experiment: before and after the experiment. At these time points, a wide range of laboratory tests was performed. The plasma total cholesterol (TC) was determined with Radox kit reagents No Cat. CH 280, Appl. No 7; LDL cholesterol (LDL-C) and high-density lipoprotein cholesterol using the Friedewald method; and triglycerides with Radox kit reagents No Cat. 1697, Appl. No 8 [17,18].

The antioxidant capacity of plasma was determined by ABTS^{•+}/K₂S₂O₈ and by CUPRAC assays in the same way as described above for garlic extracts [19,20,22,23].

2.6. Statistical analysis

Values are given as the means ± SD of 5 measurements. Where appropriate, data were tested by 2-way analysis of

variance using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, Calif), followed by Duncan new multiple range test to assess differences between groups means. Differences of $P < .05$ were considered significant.

3. Results and discussion

The use of herbs and other natural products has gained popularity, and the increase in their consumption is backed by solid scientific evidence [6,24,25]. One of these natural products is garlic, which has been used throughout the history of civilization for treatment of a wide variety of ailments [1–4]. The wide range of ailments in which this vegetable is used [7,9,10] could create an impression that garlic is a panacea, but it is not [11,13].

The most studied and reported health promoting effect of garlic is cardioprotection [5,8]. However, some authors claim that garlic is not effective in plasma lipid lowering, and the use of garlic for treatment of hypercholesterolemia is of questionable value [12,26]. It is a common knowledge that fruits and vegetables subjected to cooking temperature lose a certain part of their bioactivity. Therefore, the aims of this investigation were (a) to investigate in vitro the bioactive properties of raw and boiled garlic; (b) to determine the optimal boiling regime, which preserves the bioactivity of garlic; and (c) to study the influence of raw and boiled garlic on plasma lipid levels and plasma antioxidant activity of rats fed cholesterol-containing and cholesterol-free diets.

3.1. In vitro experiment

3.1.1. Dietary fibers

The contents of the total, soluble, and insoluble dietary fibers in the studied samples of raw garlic were as follow: 23.9 ± 2.1 , 8.6 ± 0.6 , and 14.8 ± 1.2 g/kg fresh weight (FW), respectively. There were no significant changes in the contents of the dietary fibers in the samples boiled at

100°C for 20, 40, and 60 minutes as well as those in the year of harvesting [16–18] (data not shown).

3.1.2. Minerals and Minor elements

There were no significant changes in the contents of the minor elements (Fig. 1A and B) in garlic samples boiled at 100°C for 20, 40, and 60 minutes ($P > .05$) as well as those in the year of collection [16–18].

3.1.3. Total and α -tocopherols

The contents of total and α -tocopherols in raw garlic were 103.1 ± 9.1 and 84.9 ± 6.9 $\mu\text{g}/100$ g FW, respectively. The contents of total and α -tocopherols in garlic samples boiled at 100°C for 20, 40, and 60 minutes were 101.3 ± 8.9 and 82.8 ± 6.9 , 79.9 ± 6.7 and 64.3 ± 5.9 , and 70.1 ± 6.1 and $62.4.9 \pm 5.9$ $\mu\text{g}/100$ g FW, respectively. The decrease in the content of total and α -tocopherols was significant only in garlic samples boiled at 100°C for 40 and 60 minutes ($P < .05$).

3.1.4. Total polyphenols and total antioxidant potentials

The decrease in the contents of total polyphenols and in related total antioxidant potentials of raw and boiled garlic

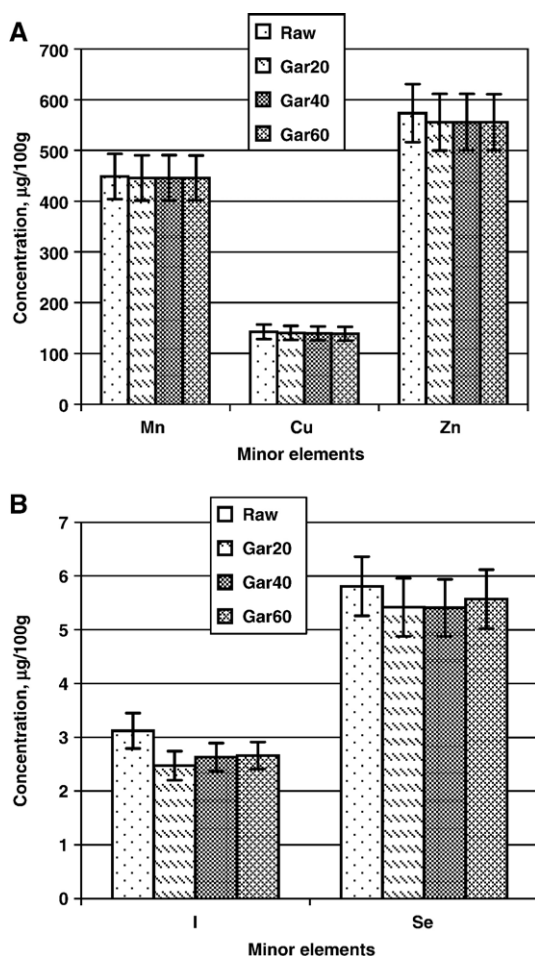


Fig. 1. Changes in the contents of minor elements in raw and boiled garlic samples ($\mu\text{g}/100$ g FW). A, Mn, Cu, and Zn; B, I and Se.

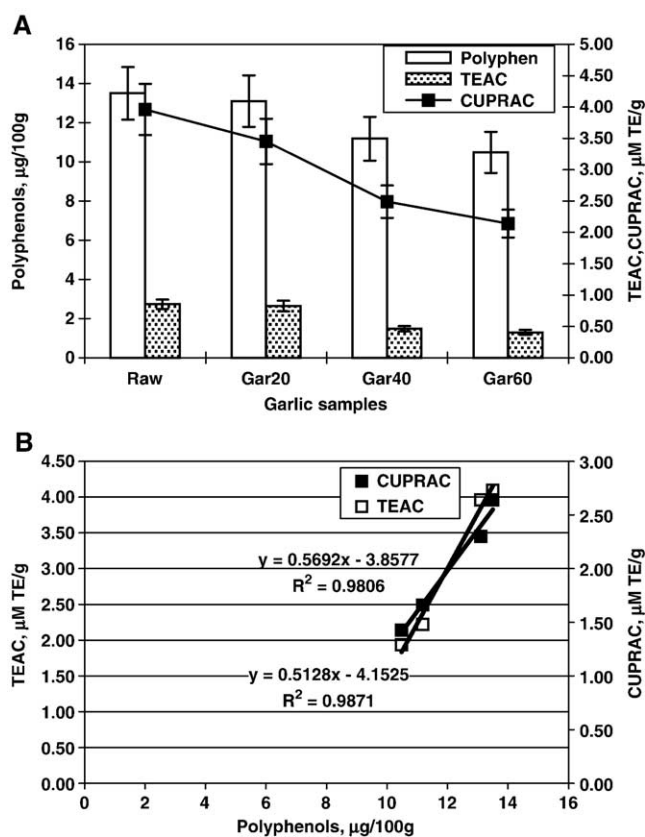


Fig. 2. A, Changes in total polyphenols and total antioxidant potentials of raw and boiled garlic samples at 100°C for 20, 40, and 60 minutes. B, Relationship, calculated by linear regression analysis for garlic extracts, between polyphenols ($\mu\text{g GAE}/100$ g, x) to TEAC ($\mu\text{M TE/g}$, y_1) indicated by \square and polyphenols ($\mu\text{g GAE}/100$ g, x) to CUPRAC ($\mu\text{M TE/g}$, y_2) indicated by \blacksquare . Polyphenols are expressed as μg gallic acid equivalent (GAE). TEAC indicates trolox equivalent antioxidant capacity.

samples (Fig. 2A) was significant only after boiling at 100°C for 40 and 60 minutes ($P < .05$). The antioxidant polyphenolic compounds showed that the highest capacities in ABTS^{++} with $\text{K}_2\text{S}_2\text{O}_8$ /CUPRAC assays were observed for catechin (2.31/2.96), caffeic acid (1.42/2.65), and gallic acid (3.11/2.42). The results with these 2 assays were very similar and corresponded with the data of other reports [19,20]. Garlic showed slightly higher results than catechin itself.

The contribution of total polyphenols to the antioxidant potentials of raw and boiled garlic determined by 2 methods was high (Fig. 2B; R^2 , 0.9806–0.9871). It was proven that the antioxidant potentials were directly related to the contents of phenolic compounds, as in other reports as well [1,27–29]. The reviewed report [27] has shown that organic and aqueous extracts of garlic had significant antioxidant potential, as measured by the decreases in free radicals and by its ability to inhibit lipid oxidation. The hydrogen-donating and hydrogen peroxide-scavenging activities as well as the reducing power of garlic samples depend on the composition and the amount of minor elements that participate in the complex of polyphenols, the main dietary antioxidants. The investigated complex of garlic bioactivity including selenium, copper, and

other metals is very important for the overall antioxidant activity of garlic samples, which was similar to the data of others [2,4,30] and nearly repeated the results of the previous years of collection [16-18]. Some differences in the contents of selenium, total tocopherols, polyphenols, and dietary fibers obtained in this report and data published by others [4,27-29] may be because of the growing conditions and year of harvesting.

The results of in vitro studies have shown that minerals and minor elements, as well as tocopherols, dietary fibers, polyphenols and antioxidants in raw garlic, its aqueous extracts [17], and samples boiled for a short time, were relatively high and were comparable ($P > .05$) and stand in the same line with other reports [25,28]. However, the contents of tocopherols, phenolics, and the total antioxidant potential in garlic boiled at 100°C for 40 and 60 minutes were significantly less than in raw samples ($P < .05$).

3.2. In vivo experiment

Addition of raw or boiled garlic and/or cholesterol to the diets did not affect feed intake, body weight gains, or feed efficiencies (data not shown).

At baseline, the 10 diet groups did not differ from one another in plasma lipid concentration. The changes in the plasma lipid levels after completion of the trial are shown in

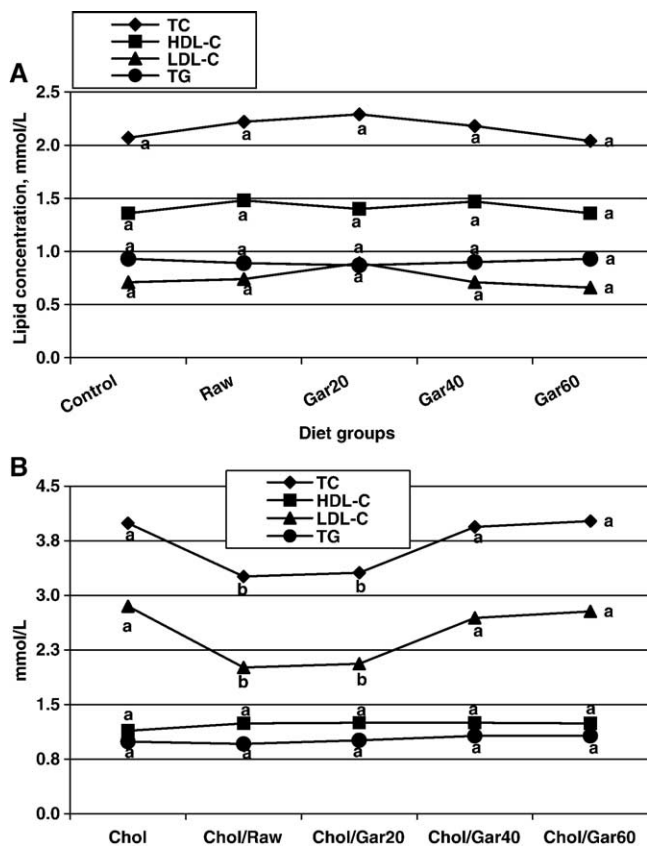


Fig. 3. Changes in lipid levels in rats fed cholesterol-free diets (A) and cholesterol-containing diets (B). Values are means (n = 7). Data with different letters are significantly different ($P < .05$).

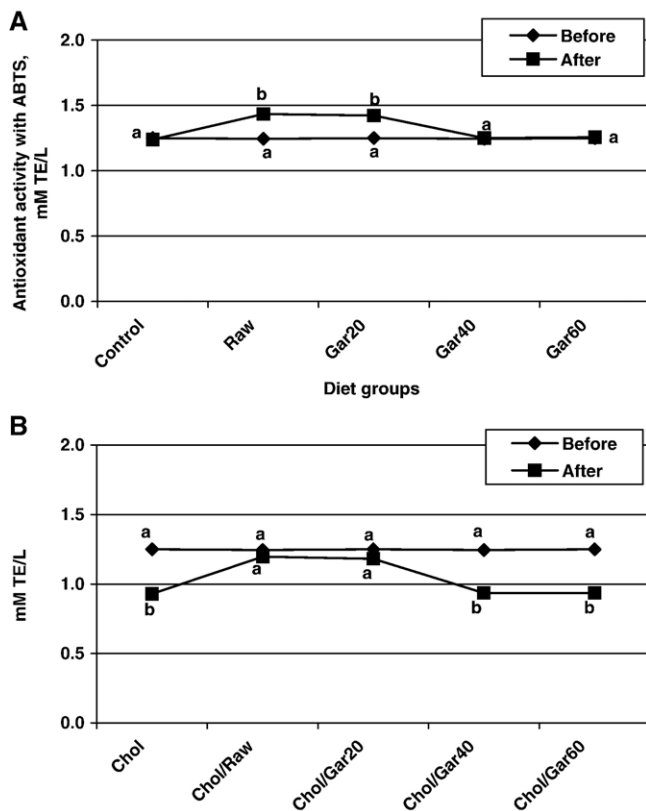


Fig. 4. Plasma antioxidant activity in rats fed cholesterol-free diets (A) and cholesterol-containing diets (B). Values are means (n = 7). Data with different letters are significantly different ($P < .05$).

Fig. 3. As can be seen (Fig. 3A), no significant changes in the lipid levels after completion of the experiment in all groups of rats fed cholesterol-free diets were found ($P > .05$). In the group of rats fed cholesterol-containing diets (Fig. 3B), only garlic samples of raw and boiled at 100°C for 20 minutes (Chol/Raw and Chol/Gar20) significantly hindered the rise of TC and LDL-C ($P < .05$). Therefore, the raw garlic, and to less degree garlic boiled at 100°C for 20 minutes, significantly hindered the cholesterol-induced increase in plasma TC and LDL-C.

Diets supplemented with garlic in rats fed without cholesterol did not affect the lipid levels ($P > .05$). In addition, others have demonstrated that hypolipidemic effect of fruits and vegetables is evident when they are added to diets of rats fed cholesterol [31].

At the end of the trial, a significant increase in the plasma antioxidant activity in the rats of Raw (14.7%) and Gar20 (13.8%) groups was found ($P < .05$), as well as an increase in the trolox values (Fig. 4A). A decrease in the plasma antioxidant activity in rats fed with added cholesterol (Chol, Chol/Raw, Chol/Gar20, Chol/Gar40, and Chol/Gar60 groups) was registered. However, only in the rats of Chol (25.7%), Chol/Gar40 (20.9%), and Chol/Gar60 (24.4%) groups, the decrease in the antioxidant activity values (Fig. 4B) was significant ($P < .05$). Therefore, the samples of raw garlic and garlic boiled at 100°C for 20 minutes

hindered the decrease in the antioxidant activity in the Chol/Raw (94.1%) and Chol/Gar20 (94.9%) groups. Results of the CUPRAC assay showed similar to ABTS/K₂S₂O₈ values of antioxidant activity. The control samples showed with CUPRAC and ABTS/K₂S₂O₈ antioxidant values of 1.01 ± 0.11 and 1.25 ± 0.13 mmol TE/L. Antioxidant capacity of plasma in experimental groups supplemented with whole garlic after boiling or with his water fractions [17] showed with CUPRAC an increase of Raw (13.9%) and Gar20 (12.5%) diets, and a decrease in Chol group (23.9%), Chol/Gar40 (19.3%), and Chol/Gar60 (21.2%) was found. The findings of CUPRAC not completely agreed with those of ABTS-persulfate but were similar. This can be explained by the method of extraction. In this report, the total antioxidant activity was determined without extraction of 2 plasma fractions: lipophilic and hydrophilic. As an advantage over other electron transfer-based assays as ABTS and Folin, CUPRAC values are acceptable in regard to its realistic pH close to the physiological pH [20,23].

At the end of the trial, a significant increase in the plasma antioxidant activity in the rats of the Raw and Gar20 diet groups and a decrease in the plasma antioxidant activity in rats fed with added cholesterol (Chol, Chol/Raw, Chol/Gar20, Chol/Gar40 and Chol/Gar 60 groups) were observed. Such results were expected. Uysal [32] observed that cholesterol-supplemented diet decreases the blood antioxidant activity. In addition, Durak et al [33] reported that cholesterol-fed animals showed a significantly impaired antioxidant system.

Therefore, the aims of this investigation were achieved.

4. Conclusions

Raw garlic and garlic boiled at 100°C for 20 minutes contain high comparable quantities of bioactive compounds and possess high total antioxidant potential. Raw garlic and garlic boiled at 100°C for 20 minutes positively influenced plasma lipid levels and antioxidant activity in all groups of rats fed cholesterol-containing diets. To preserve the important properties of garlic, this vegetable has to be added to dishes not earlier than 20 minutes before the end of the cooking process.

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