Protective effect of sweet grass and black berries beverages on ethanol-induced disturbances in brain fatty acids

Cyuńczyk M.[1*], Jarocka I.[1], Hodun T. [1], Hermanowicz J [2].

Department of Inorganic and Analytical Chemistry, Medical University of Białystok, Poland

Department of Clinical Pharmacy, Medical University of Białystok, Poland

ABSTRACT

Purpose: To investigate influence of sweet grass and blackcurrant beverages on the level of free as well as phospholipid fatty acids and MDA - lipid peroxidation product in the brain of rats intoxicated with ethanol (28 days).

Materials and methods: Fatty acids profile in the rat brain was determined by gas chromatography while MDA level was examined by HPLC.

Results: Alcohol intoxication contributed to increase in the level of the most of free as well as phospholipid fatty acids (saturated and unsaturated). The above changes are accompanied by a significant increase in the lipid peroxidation product – MDA. Similarly, drinking the sweet grass beverage as well as black currant juice leads to an increase in the level of the most of free as well as phospholipid fatty acids [saturated and unsaturated] while the increase after drinking of the black currant juice was higher than after sweet grass. Intoxication of rats drinking both natural beverages causes diminution of all fatty acids level while changes after black currant juice are more significant than after sweet grass. The level of MDA in the brain of rats intoxicated with ethanol and drinking natural beverages is lower than in the ethanol group.

Conclusions: Beverages of sweet grass and to a higher degree black berries partially prevent disturbances in the brain fatty acids level and protects lipids against peroxidation caused by chronic ethanol intoxication.

Key words: fatty acid, sweet grass, black berries

*** Corresponding author**:
Department of Inorganic and Analytical Chemistry
Medical University of Białystok
Bialystok, Poland
Tel. 857485708
Email: monika.cyunczyk@umb.edu.pl (Monika Cyuńczyk)

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INTRODUCTION

All living organisms can suffer from oxidative damage, but the animal brain is especially sensitive. One of the reasons is its large of oxygen consumption in relatively small tissue mass. Moreover, several other mechanisms in the brain metabolism promote reactive oxygen species (ROS) generation, e.g. oxidation of neurotransmitters is accompanied by ROS generation as well as microglias can participate in ROS generation [1]. The cells of nervous system contain high concentration of readily oxidazible substrate, in particular polyunsaturated fatty acids, high ratio of membrane surface area to cytoplasmic volume and extended axonal morphology prone to peripheral injury. In addition, some regions have high nonheme iron concentration. Moreover**,** the nervous cells contain low level of protective antioxidants [2] as a result of which they are especially vulnerable to oxidative damage caused by reactive oxygen species.

Due to oxygen consumption in metabolic reactions and xenobiotics metabolism including that of ethanol, the central nervous system is influenced by a substantial amount of superoxide anion [3]. Superoxide dismutase metabolizes superoxide anion into hydrogen peroxide, which can activate some neurotransmitters responsible for control of dopamine metabolism in rodents [4]**.** Hydrogen peroxide is converted to highly reactive hydroxyl radical under the influence of iron ions (II), whose content is very high in the central nervous system [5]. This radical easily reacts with all cell components, but with free as well as lipid polyunsaturated fatty acids in particular, causing their peroxidation. One group of oxidation products that may exert toxic effects on the cells are very reactive aldehydes (such as malondialdehyde and 4-hydroxynonenal [6]). Their generation and reactions disturb the structure and functions of biomembrane components as well as cytosolic protein participating in cellular signaling [7].

Due to very high lipids content, the central nervous system is extremely susceptible to hydroxyl radical action [8]. Moreover**,** hydroxyl radical can also react with ethanol forming 1-hydroxyethyl radical, which due to relatively long half-time period considerably contributes to cell damage. The hydroxyethyl radical level in the central nervous system increases also as a result of izoenzyme CYP2E1 induction by ethanol intoxication [9]. Hydroxyl radical reacts also with ethanol metabolite - acetaldehyde resulting in formation of acetyl radicals whose level increases in the central nervous system [10].

In order to prevent oxidative stress generation and its consequences potent antioxidants especially those belonging to natural products are investigated. One of such potentially health promoting plants is sweet grass (*Hierochloë odorata*), which belongs to the *Graminacae family*. Its chemical composition and biological properties have not been extensively investigated. However it was proved that extracts of sweet grass promoted retardation of lipid peroxidation [11]. This herb contains among others coumarin and its derivatives – 5,8-dihydroxycoumarin and 5-hydroxy-8-O-□-D-glucopyranosycoumarin [12]. Coumarin hydroxyl derivatives were reported to be antioxidant compounds [13,14]. Another group of antioxidant natural modulators are berries including blackcurrant berries. Blackcurrant berries contain a large amount of vitamin C and polyphenols, especially flavonoids, of which particularly important are anthocyanins whose amount is to 92–95 % of all polyphenols contained in the blackcurrant [15]. The anthocyjanins have the ability to prevent oxidative stress formation [16-18].

Therefore, the objective of our study is the measurement the influence of drinking sweet grass beverage and blackcurrant juice on the level of free as well as phospholipid fatty acids and the lipid peroxidation product – malondialdehyde in the brain of rats intoxicated with ethanol.

MATERIALS AND METHODS

Sweet grass extract used in the experiment contained coumarin (312 mg/l), 5,8-dihydroxycoumarin (4,2 mg/l) and 5-hydroxy-8-O-β -D-glucopyranosyl-benzopyranone (3.1 mg/l). The level of these compounds was analyzed using a gas chromatograph (Agilent Technologies) equipped with a MS/MS detector in the electron-impact ionization mode (GC System 7890A with GC/MS Triple Quad 7000). The coumarin peak was identified by comparison of the retention time with the standard and its mass spectrum by using the National Institute of Standards and Technology Virtual Library (NIST).

Blackcurrant (*Ribes nigrum L*) — was used as a juice (containing 28% pure blackcurrant juice). The total amount of phenols in the used blackcurrant juice was spectrophotometrically determinated with Folin-Ciocatleu's reagent [19] and was 1,269 mg of gallic acid equivalents/l. Anthocyanins concentration was determined by HPLC with a diode-array detector [19] and the concentration of the four main anthocyanins was 18.28 μmol/l, 14.06 μmol/l, 2.33 μmol/l, and 1.61 μmol/l, for delphinidin-3-rutinoside, cyaniding-3-rutinoside, delphinidin-3-glucoside and cyanidnin-3-glucoside, respectively. The level of vitamin C was determined by HPLC with UV detector [20] and was 50.03 mg/l.

Animals

12 months old male Wistar rats were used for the experiment. They were housed in groups with free access to a granular standard diet and water and maintained under a normal light-dark cycle. The rats were weighed every week of the experiment and changes in the weight of animals from different groups were not statistically significant. All experiments were approved by the Local Ethic Committee in Bialystok (Poland) referring to Polish Act Protecting Animals of 1997. The animals were divided into the following groups:

- **The control group** was treated intragastrically with 1.8 ml of physiological saline every day for 4 weeks (n=6).
- **The sweet grass group** rats received sweet grass water beverage (coumarin content 10mg/l of water) ad libitum instead of water for one week. Next they were treated intragastrically with 1.8 ml of physiological saline and received sweet grass water beverage ad libitum instead of water every day for 4 weeks (n=6).
- **The blackcurrant group** was given blackcurrant juice ad libitum instead of water for one week. Next it was treated intragastrically with 1.8 ml of physiological saline and received black tea solution ad libitum every day for 4 weeks (n=6).
- **The ethanol group** was treated intragastrically with 1.8 ml of ethanol in doses from 2.0 to 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 three days (n=6).
- **The sweet grass and ethanol** group rats received sweet grass water beverage ad libitum instead of water for one week. Next they were treated intragastrically with 1.8 ml of ethanol in doses from 2.0 to 6.0 g/kg b.w. and received sweet grass water beverage ad libitum instead of water every day for 4 weeks.
- **The blackcurrant and ethanol** group was given blackcurrant juice ad libitum instead of water for one week. Next it was treated intragastrically with 1.8 ml of ethanol in doses from 2.0 to 6.0 g/kg body weight and received blackcurrant juice ad libitum every day for 4 weeks.

Preparation of tissue

After 28 days of experiment the rats were sacrificed under ether anaesthesia (six animals in each group). Brains were removed quickly and fragments of them for fatty acids determination were frozen in liquid nitrogen immediately after they had been removed and were pulverized.

Fragments of brains for MDA determination were removed quickly and placed in iced 0.15 M NaCl solution, perfused with the same solution to remove blood cells, blotted on filter paper, weighed and homogenized in 0.15 M NaCl with addition of 6 µl 250 mM BHT (butylated hydroxytoluene) in ethanol to prevent formation of new peroxides during the assay.

Biochemical assays

Fatty acids profile in the brain was determined by gas chromatography [21]. After pulverization, lipids components were isolated by Folch extraction using chloroform/methanol mixture (2:1, v/v). Using TLC free fatty acids and the total phospholipids were separated with the mobile phase heptane - diisoprophyl ether – acetic acid (60:40:3, v/v/v). Lipid fractions were transmetylated to fatty acid methyl esters (FAMEs) with boron trifluoride in methanol reagent under nitrogen atmosphere without previous separation from the layer material at 100°C (for 30 min for phospholipids and for 2 min for free fatty acids). The FAME's were analyzed by gas chromatography with a flame ionization detector. Separation of FAME was carried out on a capillary column coated with Varian CP-Sil88 stationary phase.

Lipid peroxidation was estimated by measuring of malondialdehyde (MDA) [22]. Malondialdehyde was determined as a malondialdehyde-tiobarbituric acid adducts separated by HPLC with spectrofluorometric quantification at 532 nm exitation and 553 nm emission.

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 test for multiple comparisons, to determine significant differences between different groups. The data were analyzed separately for treatment and age groups. A P value of < 0.05 was considered significant.

RESULTS

Table 1 presents the content of fatty acids bound in phospholipids of the rat brain. In the alcohol rat group, the content of acids 20:4 and 22:5 remained at the same level as compared to the control, whereas the content of the other fatty acids was raised, with the highest increase in acids 20:0 and 20:5 (respectively 31% and 26%) in comparison to the control. An increase in the level of acids 20:0, 22:0, 18:1 and 24:1 (respectively 13%, 11%, 9% and 9%), and a decrease in the content of acids 20:4 and 22:5 (respectively 9% i 8%) was revealed in the group of rats drinking sweet grass extract in comparison to the control.

Table 1. The level of phospholipids fatty acids in the brain of rats chronically intoxicated with ethanol and rats drinking sweet grass beverage blackcurrant juice and chronically intoxicated with ethanol

Phospholipid fatty acids [µmol/g tissue]	**Groups of rats**					
	Control	**Sweet grass**	**Blackcurrant**	**Alcohol**	**Alcohol+ sweet grass**	**Alcohol+ blackcurrant**
16:0	21.4 ± 1.4	21.4 ± 1.5	24.0 ± 1.5 [a]	21.8 ± 1.3 [c]	20.9 ± 1.7	19.4 ± 1.7 [acd]
18:0	17.2 ± 1.1	17.3 ± 1.1	19.1 ± 1.1[a]	18.0 ± 1.6	17.7 ± 1.5	15.6 ± 1.3 [acd]
20:0	0.22 ± 0.01	0.25 ± 0.02[a]	0.26 ± 0.01 [a]	0.29 ±0.03 [abc]	0.24 ± 0.02[d]	0.21 ± 0.02 [cd]
22:0	0.18 ± 0.01	0.20 ± 0.02[a]	0.20 ± 0.01 [a]	0.23 ±0.02 [abc]	0.20 ± 0.02[ad]	0.16 ±0.01 [acd]
Total saturated	**39.00 ± 2.52**	**39.15 ± 2.64**	**43.56 ± 2.62** [a]	**40.32 ± 3.65**	**39.04 ± 3.24**	**35.37±3.03**[a] [cd]
16:1	0.48 ± 0.03	0.49 ± 0.02	0.54 ± 0.03 [a]	0.52 ± 0.04 [ab]	0.48 ± 0.04[d]	0.48 ± 0.03 [cd]
18:1	13.2 ± 0.9	14.4 ± 0.8[a]	15.0 ± 0.8 [a]	13.8 ± 1.2 [c]	14.2 ± 1.2[a]	12.3 ± 1.0 [acd]
18:2	1.30 ± 0.08	1.42 ± 0.09	1.36 ± 0.08	1.44 ± 0.12 [ac]	1.38 ± 0.11[a]	1.14± 0.10 [acd]
20:4	14.3 ± 0.9	13.1 ± 0.9[a]	15.2 ± 0.9 [a]	14.3 ± 1.3 [bc]	13.2 ± 0.11[ad]	12.5 ±0.10 [acd]
20:5	0.84 ± 0.03	0.86 ± 0.07	0.95 ± 0.05 [a]	1.06 ± 0.09[abc]	0.91 ± 0.07[abd]	0.68± 0.04 [acd]
22:5	0.26 ± 0.01	0.24 ± 0.02[a]	0.27 ± 0.02	0.26 ± 0.02[b]	0.23 ± 0.02[ad]	0.20 ±0.02 [acd]
22:6	29.2 ± 2.0	30.5 ± 2.1	32.7 ± 2.2 [a]	31.6 ± 2.6[a]	28.9 ± 2.5b[d]	27.2 ± 2.2 [acd]
24:1	0.74 ± 0.03	0.81 ± 0.06[a]	0.82 ± 0.05 [a]	0,82 ± 0.06[a]	0.70 ± 0.05[bd]	0.58 ±0.03 [acd]
Total unsaturated	**60.32 ± 3.98**	**61.82 ± 4.06**	**66.84 ± 4.13** [a]	**63.8 ± 5.43**	**60.00 ± 4.10**	**55.08±3.52**[a] [cd]

Data points represent mean ± SD; n=6; ([a] $p<0.05$ in comparison with control group; [b] $p<0.05$ in comparison with sweet grass group; [c] $p<0.05$ in comparison with blackcurrant group; [d] $p<0.05$ in comparison with alcohol group).

A decrease of 7.8% and 11.5% in the content of acids 20:4 and 22:5 and an increase in the content of acids 20:0, 22:0 and 18:1 (respectively of 9%, 11% and 7,5%) was found in the group of rats drinking both alcohol and sweet grass extract. The level of acid 16:1 was not changed in the groups of rats drinking alcohol and sweet grass extract as well as ingesting sweet grass extract in comparison to the control group.

An increase in all fatty acids was revealed in the fraction of fatty acids bound in phospholipids of the rat brain, in a group of rats consuming black currant juice compared to the control, with an increase of more than 10% percent for saturated fatty acids 16:0, 18:0, 20:0 and 22:0 and unsaturated ones 16:1, 18:1, 20:5, 22:6, 24:1. Simultaneously, a decrease in the content of all fatty acids was observed in the group of rats consuming alcohol and black currant juice in comparison to the control and to that drinking black currant juice.

In table 2, it has been shown that the response to chronic ethanol consumption causes statistically significant increase in the total level of saturated free fatty acids (by about 12%), notably in the level of free behenic acid (22:0), by about 58%, while free palmitic acid (16:0) was increased (by about 12%) in the same group.

Table 2. The level of free fatty acids in the brain of rats chronically intoxicated with ethanol and rats drinking sweet grass beverage and blackcurrant juice and chronically intoxicated with ethanol

Free fatty acids [nmol/g tissue]	**Groups of rats**					
	Control	**Sweet grass**	**Black currant**	**Alcohol**	**Alcohol+ sweet grass**	**Alcohol+ blackcurrant**
16:0	347 ± 24	325 ± 22	518 ± 34 [a]	390 ± 30[abc]	372 ± 30[abc]	461 ± 30 [acd]
18:0	523 ± 33	449 ± 32[a]	702 ± 52 [a]	511 ± 41[bc]	500 ± 39[b]	615 ± 44 [acd]
20:0	25 ± 2	24 ± 2	27 ± 2	27 ± 2	26 ± 2	24 ± 2 [cd]
22:0	59 ± 3	82 ± 5[a]	153 ± 9 [a]	93 ± 7[abc]	95 ± 7[ab]	97 ± 7 [ac]
Total saturated	**954 ± 62**	**880 ± 61**	**1400 ± 97** [a]	**1021 ± 80[abc]**	**993 ± 78[b]**	**1197 ± 83** [acd]
16:1	18 ± 1	18 ± 1	23 ± 1 [a]	20 ± 2	20 ± 2	20 ± 2
18:1	259 ± 21	308 ± 25[a]	411 ± 29 [a]	313 ± 26[ac]	340 ± 30[a]	361 ± 31 [acd]
18:2	57 ± 3	129 ± 10[a]	64 ± 4 [a]	140 ± 12[ac]	123 ± 10[ac]	55 ± 3 [cd]
20:4	478 ± 32	420 ± 30[a]	621 ± 48 [a]	501 ± 43[bc]	490 ± 38[b]	483 ± 37 [c]
20:5	85 ± 6	81 ± 5	84 ± 5	92 ± 7 [abc]	89 ± 6[b]	82 ± 6 [d]
22:5	12.1 ± 0.9	11.9 ± 1.0	14.1 ± 1.0 [a]	15.2 ± 1.3[ab]	12.5 ± 1.0[c]	12.9 ± 1.0 [acd]
22:6	253 ± 19	256 ± 17	338 ± 24 [a]	296 ± 25[bc]	271 ± 22	283 ± 23 [ac]
24:1	3.2 ± 0.2	4.4 ± 0.3[a]	7.6 ± 0.3 [a]	5.8 ± 0.5[abc]	7.4 ± 0.6[abc]	6.2 ± 0.6 [ac]
Total unsaturated	**1165.3 ± 82**	**1228.3 ± 89**	**1562.7 ± 112** [a]	**1383 ± 117[abc]**	**1352.9 ± 110[a]**	**1303.1 ± 104** [ac]

Data points represent mean ± SD; n=6; ([a] $p<0.05$ in comparison with control group; [b] $p<0.05$ in comparison with sweet grass group; [c] $p<0.05$ in comparison with blackcurrant group; [d] $p<0.05$ in comparison with alcohol group).

In the rest of experimental groups, changes in the total level of saturated free fatty acids were not statically significant. Administration of alcohol and drinking sweet grass beverage caused a slight decrease in a the concentration of the examined free saturated fatty acids, while statistically significant decrease (by about 5%) was observed for the palmitic acid. Ethanol intoxication influences also the level of free unsaturated fatty acids. Significant increase in the total concentration of unsaturated free fatty acids (by about 19%) was observed after alcohol intake. The level of linoleic acid (18:2) in the brain of rats intoxicated with ethanol was almost 2.5 fold higher than in control group. An increase of about 81.26 and 21% was observed in the content of nervonic (24:1), clupanodonic (22:5) and oleic (18:1) acids, respectively. A significant decrease in the level of linoleic and clupanodonic acids (by about 12 and 18% respectively) was found in rats which had drunk alcohol and sweet grass beverage in comparison with the ethanol group. However the total content of the brain unsaturated fatty acids after administration of ethanol to rats drinking sweet grass solution was higher (by about 17%) in comparison with the control group.

The level of linoleic acid (18:2) was over 2-fold higher after sweet grass administration in comparison to the control, while the concentration of arachidonic (20:4) acid was significantly decreased by about 12%. In fact, the total level of unsaturated free fatty acids in the brain of rats drinking sweet grass solution was closed to the control group. Blackcurrant juice consumption increased both saturated and unsaturated free fatty acids levels in the rats brains; saturated: palmitic (16:0), stearic acid (18:0) (58%, 41% respectively), and behenic acid more than 2.5 fold; unsaturated: oleic (18:1), arachidonic (20:4), docosahexaenoic (22:6) and nervonic acid (24:1) (40%, 30%, 34% and 2,4 times respectively). Rats receiving alcohol and drinking black currant juice showed an increase in the level of stearic acid (18:0) in comparison to the alcohol group (about 20%), and decrease of 12% in comparison to the blackcurrant group. The content of nervonic acid (24:1) was almost 2 fold higher than in the control group after ethanol admini-stration together with blackcurrant juice.

Changes in the level of fatty acids after ethanol intoxication are accompanied by an increase in lipid peroxidation in the rat brain. That is manifested by a significant increase in the level of malondialdehyde (by about 93%) in the brain in comparison with the control group (Figure 1).

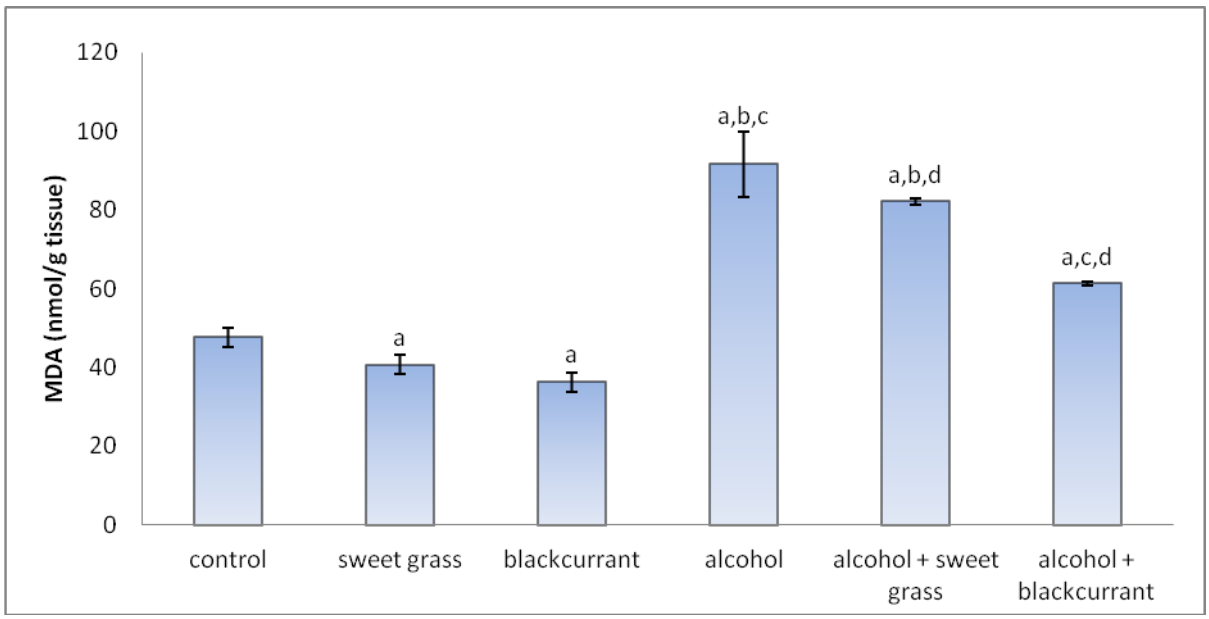

Figure 1. MDA in the brain of rats chronically intoxicated with ethanol and rats drinking sweet grass beverage and blackcurrant juice and chronically intoxicated with ethanol

Data points represent mean ± SD; n=6; ([a] $p<0.05$ in comparison with control group; [b] $p<0.05$ in comparison with sweet grass group; [c] $p<0.05$ in comparison with blackcurrant group; [d] $p<0.05$ in comparison with alcohol group).

Administration of sweet grass solution causes a significant decrease in the level of MDA (by about 14%). Sweet grass given to ethanol intoxicated rats leads to a smaller increase in the level of this aldehyde in the brain, however the level of MDA is still significantly increased (by about 72%) in comparison with the control group. Administration of blackcurrant juice causes a significant decrease of this aldehyde (by about 25%) in comparison with the control group. However, blackcurrant juice given with ethanol resulted in a significant decrease in the level of MDA (by about 33%) in comparison with the ethanol group.

DISCUSSION

Ethanol metabolism in the central nervous system (CNS) is accompanied by enhanced free radical formation [23]. Independently from free radical generation ethanol intoxication causes a decrease in the activity of antioxidant enzymes [24, 25]. Such a situation leads to shift in the redox balance into oxidative direction. In such a situation ROS react with free as well as phospholipid polyunsaturated fatty acids resulting in the formation of a number of peroxidation products. Lipid peroxides and low molecular aldehydes are the first to be produced [6]. The aldehydes, mainly 4-hyroxynonenal and malondialdehyde live longer than ROS and can diffuse from the formation site**.** What is more, they are very reactive compounds so cause greater cell damages. Lipid peroxidation aldehydes may act as "secondary toxic messengers" of the primary free radical event. That property can be attributed to their structure, which gives these biogenic aldehydes strong electrophilic properties and reflects the ability to form adducts with nucleophilic sulfhydryl, amino and histydyl groups of proteins and nucleic acids, what in consequence causes changes in their structure and functions. It has been proved that these aldehydes inhibit one of the main antioxidant enzymes - glutathione peroxidase in this way [26]. Glutathione peroxidase activity inhibition is additionally diminished by specific reactions between malondialdehyde or 4-hydroxynonenal with selenocysteine residue of the active center of glutathione peroxidase [26, 27]. It should be emphasized that selenium deficiency in the rat brain may increase susceptibility to damage, particularly to glutamate–induced excitotoxicity [28]. An increase in the level of MDA in the brain observed in this study may suggest that all these damages are possible in ethanol intoxication.

The increase in lipid peroxidation product is accompanied by a fall in the level of unsaturated and a rise in the level of saturated fatty acids which was observed after chronic ethanol intoxication.

Alcohol consumption was generally found to decrease unsaturated fatty acids level, probably by peroxidation of nonsaturated bonds and inhibiting the elongation and desaturation of n-3 and n-6 precursors [29]. However, some studies have reported that alcohol abuse leads to increases in the brain level of fatty acid 22:5n-6 [30]. Ethanol intoxication may affect fatty acids catabolism and anabolism. The increased level of brain MDA indicates enhanced fatty acids catabolism, however, the level of fatty acids is also increased. This can be explained by the main effect of ethanol which stimulates fatty acid anabolism**,** in situation fatty acids anabolism is stimulated in vivo by a feedback mechanism in an adaptive response that serves to maintain tissue fatty acids concentrations. This may be connected with changes in the activity of enzymes participating in lipid as well as fatty acid metabolism – phospholipases and desaturases [31-33]. This hypothesis has gained direct support from controlled dietary experiments on *in vivo* fatty acid metabolism in both cats and rhesus monkey [30, 34].

It has been shown that drinking beverages prepared from sweet grass as well as black berries by ethanol intoxicated rats leads to partial normalization of fatty acids as well as MDA level in their brains. Each of the above beverages contains compounds that possess antioxidant properties. Some previous studies have demonstrated that coumarin is metabolized to a number of hydroxycoumarins [35] that exhibited various antioxidant properties [36]. It has been proved that coumarin derivatives have the ability to prevent oxygen radicals formation, via inhibiting the activity of enzymes participating in their generation [37]. In addition, the coumarin derivatives have been revealed to inhibit the activity of xanthine oxidase [38]. What is more, sweet grass extract administration may diminish free radicals formation and contribute to their effective removing [11]. It has been suggested that the scavenging abilities of the sweet grass containing at least two hydroxycumarins, whose scavenging ability has been also proved (especially of dihydroxycumarins), are nonproportionally higher than the scavenging abilities of isolated compounds [39]. The sweet grass reducing the radicals levels may prevent their reactions with cell components including fatty acids. Reactions of free radicals formation as well as peroxidation involve transition metal ions action. Long-term ethanol exposure leads to an increase in the concentration of free iron ions, which catalyze the Haber-Weiss reaction what finally leads to an increase in lipid peroxidation [40]. Coumarin derivates may diminish the prooxidative action of the transition metal ions by their chelate formation [13]. The present study has demonstrated that sweet grass given to rats I ntoxicated with ethanol prevents

enhancement of lipid peroxidation. Also, this antiperoxidative activity of coumarin derivatives has been recently proved [14].

The biological activity of blackcurrant berries is connected with antioxidant properties of many their components, mainly vitamin C (blackcurrant fruits are the richest source of vitamin C among all berry fruits species) and polyphenols, especially flavonoids, of which particularly important are anthocyanins [41]. Strong antioxidant properties of black currant juice are connected whit mutual synergetic effect of antioxidant compounds. Anthocyanins possess ability to protect ascorbic acid from oxidation and scavenge the active forms of oxygen [42,43]. Consumed anthocyanins are distributed to different tissue (e.g. liver, brain) which could protect against oxidative damage [44-46]. Antioxidant properties of anthocyjanins revealed their abilities to scavenge the reactive oxygen forms such as hydroxyl radicals and superoxide radicals. Since the anthocyanin radicals are stable (their half-life is longer than that of other radicals) they can react with more reactive radicals e.g. superoxide ones [18]. The ability to scavenge free radicals by anthocyanins is strictly connected with the structure of the particular aglycones. Two main aglycones of blackcurrant anthocyanins (delphinidin and cyanidin) are the strongest antioxidants in the water phase among all aglycones due to the greater number of hydroxyl groups in the B ring [16]. Cyanidins and delphinidins are able to chelate ions of metal e.g., Fe, Cu, so they do not participate in free radical reactions e.g. Fenton reaction [17]. Moreover since the anthocyanins are localized in the surface layers of biological membranes, they may act as antioxidants both in the hydrophilic and hydrophobic layer and prevent free radicals generation in both phases [16] as well as protect membrane components including fatty acids. Additionally, the anthocyanins as donors of the hydrogen atom cause termination of the lipids oxidation process because the O–H bond in the molecule of anthocyanins is weaker than the C–H one in lipids.

CONCLUSIONS

Sweet grass and to a greater degree black berries partially prevent disturbances in the brain fatty acids level and protect lipids against peroxidation caused by chronic ethanol intoxication. Substantial and still growing evidence for the bioactivity of the sweet grass and black berries in vivo suggests their role in health protection and disease prevention.

Conflicts of interest

We declare that we have no conflicts of interest.

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