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RESEARCH ARTICLE

Age-dependent changes in the proteolytic–antiproteolytic balance after alcohol and black tea consumption

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Abstract

Aging is accompanied by changes in the redox balance that is additionally modified by alcohol. Ethanol metabolism is connected with generation of free radicals which can damage cell components especially when antioxidant mechanisms are not able to neutralize them. In connection with the necessity of prevention against oxidative consequences, natural antioxidants are looked for. A natural and commonly used component of the diets with antioxidant properties are teas, especially the black tea. This study provides evidence of the role of black tea in the protection of rat plasma proteins and lipids against oxidative stress caused by aging and ethanol intoxication. For 5 weeks, the rats (2-, 12-, and 24-months old) used for the experiment received a black tea beverage (3 g/l) without or with alcohol (given for 4 weeks). The decrease in antioxidant abilities determined as total antioxidant status during aging and ethanol intoxication resulted in enhanced lipid and protein oxidation (determined as malondialdehyde, carbonyl groups, dityrosine, tryptophan and sulfhydryl groups level). In consequence the decrease in anti-proteases (alpha-1-antitrypsin, alpha-2-macroglobulin) activity and the increase in proteases (elastase and cathepsin G) activity were observed. Black tea protected the plasma antioxidants and prevented oxidative modifications of lipid and protein observed during aging as well as ethanol intoxication. The results indicate that a shift into plasma proteolytic activity results from a decrease in antioxidant abilities, so the use of black tea appears to be beneficial in reducing oxidative stress caused by ethanol and/or aging.

Keywords: *Ethanol; proteases; anti-proteases; lipid peroxidation; protein oxidative modifications*

Introduction

The free radical theory of aging suggests that this unpleasant yet inevitable chain of changes is caused by a shift in the balance between the pro-oxidative and anti-oxidative processes in the direction of the pro-oxidative state. The oxidative state is dependent on the level of reactive oxygen species (ROS) as well as the activity of antioxidant enzymes and the level of non-enzymatic antioxidants as well as the factors that regulate their biosynthesis and degradation. However, it has been shown that, during aging, the antioxidant system is not able to prevent ROS action (Leutner et al. 2001). The oxidative state, which is changed during aging, may additionally be modified by diet. Food components may increase, as well as decrease, cellular antioxidant abilities. One such diet component, which influences ROS generation and antioxidant status, is alcohol (Luczaj and Skrzydlewska 2004; Kapaki et al. 2007). It has been postulated that ethanol consumption, as well as the aging process, results from an increase in the oxidative modifications of lipids, proteins, or DNA, and the accumulation of unrepaired damages (Fukagawa 1999). Oxidation is believed to be one of the most important modifications relevant to aging because of its ubiquitous effect.

Proteins are known to continuously undergo a variety of reversible or post-translational modifications, including oxidation, which modulate their biological activity. Protein oxidation is mainly dependent on physiological and environmental factors responsible for ROS as well as lipid and carbohydrate derivative carbonyl compounds, whose levels are increased in aging as well as during ethanol metabolism (Pérez et al. 2009). The intensity of oxidation is also dependent on the availability of redox active iron and copper, the intracellular redox potential, the availability of NADPH and NADH, oxygen tension, etc. (Stocker and Keaney Jr. 2004). Aromatic and sulfhydryl protein amino acid residues are

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the most susceptible to oxidation (Davies et al. 1987). It has also been proven that a decrease in the amount of protein sulfhydryl groups is a sensitive marker of oxidative modifications of proteins in aging as well as during ethanol intoxication (Rizvi and Maurya 2007). The susceptibility of amino acid residues to ROS also depends on their position in the polypeptide chain. The oxidation of the side chains of amino acids residues has been shown to yield carbonyl derivatives. An increase in protein carbonyl derivatives formation with age and during ethanol consumption has also been reported (Poon et al. 2004).

In such a situation potent antioxidants are particularly useful, especially those from natural products. One such potentially health-promoting beverage is black tea, which is the most generally consumed drink in the world. Black tea anti-oxidative properties are connected with it containing relatively large amounts of polyphenols, mainly catechins, teaflavins, and their derivatives (Łuczaj and Skrzydlewska 2004).

The aim of the present study was to evaluate the influence of aging on the proteases and their inhibitors activity in the plasma of rats intoxicated with ethanol and consuming black tea. To estimate the level of oxidative stress, the total antioxidant status (TAS) and the level of the main lipid peroxidation product (MDA) was investigated.

Material and methods

Black tea

Black tea—*Camellia sinensis* (*Linnaeus*) *O. Kuntze* (standard research blends—lyophilized extract)—was provided by TJ Lipton (Englewood Cliffs, NJ) and was dissolved in drinking water at a concentration of 3 g/l. The tea was prepared three times per week and stored at 4°C until use. The drinking vessels were replenished every day. The black tea extract contained catechins (epigallocatechin gallate [EGCG]: 14.53 mg/l; epigallocatechin [EGC]: 2.21 mg/l; epicatechin [EC]: 2.83 mg/l) and theaflavins (theaflavin [TF_1]; theaflavin 3-gallate [TF_2A]; theaflavin 3'-gallate [TF_2B]; theaflavin 3,3'-digallate [TF_3] in the amount of 156.16 mg/g of dried extract for all four TFs). The levels of catechins and TFs were determined using modified high-performance liquid chromatography (HPLC) methods (Maiani et al. 1997; Lee et al. 2000; Matilla et al. 2000).

Animals

Two (200–220 g b.w.), 12 (520–550 g b.w.), and 24 month-old (750–780 g b.w.) male Wistar rats were used for the experiments. All experiments were approved by the Local Ethics Committee in Białystok (Poland) with resolution no 14/2007 in the matter of application no 2007/13 of 28 March 2007 with reference to the Polish Act on Protecting Animals of 1997.

The animals from each age group were divided into the sub-groups that were housed with free access to a standard granular diet and water and maintained under a normal light–dark cycle. One-aged rats were divided into the following sub-groups:

- The control group was treated intragastrically with 1.8 ml of physiological saline every day for 4 weeks ($n = 6$).
- The black tea group was given black tea solution ad libitum instead of water for 1 week. Next it was treated intragastrically with 1.8 ml of physiological saline and received black tea solution ad libitum instead of water every day for 4 weeks ($n = 6$).
- The ethanol group was treated intragastrically with 1.8 ml of ethanol in doses from 2.0–6.0 g/kg body weight every day for 4 weeks. The dose of ethanol was gradually increased by 0.5 g/kg body weight every 3 days ($n = 6$).
- The ethanol and black tea group was given black tea solution ad libitum instead of water for 1 week. Next it was treated intragastrically with 1.8 ml of ethanol in doses from 2.0–6.0 g/kg body weight and received black tea solution ad libitum instead of water every day for 4 weeks.

Preparation of tissue

After the procedure described above, from rats under ether anesthesia the blood was collected into EDTA tubes by cardiac puncture. The blood was centrifuged at 3000 x g (4°C) to obtain the plasma.

Biochemical assays

Proteolytic enzymes

The activities of cathepsin G and elastase were determined with specific synthetic substrates. Cathepsin G activity was examined with Suc-Phe-Pro-Phe-pNA (Bachem, Switzerland) at pH 7.6 (Woodbury and Nerath 1980), while elastase activity was determined using Suc-Ala-Ala-Val-pNA (Bachem, Switzerland) at pH 8.5 (Bieth et al. 1974). The activities of these proteases were measured according to the quantity of released p-nitroaniline after 2 h of incubation of the plasma with substrates (74 mM) mixed in a ratio of 1:9 (v:v) at 37°C. The released p-nitroaniline was determined by measuring the absorbance at 410 nm (Erlanger et al. 1961). The protein concentration was determined by the biuret method (Gornall et al. 1949).

Inhibitors of proteolytic enzymes

The activity of alpha-1-antitrypsin was determined by estimating the inhibition by plasma inhibitor trypsin activity. Trypsin activity was measured using hemoglobin as a substrate; 0.1 ml of 16-fold diluted with buffer (0.05M Tris-HCl, pH 7.4) plasma was added to 0.1 ml of trypsin (0.025%), and this mixture was incubated for 5 min at 37°C. Control trypsin activity without plasma was also determined. Next, 0.3 ml of 2% hemoglobin was added to the mixture. The solution was incubated at 37°C for 10 min, and the reaction was terminated by adding 1.25 ml of 20% trichloroacetic acid. Tyrosine was determined in the filtrate by the Folin-Ciocalteu method (Barrett 1977). Alpha-2-macroglobulin was measured with the Unitest™ test of Unicorn Diagnostics Ltd. (London, UK) (Gallimore et al. 1983). The plasma was 160-fold diluted with buffer (0.05 M Tris, 0.1 M NaCl, pH 8.0) to determine the activity of alpha-2-macroglobulin. The diluted plasma was

incubated with porcine trypsin (20 mg/l) for 2 min at 37°C, next, after the addition of soybean trypsin inhibitor, it was incubated for 2 min at 37°C. After adding Bz-Val-Gly-Arg-pNA (1 mM), the mixture was incubated once more for 2 min at 37°C, and then the released p-nitroaniline was measured spectrophotometrically (at 405 nm).

Products of protein oxidative modification

The protein fraction of rat plasma were used to assay protein oxidation. The protein fractions were obtained by protein precipitation with 95% ethanol. After 24 h (4°C), the protein precipitate was centrifuged and dissolved in water and diluted (100-fold) in 5 mM phosphate buffer (pH 7.5) with protease inhibitors (leupeptin 0.5 mg/ml, aproteinin 0.5 mg/ml, pepstatin 0.7 mg/ml). Protein oxidative modifications were examined by determining the carbonyl groups, dityrosine, tryptophan, sulfhydryl groups, and the amino groups level. The carbonyl groups were determined spectrophotometrically using 2,4-dinitrophenylhydrazine (Levine et al. 1990). The dityrosine content was estimated by fluorescence spectrophotometry at 325 nm excitation and 420 nm emission, while fluorescence emission at 338 nm and excitation at 288 nm were used as a reflection of the tryptophan content (Rice-Evans et al. 1991). The signal intensity was calibrated against 0.1 mg/ml quinine sulfate solution in sulfuric acid, whose fluorescence was assumed as a unit. The free amino groups were assayed by reaction with ninhydrin (Devenyi and Gregely 1968), while the sulfhydryl groups were determined by the Ellman reaction (Ellman 1959).

Total antioxidant status (TAS)

Total antioxidant status (TAS) was measured with ABTS reagent (2,2'-azino-di-[3-ethylbenzthiazoline sulfonate) which was incubated with peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$, measured spectrophotometrically at 660 nm. Antioxidants in the added sample cause suppression of color production. The total antioxidant capacity concentration, which was expressed in μmoles of Trolox/ml, was compared to the equivalent antioxidant capacity of Trolox. This method was elaborated by Miller et al. (1993).

Product of lipid peroxidation

Lipid peroxidation was assayed by HPLC measurement (with fluorimetric detection) of malondialdehyde (MDA) as malondialdehyde-thiobarbituric acid adducts (Londero and Greco 1996).

Statistical analysis

Data obtained in the current study are expressed as mean ± SD. These data were analyzed by using standard statistical analyses, one-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons, to determine significant differences between the different groups. A p-value of < 0.05 was considered significant.

Results

The total antioxidant status (TAS) level in the plasma was statistically decreased in the 24-month-old rats in comparison to the 2- and 12-month-olds. Ethanol intoxication additionally enhanced the TAS level changes in the all rat age groups (Figure 1).

However, the administration of black tea caused a significant increase in the TAS level in all rat age groups. The treatment of intoxicated rats with black tea caused a significant reduction of the changes in the plasma antioxidant abilities.

The diminution of the antioxidant status during aging, as well as after ethanol intoxication, was accompanied by changes in the level of protein oxidation markers. This was manifested by a statistically significant increase in the carbonyl groups and the dityrosine concentration (Table 1) as well as by a decrease in the sulfhydryl and amino groups and the tryptophan concentration (Table 1) in the blood plasma of the aged rats which had received ethanol. The carbonyl groups and the dityrosine content increased with age by ~ 10% in 12-month-old rats and by ~ 40% in 24-month-old rats in comparison to 2-month-old rats, whereas the level of the sulfhydryl and amino groups was statistically significantly decreased in the blood plasma of 24-month-old rats in comparison to 2-month-old rats. Chronic ethanol ingestion caused a significant increase in the level of the carbonyl groups (up to ~ 20% in 12-month-old rats and by ~ 30% in 2- and 24-month-

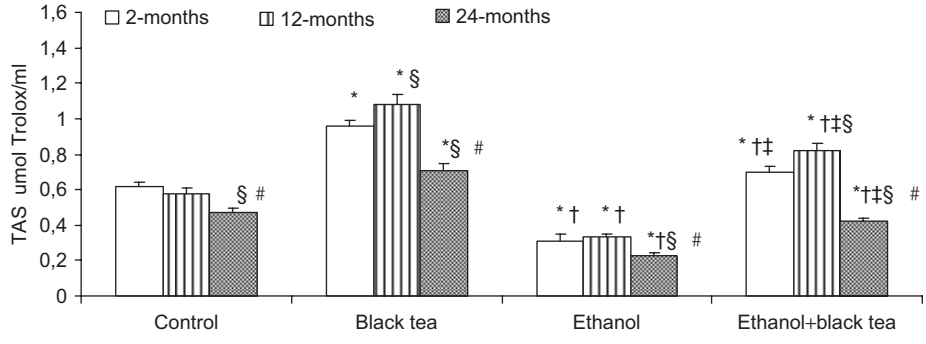

Figure 1. Total antioxidant status in the blood plasma of 2-, 12-, and 24-month-old rats drinking black tea, chronically intoxicated with ethanol, and rats chronically intoxicated with ethanol and drinking black tea. Data points represent mean ± SD; $n = 6$. *$p < 0.05$ in comparison with control group; †$p < 0.05$ in comparison with black tea group; ‡$p < 0.05$ in comparison with ethanol group; §$p < 0.05$ in comparison with 2-months group; #$p < 0.05$ in comparison with 12-months group

Table 1. The level of protein oxidative modification markers: carbonyl group, dityrosine, tryptophan, sulfhydryl, and amino groups in the blood plasma of 2-, 12-, and 24-month-old rats drinking black tea, chronically intoxicated with ethanol and rats chronically intoxicated with ethanol and drinking black tea.

		Group of rats			
Analyzed parameter	Age of rats	Control	Black tea	Ethanol	Ethanol + black tea
Carbonyl groups (nmol/mg protein)	2-months	1.51 ± 0.08	1.29 ± 0.07*	1.92 ± 0.13*†	1.59 ± 0.12†‡
	12-months	1.73 ± 0.07§	1.60 ± 0.08*§	2.05 ± 0.16*†	1.78 ± 0.12†‡§
	24-months	2.11 ± 0.12§#	2.02 ± 0.10§#	2.72 ± 0.19*†§#	2.37 ± 0.15*†‡§#
Dityrosine (U/mg protein)	2-months	0.42 ± 0.02	0.38 ± 0.02*	0.54 ± 0.04*†	0.45 ± 0.03†‡
	12-months	0.47 ± 0.03§	0.41 ± 0.03*	0.73 ± 0.06*†§	0.52 ± 0.04*†‡§
	24-months	0.62 ± 0.05§#	0.59 ± 0.04§#	0.99 ± 0.08*†§#	0.81 ± 0.06*†‡§#
Tryptophan (U/mg protein)	2-months	7.57 ± 0.28	7.54 ± 0.28	7.43 ± 0.30	7.50 ± 0.30
	12-months	7.49 ± 0.32	7.55 ± 0.33	7.27 ± 0.33	7.38 ± 0.32
	24-months	7.32 ± 0.36	7.39 ± 0.35	6.95 ± 0.38§	7.18 ± 0.35
Sulfhydryl groups (nmol/mg protein)	2-months	4.21 ± 0.19	4.16 ± 0.17	4.05 ± 0.19	4.17 ± 0.19
	12-months	4.10 ± 0.22	4.18 ± 0.20	3.82 ± 0.20*†	4.02 ± 0.21
	24-months	3.85 ± 0.27§	3.93 ± 0.23	3.59 ± 0.21†§	3.76 ± 0.20§
Amino groups (Tyr, nmole/mg protein)	2-months	48.2 ± 2.3	48.7 ± 2.2	45.1 ± 2.4*†	47.8 ± 2.3
	12-months	46.7 ± 2.6	47.5 ± 2.5	42.9 ± 2.7*†	45.6 ± 2.6
	24-months	43.5 ± 2.7§	43.7 ± 2.7§#	38.9 ± 2.3*†§#	40.9 ± 2.5§#

Data points represent mean ± SD; $n = 6$.

* $p < 0.05$ in comparison with control group; † $p < 0.05$ in comparison with black tea group; ‡ $p < 0.05$ in comparison with ethanol group; § $p < 0.05$ in comparison with 2-months group; # $p < 0.05$ in comparison with 12-months group.

old rats) and dityrosine (up to ~ 30% in 2-month-old rats and by ~ 60% in 12- and 24-month-old rats). The sulfhydryl groups concentration was statistically significant decreased in 12-month-old rats, while the amino groups were statistically significantly decreased in 2-, 12-, and 24-month-old rats chronically intoxicated with ethanol in comparison to the controls. Black tea given to 2- and 12-month-old rats caused a significant decrease in dityrosine, but it did not change the level of this marker as well as the level of the carbonyl groups when given to 24-month-old rats in comparison to the control group. Black tea did not cause significant changes in the level of the sulfhydryl groups, amino groups, and tryptophan compared to the controls. Black tea given to the rats (2-, 12-, and 24-month-old) intoxicated with ethanol caused a significant decrease in dityrosine as well as the carbonyl groups content in comparison to ethanol groups. Black tea given with ethanol did not cause significant changes in the level of the sulfhydryl groups, amino groups and tryptophan compared to the control, black tea, and ethanol groups.

Aging and chronic ethanol consumption were accompanied by enhanced lipid peroxidation estimated as MDA level (Figure 2). This was manifested by a statistically significant increase in the level of this marker with age (up to ~ 30% in 12-month-old rats and 60% in 24-month-old rats compared to 2-month-old rats). Chronic ethanol ingestion caused a significant increase in the MDA level in young as well as aged rats (up to 2-fold in 2-month-old rats and by ~ 30% in 12- and 24-month-old rats compared to the controls). However, black tea improved the plasma antioxidant abilities, especially in 12-month-old rats.

The activities of proteases and their inhibitors were also changed during aging and after ethanol intoxication. Cathepsin G and elastase activity were significantly increased during aging in 24-month-old rats (Table 2). Ethanol intoxication caused additional, statistically significant, increase in the plasma activity of cathepsin G in the 2-, 24-month-old rats and elastase in the 2-, 12-, 24-month-old rats in comparison to the controls. Ingestion of black tea did not change the activity of the examined enzymes. The treatment of intoxicated rats with black tea significantly reduced changes observed after ethanol intoxication with the exception of cathepsin G in 12-month-old rats.

Table 3 presents the changes in the activities of α_1-antitrypsin and α_2-macroglobulin in the plasma of rats intoxicated with ethanol. The changes in α_1-antitrypsin activity are estimated by changes not inhibited by inhibitor trypsin activity. The activities of protease inhibitors during aging and after ethanol intoxication were significantly reduced. Only the activity of α_1-antitrypsin in 12-month-old rats was slightly increased in comparison to 2-month-old rats. α_2-macroglobulin activities were similar to those in the control groups after the administration of black tea together with ethanol, both in the case of 2- and 12-month-old rats. Also, these same changes were observed in the case of α_1-antitrypsin activities in 12-month-old rats as well.

Discussion

In physiological conditions the activity of cellular proteases is very low in the blood stream and remains in balance with protease inhibitors (Sottrup-Jensen 1989; Korkmaz et al. 2008) that effectively regulate the proteases activities and prevents proteins from protease attack. This study has shown that during aging and additionally during ethanol intoxication, the activities of protease inhibitors (α_1-antitrypsin, α_2-macroglobulin) are decreased, while the activities of proteases (cathepsin G and elastase) are enhanced. The diminished inhibitors activity may be connected with chemical

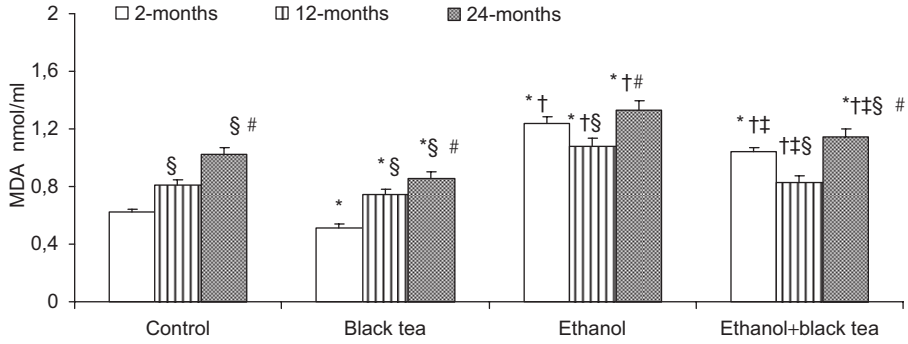

Figure 2. The level of malondialdehyde in the blood plasma of 2-, 12-, and 24-month-old rats drinking black tea, chronically intoxicated with ethanol, and rats chronically intoxicated with ethanol and drinking black tea. Data points represent mean ± SD; $n = 6$. *$p < 0.05$ in comparison with control group; †$p < 0.05$ in comparison with black tea group; ‡$p < 0.05$ in comparison with ethanol group; §$p < 0.05$ in comparison with 2-months group; #$p < 0.05$ in comparison with 12-months group.

Table 2. Activity of elastase and cathepsin G in the blood plasma of 2-, 12-, and 24-month-old rats drinking black tea, chronically intoxicated with ethanol, and rats chronically intoxicated with ethanol and drinking black tea.

		Group of rats			
Analyzed parameter	Age of rats	Control	Black tea	Ethanol	Ethanol + black tea
Elastase (nmol pNA/ml/2 h)	2-months	13.5 ± 0.6	14.0 ± 0.7	16.3 ± 1.0*†	14.5 ± 0.9*‡
	12-months	13.9 ± 0.6	14.2 ± 0.8	17.5 ± 1.1*†	15.1 ± 1.0*‡
	24-months	14.9 ± 0.6$^{\S\#}$	15.0 ± 0.7§	19.9 ± 1.0$^{*\dagger\S\#}$	14.5 ± 0.9‡
Cathepsin G (nmol pNA/ml/2 h)	2-months	127 ± 5	121 ± 7	150 ± 9*†	135 ± 7*†‡
	12-months	134 ± 7	129 ± 7	142 ± 9†	136 ± 8
	24-months	139 ± 8§	135 ± 8§	169 ± 12$^{*\dagger\S\#}$	151 ± 11$^{\dagger\ddagger\S\#}$

Data points represent mean ± SD; $n = 6$.
* $p < 0.05$ in comparison with control group; † $p < 0.05$ in comparison with black tea group; ‡ $p < 0.05$ in comparison with ethanol group; § $p < 0.05$ in comparison with 2-months group; # $p < 0.05$ in comparison with 12-months group.

Table 3. Activity of protease inhibitors (α_1-antiprotease and α_2-macroglobulin) in the blood plasma of 2-, 12-, 24-month-old rats drinking black tea, chronically intoxicated with ethanol, and rats chronically intoxicated with ethanol and drinking black tea.

		Group of rats			
Analyzed parameter	Age of rats	Control	Black tea	Ethanol	Ethanol + black tea
α_1-antiproteinase (nmol pNA/ml/min)	2-months	47.8 ± 2.7	46.9 ± 2.9	60.2 ± 4.2*†	52.1 ± 3.5*†‡
	12-months	49.2 ± 2.4	48.2 ± 2.8	58. 9 ± 3.9*†	51.3 ± 3.2‡
	24-months	55.9 ± 2.9$^{\S\#}$	54.8 ± 3.0$^{\S\#}$	82.2 ± 4.8$^{*\dagger\S\#}$	67.4 ± 3.9$^{*\dagger\ddagger\S\#}$
α_2-macroglobulin (nmol pNA/ml/min)	2-months	31.5 ± 2.1	31.9 ± 2.0	34.7 ± 2.5*	32.7 ± 2.2
	12-months	34.3 ± 2.0§	33.6 ± 2.2	38.8 ± 2.8*†§	35.1 ± 2.1 ‡
	24-months	41.2 ± 2.6$^{\S\#}$	41.9 ± 2.2$^{\S\#}$	59.7 ± 2.9$^{*\dagger\S\#}$	51.4 ± 3.4 $^{*\dagger\ddagger\S\#}$

Data points represent mean ± SD; $n = 6$.
* $p < 0.05$ in comparison with control group; † $p < 0.05$ in comparison with black tea group; ‡ $p < 0.05$ in comparison with ethanol group; § $p < 0.05$ in comparison with 2-months group; # $p < 0.05$ in comparison with 12-months group.

modifications of the protein molecular structure mainly caused by ROS. Their concentration is elevated as a result of both aging and intoxication with ethanol. An increase in ROS generation can be the reason of the impairment of protein molecules, but only in the case when the antioxidant defense system is not capable of destroying ROS (Gębicki and Gębicki 1993). The present study has demonstrated that the plasma total antioxidant status (TAS) is significantly decreased during aging as well as during ethanol intoxication. This indicates a possible shift into the oxidation direction when ROS may cause the oxidation of proteins including protease inhibitor molecules. All protein amino acids residues are susceptible to ROS attack, but some are more vulnerable than others (Gębicki and Gębicki 1993; Stadtman and Levine 2003). The structure and function of alpha-2-macroglobulin is dependent on the existing disulfide bridges between cysteine residues (Catanescu et al. 2008), while cysteine and methionine belong to the amino acid residues that are extremely sensitive to ROS (Davies et al. 1987; Stadtman and Levine 2003**).** It was shown that the cysteine/cystine content of the proteins subjected to oxidation is altered while the decrease in protein sulfhydryl groups has been observed in this paper during aging and after ethanol intoxication so this may be the reason of a decrease in the α_2–macroglobulin activity. Moreover, oxidants may modify methionyl residues of the α_1-antitrypsin molecule to dimethyl sulfoxide including one in the reactive center of α_1-antitrypsin (Johnson and Travis 1979; Luo and Levine 2009), so it may lead to a decrease in its activity. Other

amino acid residues (such as tyrosine or phenylalanine) are also modified by free radicals, which induced changes of the protein structure (Stadtman and Levine 2003; Fedorova et al. 2009). As a consequence of these modifications of amino acid residues the secondary and the tertiary structure of protein can be changed, leading to the loss of the biological activity (Davies and Delsignore 1987). The activity of the examined inhibitors may also be decreased by hydrolysis of their molecules by proteases (Seo et al. 2000; Gupta and Gowda 2008). Moreover, the diminution of the α_1-antitrypsin and α_2-macroglobulin activities are also caused by acetaldehyde, which is the main ethanol metabolite as well as by products of lipid peroxidation, whose generation is enhanced during aging as well as during ethanol metabolism, as has been shown in this paper. Acetaldehyde reacts most easily with the epsilon-amino groups of lysine residues of proteins, but it also may react with other residues (Mauch et al. 1986; Kapaki et al. 2007). As a result, intra- and intermolecular bonds are formed which cause changes in the structural and physicochemical properties as well as in the function of the proteins (Mauch et al. 1986; Tyulina et al. 2006).

The decrease in the antioxidant plasma potential observed during aging as well as during ethanol intoxication is conducive to enhancing the membrane lipid peroxidation. The augmentation of the lipid peroxidation product level, such as malondialdehyde (MDA), shown in this paper, indicates that the reaction of ROS with polyunsaturated fatty acids in phospholipids membranes is intensified. ROS reacting with phospholipids produce superoxide and low molecular aldehydes (Esterbauer et al. 1991). They have a longer life time than ROS, can diffuse from the formation site, and may be considered a secondary transmitter of lipid peroxidation. Lipid peroxidation products also react with the amino acids residues of proteins changing their structure (Esterbauer et al. 1991; Soszynski and Bartosz 1997), including membrane proteins and, in this way, they additionally promote perturbation in the structure and function of biological membranes, including changes in their permeability for cellular compounds (Murin et al. 2001). Therefore, the release of cell constituents, e.g. proteolytic enzymes, from the cells into the extracellular space and the blood stream is likely to occur. The increased MDA level observed in this and the previous paper (Łuczaj and Skrzydlewska 2004) indicate such a possibility and the increased activity of cellular enzymes—cathepsin G and elastase in the blood—confirms this suggestion.

In physiological conditions, α_1-antitrypsin and α_2-macroglobulin inhibit, e.g. cathepsin G and elastase, forming inactive and stable complexes (Allen and Tracy 1995) preventing in this way possible damage to the tissues caused by those proteases (Dollery and Libby 2006). The activity of these enzymes is manifested if the mentioned inhibitors are inactivated, as in the situation observed in this paper. This can lead to the destruction processes, because an uncontrolled proteolysis can occur. Moreover, as a consequence of phospholipids membrane destruction, proteases may pass into the intracellular spaces and may cause activation of metalloproteases, which possess large substrate specificity and can degrade collagen, elastin, proteoglycans, complements, immunoglobulins, and other proteins of the extracellular matrix (Capodici and Berg 1989; Murphy and Reynolds 1993).

The changes in the proteolytic–antiproteolytic balance during aging as well as after ethanol intoxication are mainly connected with ROS reactivity that indicates the necessity to find protective, antioxidant compounds. Therefore, the ingestion of a black tea beverage characterized by antioxidant properties and commonly used world-wide seems to be more than advisable. The protective effect of black tea is connected with its components which possess antioxidant properties (Łuczaj and Skrzydlewska 2004). The black tea components with proved antioxidant abilities are theaflavins and catechins. They possess the ability to prevent ROS formation via the inhibiting activity of the enzymes participating in their generation, as well as to scavenge ROS and to chelate transition metal ions increasing radical reactions (Ojo et al. 2007). It has been shown that theaflavins and catechins inhibit the activity of xanthine oxidase, an enzyme which generates the main and first of all radicals—superoxide anion (Frei and Higdon 2003). Moreover, polyphenols are able to scavenge the superoxide anion, singlet oxygen, and hydroxyl radical (Stangl et al. 2007). Theaflavins and catechins, by theirs chelating effect and also by a decrease in iron absorption from the digestive tract, decrease the metal ions level (Rietveld and Wiseman 2003). In consequence, the above effects diminish free radicals generation and decrease the possibility of their reaction with integral cell components, especially proteins and lipids. The results of this paper indicate the protective action of black tea on plasma proteins. This may explain the preventative changes in the proteolytic–antiproteolytic balance observed in our studies. The present study has also proved that giving black tea to rats intoxicated with ethanol prevents enhancement of lipid peroxidation. Moreover, black tea prevents the lipophilic antioxidant–α-tocopherol, and hydrophilic-ascorbate that repairs the tocopheroxyl radical (Ojo et al. 2007). It is likely that the black tea components are mostly localized near the surface of the membranes where the main aqueous lipid oxidation initiator hydroxyl radical is easily trapped and is accessible to chain-initiating peroxyl radicals, more readily than α-tocopherol. In such a way, black tea decreases lipid peroxidation when membrane phospholipids are exposed to oxygen radicals from the aqueous phase. The oxidative attack from the aqueous phase seems to be an important reaction for initiating the membrane lipid peroxidation. Apart from the antioxidant effect of the black tea components, the membrane prevention may be connected with the fact that theaflavins and catechins reveal a partially lipophylic character and can penetrate the interior of the membranes where they exert a membrane stabilizing effect by modifying the lipid packing order (Arora et al. 2000).

In conclusion, black tea prevents a shift in the proteolytic–antiproteolytic balance and in consequence protects proteins from degradation, which may cause the development of a wide range of diseases. Thus, black tea plays an important role in health protection and disease prevention.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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