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**GREEN TEA MODULATION OF THE BIOCHEMICAL AND  
ELECTRIC PROPERTIES OF RAT LIVER CELLS THAT WERE  
AFFECTED BY ETHANOL AND AGING**

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**Abstract:** The oxidative stress induced by chronic ethanol consumption, particularly in concert with the aging process, has been implicated in changes in the structure and functions of liver cell components including membrane phospholipids. To counteract such changes, particularly those resulting from lipid peroxidation, antioxidants may be applied. Green tea contains large amounts of polyphenols, mainly catechins, which possess antioxidant properties. The aim of this study was to estimate the efficacy of green tea's influence on the physicochemical and biochemical properties of the rat liver as affected by the aging process and/or chronic ethanol intoxication. Several methods were used to evaluate this effect. Antioxidant properties were evaluated by vitamin E and antioxidant status determination. The liver triglyceride and cholesterol levels were also estimated. The extent of lipid peroxidation was determined by measuring the level of lipid peroxidation products as thiobarbituric reactive substances (TBARS). The surface charge density of the rat liver cells was measured using electrophoresis. The concentration of the marker enzymes of liver damage (alanine aminotransferase and aspartate aminotransferase) in the blood serum was also evaluated. Relative to the controls, aging was found to cause a decrease in the liver's antioxidant abilities and provoke an increase in the level of lipid peroxidation; it also increased the surface charge density of the rat liver cell membrane. Ethanol significantly aggravated these changes. This might have resulted in the liver cell membrane damage visible as a leak of alanine aminotransferase and aspartate aminotransferase into the blood. The ingestion of green tea with ethanol partially prevented these aging and/or ethanol-induced changes. Long-term drinking of green tea partially prevents the changes in the structure and function of the cell membrane caused by chronic ethanol intoxication.

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## INTRODUCTION

The mechanism underlying aging is still an area of significant controversy. One of the aging theories is the free radical theory, which suggests that aging is caused by a shift in the balance between pro-oxidative and antioxidative processes in the direction of the pro-oxidative state [1]. It is postulated that aging results from an increase in the levels of oxidatively damaged lipids, proteins or DNA, and the accumulation of unrepaired amounts of these cellular components [2]. The oxygen-based theory is sometimes referred to the lipid peroxidation theory of aging because lipid peroxidation has traditionally been regarded as the major process that produces oxygen-radical damage. The cellular redox state, which is changed during aging additionally or/and independently, may be modified by diet. Food components may cause an increase or a decrease in cellular antioxidative ability. One such food component which influences ROS generation and antioxidant status is alcohol. Ethanol is rapidly absorbed from the gastrointestinal tract, and about 90% of it is metabolized in the liver [3]. There, ethanol is oxidized into acetaldehyde and next into acetate; these processes are accompanied by free radical generation [4]. Electrophilic free radicals and acetaldehyde readily react with the nucleophile groups of proteins, phospholipids and nucleic acids to produce adducts, some of which have been detected in the tissues of alcoholic patients [5, 6]. As a consequence of both aging and ethanol intoxication, conducive changes in the properties of the liver, including cell membrane properties, take place. The biological membrane functions as a selective barrier and is essential for transport, ion permeability, enzyme activity and receptor responsiveness [7]. All of these membrane functions require structural integrity and the correct electric properties, which may be modified by changes in membrane composition.

Because most of the changes caused by aging and alcohol are directly or indirectly linked with lipid peroxidation, potent antioxidants are looked for, especially those found in natural products. One such potentially health-promoting beverage is green tea. It is generally believed that tea has an influence on the effects of various biological and pharmaceutical processes although many of them remain to be investigated. Green tea contains relatively large amounts of polyphenols [8]. Tea polyphenols comprise mainly catechins and catechin derivatives, which are reported to have antioxidant properties. Under *in vitro* conditions they can inhibit free radical generation, scavenge hydroxyl and superoxide radicals, reduce lipid peroxy radical formation and inhibit lipid peroxidation [9]. Catechin incorporation into cell membranes was shown to prevent or reduce the morphological and biochemical alterations of hepatocytes induced by hepatotoxic agents [10, 11]. It has been shown that catechins and their derivatives can be absorbed and metabolized, and the catabolites of tea polyphenols have been found in rat and human urine and plasma [12, 13]. These

results indicate that ingested catechins are absorbed and can have biological effects.

The aim of this study was to estimate the biochemical and electric properties of aged rat liver cells after chronic ethanol intoxication. In addition, the efficacy of green tea in terms of its protective action was investigated.

## MATERIALS AND METHODS

### Animals

2- (200-220g b.w.), 12- (520-550g b.w.) and 24-month old (750-780g b.w.) male Wistar rats were used for all the experiments. The rats were fed a liquid diet for 5 weeks before death. The dietary intake was comparable in all the groups, with all the rats demonstrating consistent weight gain throughout the 5-week feeding period. The ethanol-fed rats had slightly decreased rates of weight gain (about 10% less, relative to the control rats), which is consistent with the well-studied effects of isocaloric ethanol feeding on the intermediate metabolism. All the experiments were approved by the Local Ethic Committee in Białystok, Poland (the Polish Protection of Animals Act, 1997). The Rats were housed in individual cages and pair-fed with either a nutritional control-adequate liquid Lieber DeCarli diet with 47% of the total energy intake as carbohydrate, 18% as protein, and 35% as lipid, or an identical diet with ethanol substituted isocalorically for carbohydrate (36% of the total energy intake) [14]. A liquid diet (control and ethanol) containing 7 g green tea extract/l diet was also prepared. The green tea – *Camellia sinensis* (Linnaeus) O. Kuntze (standard research blends – lyophilized extract) – was provided by TJ Lipton (Englewood Cliffs, NJ), and its extract contained epigallocatechin gallate (97 mg/g dried extract), epigallocatechin (82 mg/g dried extract), epicatechin (90 mg/l), epicatechin gallate (15 mg/g dried extract) and caffeic acid (10 mg/g dried extract). The composition was determined via HPLC [15].

The animals from each age group were divided into the following groups:

- the control group (n=6) was fed on a control Lieber DeCarli liquid diet for 5 weeks;
- the green tea group (n=6) was fed on a control liquid Lieber DeCarli diet containing green tea (7 g/l) for 5 weeks;
- the ethanol group (n=6) was fed on a control liquid Lieber DeCarli diet for one week and for the next 4 weeks, on an ethanol Lieber DeCarli liquid diet;
- the ethanol and green tea group was fed on a control Lieber DeCarli liquid diet containing green tea (7 g/l) for one week, and for the next 4 weeks, with an ethanol Lieber DeCarli liquid diet also containing green tea (7 g/l).

### Preparation of tissues for biochemical assays

After the fifth week of the experiment all rats were killed under ether anaesthesia (six animals in each group). The liver was removed quickly and placed in an iced 0.15 M NaCl solution, perfused with the same solution to remove blood cells, blotted on filter paper, weighed, diluted 10% w/v in ice-cold 0.15 M NaCl

containing 167  $\mu\text{M}$  BHT in ethanol to prevent the formation of new peroxides during the assay. The homogenization procedure was performed under standardized conditions. A glass homogenizer with a rotatory speed of 1500 piston/min was used, and three downwards and three upwards shifts were performed. The homogenates were centrifuged at 10,000  $\times$  g for 15 min at 4°C. The supernatants were used for biochemical analysis.

### Biochemical assays

The total antioxidant status (TAS) was measured via exploration of the peroxidase activity of metmyoglobin combined with its interaction with a phenothiazine compound to form a radical cation intermediate. This method is based on the observation of the formation of a radical  $\text{ABTS}^{\text{R}+}$  cation after the incubation of 2,2'-azino-di-[3-ethylbenzthiazoline] sulphonate ( $\text{ABTS}^{\text{R}}$ ) with a peroxidase (metmyoglobin) and  $\text{H}_2\text{O}_2$ . A large number of free radicals also react with ABTS to form this species. It has a relatively stable blue-green colour, which is measured at 660 nm. Antioxidants in the added sample suppressed colour production to a degree which is proportional to their concentration. This method was developed by RANDOX Laboratories LTD Aldmore, UK [16]. The concentration of TAS is defined in comparison to the concentration of Trolox having the equivalent antioxidant capacity. HPLC methods were used to determine the level of vitamin E (De Leenher *et al.* 1979). The extent of lipid peroxidation was assayed as the amount/concentration of thiobarbituric acid reactive substances (TBA-rs) according to the method described by Salaris and Babs [17]. Triglyceride and cholesterol concentrations were measured with kits from BioMerieux in an Express Plus biochemical analyzer (Ciba-Corning Diagnostics, Medfield, USA). The diagnostic Cornway test was used for the assessment of blood serum alanine (ALT) and aspartate aminotransferase (AST) activities.

### Electrochemical methods

In order to determine the surface charge density of the cell membrane, rat liver tissue was exposed to the action of trypsin. The cells obtained were put into a measuring vessel, and then the electroforetic mobility was measured using a DTS5300 ZETASIZER 3000 apparatus (MALVERN INSTRUMENTS).

The surface charge density was determined using the equation:  $\sigma = \eta u / d$ ; here  $u$  – electrophoretic mobility,  $\eta$  – the viscosity of the solution,  $d$  – diffuse layer thickness [18].

The diffuse layer thickness was determined from the formula

$$d = \sqrt{\frac{\varepsilon \cdot \varepsilon_0 \cdot R \cdot T}{2 \cdot F^2 \cdot I}} \quad [19],$$
 where  $R$  is the gas constant,  $T$  is the temperature,  $F$  is

the Faraday number,  $I$  is the ionic strength of 0.9% NaCl, and  $\varepsilon\varepsilon_0$  is the permeability of the electric medium.

### Statistical analysis

The data obtained in this study is expressed as mean  $\pm$  SD. The data was analyzed by use of standard statistical analyses; the one way ANOVA with Scheffe's F test for multiple comparisons to determine significance between different groups. Values of  $p < 0.05$  were considered significant.

### RESULTS

Fig. 1 shows changes in the surface charge density of liver cell membranes from the control rats (2-, 12- and 24-months) and from the animals that received ethanol or ethanol and green tea. Ethanol intoxication of the 2-, 12- and 24-month old rats caused a respective increase in the surface charge density of the liver cell membrane of about 35%, 35% and 45% compared with the control group. In rats receiving both ethanol and green tea, the increase in surface charge density was smaller by about 20% for every age group relative to the result for the equivalent ethanol groups.

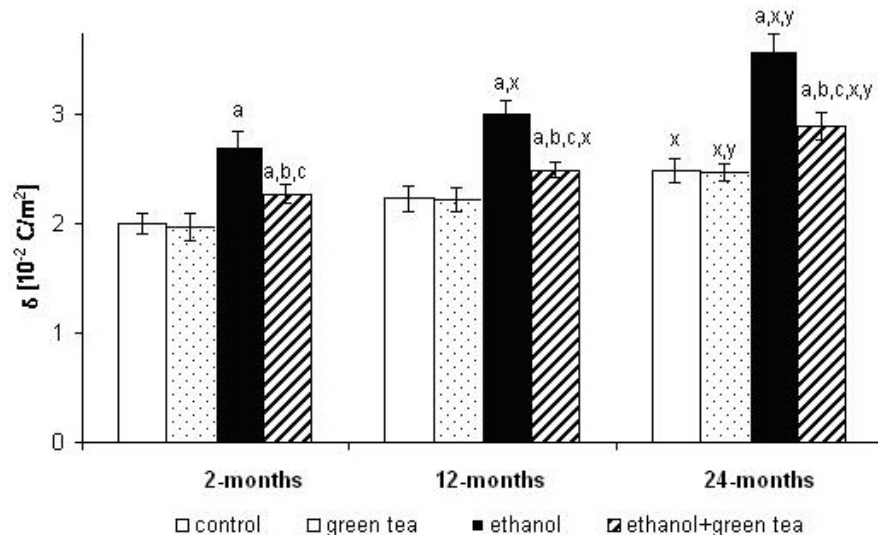


Fig. 1. The effect of green tea on the surface charge density of liver cell membranes from rats of different ages intoxicated with ethanol. a – significantly different from the control ( $p < 0.05$ ); b – significantly different from the green tea group ( $p < 0.05$ ); c – significantly different from the ethanol group ( $p < 0.05$ ); x – significantly different from the 2-month old group ( $p < 0.05$ ), y – significantly different from the 12-month old group ( $p < 0.05$ ).

Ethanol intoxication and aging both cause decreases in the antioxidant abilities of the rat liver (Tab. 1). It has been demonstrated that during aging, the vitamin E level consistently diminishes to up to 65% of the value observed for young rats. Ethanol significantly enhanced these changes. Green tea was given to the green tea group rats and seen to increase their level of vitamin E. The green tea given to the ethanol intoxicated rats partially but significantly prevented changes

observed after ethanol administration. The trend for changes in the total antioxidant status of the rat liver observed during the aging process and after ethanol intoxication is the same as that in the case of vitamin E. Green tea was more effective in preventing the decrease observed during aging and after ethanol consumption.

Tab. 1. Antioxidant ability of the liver of rats of different ages receiving ethanol and/or green tea.

Analyzed parameter	Age of rats	Group of rats			
		Control	Green tea	Ethanol	Green tea + ethanol
Vitamin E (nmol/g tissue)	2 months	30.8 ± 1.9	33.1 ± 1.8	15.4 ± 1.4 <sup>a</sup>	18.4 ± 1.1 <sup>abc</sup>
	12 months	26.0 ± 1.4 <sup>x</sup>	27.3 ± 1.3 <sup>x</sup>	20.2 ± 1.2 <sup>ax</sup>	24.4 ± 1.2 <sup>bcx</sup>
	24 months	20.3 ± 1.3 <sup>xy</sup>	24.1 ± 1.4 <sup>axy</sup>	14.7 ± 1.3 <sup>ay</sup>	18.3 ± 1.3 <sup>abcy</sup>
TAS (mmol/g tissue)	2 months	29.3 ± 1.3	36.7 ± 2.4 <sup>a</sup>	22.1 ± 1.1 <sup>a</sup>	30.2 ± 1.8 <sup>bc</sup>
	12 months	27.8 ± 1.5	31.1 ± 2.0 <sup>ax</sup>	21.9 ± 1.4 <sup>a</sup>	26.4 ± 1.9 <sup>bcx</sup>
	24 months	22.9 ± 1.5 <sup>xy</sup>	24.4 ± 1.9 <sup>xy</sup>	17.8 ± 1.5 <sup>axy</sup>	19.7 ± 1.8 <sup>abxy</sup>

a – significantly different from the control (p<0.05); b – significantly different from the green tea group (p<0.05); c – significantly different from the ethanol group (p<0.05); x – significantly different from the 2-month old group (p<0.05), y – significantly different from the 12-month old group (p<0.05).

Tab. 2. Liver cholesterol, triglyceride and malondialdehyde levels, measured as tiobarbituric reactive substances (TBARS), in rats of different ages receiving ethanol and/or green tea.

Analyzed parameter	Age of rats	Group of rats			
		Control	Green tea	Ethanol	Green tea + ethanol
Cholesterol (mg/g tissue)	2 months	2.07 ± 0.20	2.35 ± 0.25	3.64 ± 0.33 <sup>a</sup>	3.32 ± 0.27 <sup>ab</sup>
	12 months	2.53 ± .21 <sup>x</sup>	2.78 ± 0.22	4.64 ± 0.41 <sup>ax</sup>	4.09 ± 0.33 <sup>abx</sup>
	24 months	3.82 ± 0.30 <sup>xy</sup>	4.16 ± 0.31 <sup>xy</sup>	7.62 ± 0.59 <sup>axy</sup>	7.12 ± 0.54 <sup>abxy</sup>
Triglycerides (mg/g tissue)	2 months	3.17 ± 0.15	3.27 ± 0.19	4.65 ± 0.35 <sup>a</sup>	4.23 ± 0.32 <sup>ab</sup>
	12 months	3.42 ± 0.24	3.30 ± 0.22	5.95 ± 0.42 <sup>ax</sup>	5.02 ± 0.34 <sup>abcx</sup>
	24 months	4.48 ± 0.32 <sup>xy</sup>	4.62 ± 0.36 <sup>xy</sup>	7.75 ± 0.56 <sup>axy</sup>	6.97 ± 0.60 <sup>abxy</sup>
TBARS (nmol/g tissue)	2 months	31.2 ± 1.9	22.1 ± 1.7 <sup>a</sup>	55.2 ± 4.5 <sup>a</sup>	41.0 ± 3.0 <sup>abc</sup>
	12 months	47.8 ± 2.5 <sup>x</sup>	39.5 ± 2.7 <sup>ax</sup>	81.5 ± 5.4 <sup>ax</sup>	63.1 ± 4.6 <sup>abcx</sup>
	24 months	69.0 ± 5.1 <sup>xy</sup>	64.4 ± 4.7 <sup>xy</sup>	131.9 ± 8.7 <sup>axy</sup>	105.9 ± 8.2 <sup>abcxy</sup>

a – significantly different from the control (p<0.05); b – significantly different from the green tea group (p<0.05); c – significantly different from the ethanol group (p<0.05); x – significantly different from the 2-month old group (p<0.05); y – significantly different from the 12-month old group (p<0.05).

A significant increase in the level of triglycerides and cholesterol is observed post-ethanol intoxication of young rats and, to a higher degree, old rats (Tab. 2). Green tea also causes an increase in the levels of these parameters. As a consequence, the level of triglycerides and cholesterol is not protected against ethanol action. This decrease in the antioxidant ability of the liver is accompanied by a significant increase in the level of lipid peroxidation. The level of the final products of this process – aldehydes, measured as thiobarbituric reactive substances – increased after ethanol intoxication. Aging significantly enhanced this effect.

The consequence of changes in membrane phospholipid composition caused by the peroxidation process is an increase in the degree of membrane permeability, visible in the increase in liver damage marker activity (Tab. 3). The serum activity of ALT and AST increases by about 100% both after ethanol intoxication and during the aging process.

Tab. 3. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in rats of different age receiving ethanol and/or green tea.

Analyzed parameter	Age of rats	Group of rats			
		Control	Green tea	Ethanol	Green tea + ethanol
ALT (U/l)	2 months	35.7 ± 2.5	33.2 ± 2.4	65.9 ± 5.1 <sup>a</sup>	48.2 ± 3.2 <sup>abc</sup>
	12 months	39.4 ± 2.8 <sup>x</sup>	37.1 ± 2.9 <sup>x</sup>	73.1 ± 7.2 <sup>a</sup>	59.6 ± 4.1 <sup>abcx</sup>
	24 months	44.2 ± 3.4 <sup>xy</sup>	40.5 ± 3.3 <sup>x</sup>	92.6 ± 9.6 <sup>axy</sup>	76.9 ± 6.4 <sup>abcxy</sup>
AST (U/l)	2 months	162 ± 11	153 ± 11	246 ± 18 <sup>a</sup>	183 ± 14 <sup>abc</sup>
	12 months	175 ± 13	167 ± 11	302 ± 27 <sup>ax</sup>	225 ± 19 <sup>abcx</sup>
	24 months	197 ± 17 <sup>xy</sup>	186 ± 15 <sup>xy</sup>	354 ± 31 <sup>axy</sup>	287 ± 24 <sup>abcxy</sup>

a – significantly different from the control ( $p < 0.05$ ); b – significantly different from the green tea group ( $p < 0.05$ ); c – significantly different from the ethanol group ( $p < 0.05$ ); x – significantly different from the 2-month old group ( $p < 0.05$ ); y – significantly different from the 12-month old group ( $p < 0.05$ ).

## DISCUSSION

The cell membrane is an integral part of the cell, and it plays an essential role in cell life processes. It makes the cell an isolated system and determines its functions. The correct course of many metabolic processes in which the membrane is involved, such as the transport of compounds participating in metabolic transformations, and information transmission mediated by ion pumps, carriers and membrane channels, depends on the conservation of its composition, structure, and electric properties. An important electric properties of the biological membrane is its electrokinetic potential, i.e. the potential difference between the membrane and its environment and its surface charge. The results of this paper have shown that the surface charge density of liver cells is affected both during aging and by ethanol intoxication. At physiological pH,

the cell membranes have a negative total charge [7, 20]. The electric charge is determined by the amount of negative charge carriers, e.g. of some phospholipids, like phosphatidylserine, sialic acid and glycoporphins, and the free carboxyl groups of polypeptide chains, as well by the amount of positive charge carriers, such as the free amino groups of proteins and aminophospholipids. [7]. The cell membrane charge is an indication of cell "form", e.g. tumour formation is accompanied by an increase in the membrane surface charge, while cell necrosis provokes a decrease in charge [21]. It is suggested that the changes in cell membrane charge are connected with changes in membrane composition. It is known that aging and ethanol intoxication are accompanied by changes in the composition of liver phospholipids, fatty acids and cholesterol, which leads to changes in membrane symmetry, fluidity and other properties [22, 23]. Changes in membrane properties may also be connected with cell component modifications. It is known that aging and ethanol intoxication are characterized by oxidative modifications of the lipids and proteins that are the main membrane components [24, 25]. That is mainly due to the reactions of reactive oxygen species with the above compounds. It was proved that the generation of reactive oxygen species is enhanced during aging and after ethanol intoxication, and that they play a major role in the creation of oxidative stress, which may additionally be enhanced by depletion in the antioxidant defense system and, in consequence, by an imbalance between prooxidants and antioxidants [26]. Previous studies have indicated that aging and the chronic consumption of ethanol cause significant decreases in the antioxidant status of rat tissues [27-29]. The consequence of the above was observed in this and earlier studies: an increase in the extent of membrane phospholipid peroxidation. The reactions with lipids result in the formation of small molecular aldehydes, measurable as thiobarbituric reactive substances, as observed in this study. The cellular oxidative damage is markedly potentiated by the presence of free iron. Ethanol metabolism affects the release of iron from bound intracellular reserves [30]. It has been suggested that the release of free iron may be the primary mechanism for ethanol-induced lipid peroxidation. In addition, the reactions participate in the decomposition of lipid hydroperoxides to aldehydes like 4-hydroxynonenal and malondialdehyde [31], which are long lived and can therefore diffuse from the site of their generation. They can alter proteins, DNA and other biomolecules, thus disrupting their point of origin, and reach and attack intracellular and extracellular targets [32, 33].

Both reactive oxygen species and small molecular aldehydes (including the ethanol metabolite – acetaldehyde) can react with proteins and modify their structure [32]. ROS reactions with proteins lead to peroxy radical and hydroperoxide formation. The reaction of lipid peroxidation products with proteins leads to carbonyl group formation. Moreover, as a result of reactive oxygen species reaction or lipid peroxidation product reactions with proteins, their fragmentation occurs, resulting in new functional groups which may alter membrane electric charge. Additionally, acetaldehyde can react with amino, sulfhydryl and another groups of peptides and proteins [34, 35]. These reactions

lower the positive charge of proteins thereby increasing the negative charge of the membrane surface. The conformation of membrane protein is maintained by a hydrophobic and hydrogen bond, and requires a hydrophobic lipid environment for the formation and stability of the secondary structure of hydrogen bonds [36, 37]. The perturbation of the protein structure may greatly affect lipid-protein interactions [38]. Changes in membrane structure caused by the modification of lipid and protein structure lead to impairment in the structure of the hepatocyte membrane skeleton. One such known alteration is the change in distribution of phosphatidylserine, which is a component of the skeleton, from the internal to the external side of membrane [39]. This could also cause an increase in the negative charge density of the membrane. As a result of structural changes, alterations in membrane fluidity, a fall in membrane potential, and an increase in membrane permeability to  $H^+$  and other ions have been demonstrated in different experimental models [40]. The increase in membrane permeability and in the terminal situation disruptions of these membranes may cause the translocation of the liver enzymes ALT and AST, the markers of liver damage, into the blood, as observed in this study.

Because most of the above changes appearing in liver membrane cells may be linked to the increased oxidative stress caused by an imbalance in the generation and neutralization of free radicals, these negative results may be compensated for by the addition of exogenous substances possessing antioxidant properties, such as green tea. The results of this study indicate that green tea partially protects liver cells from the changes caused by ethanol consumption and the aging process. Another paper also indicated that green tea enhanced antioxidative abilities in the liver, brain and serum of rats and protected liver cells and their organelles (morphological examinations) against ethanol action [29]. Moreover, it was shown that green tea also prevented changes in the serum antioxidant parameters during the aging of ethanol intoxicated rats [28]. Green tea is a natural product containing large amounts of polyphenols, which are strong antioxidants. Tea polyphenols mainly comprise catechins and catechin derivatives, which are absorbed, metabolised and distributed into all organs [41]. These results indicate that ingested catechins can have biological effects. Catechins are considered to exert protective effects against oxidative stress [42], thus green tea induces the antioxidant abilities of the organism, as also shown in this study. The protective action of green tea is connected with the ability of its catechins to prevent oxygen radical formation and to scavenge free radicals such as hydroxyl, peroxy and lipid radicals and superoxide anions [43-45]. Scavenging the most active hydroxyl radical that may initiate lipid peroxidation protects membrane phospholipids. The efficiency of green tea in preventing lipid peroxidation was revealed earlier [28, 29]. Green tea catechins decrease the level of lipid peroxidation occurring when membrane phospholipids are exposed to oxygen radicals from the aqueous phase [46]. Oxidative attack from the aqueous phase seems to be an important reaction for initiating membrane lipid peroxidation. Perohydroxyl radicals, which are produced by the promotion of

superoxide, and whose concentration is enhanced during alcohol intoxication, are regarded as the most feasible radicals for initiating lipid peroxidation *in vivo*. Consequently, the slower pace of free radical reactions leads to an inhibition of lipid peroxidation and thus to a decrease in membrane fluidity [47]. However, catechins, which are water-soluble antioxidants, could also reduce the mobility of free radicals in the lipid bilayer. Their hydrophobic fragments can penetrate the lipid bilayer influencing antioxidant capability in biomembranes. Catechins preferentially enter the hydrophobic core of the membrane where they exert a membrane-stabilising effect by modifying the lipid packing order [48]. Furthermore, catechins can also interact with phospholipid head groups, particularly with those containing hydroxyl groups, so they could decrease the fluidity in the polar surface of the phospholipid bilayer [49]. Additionally, catechins may chelate metal ions, especially iron and copper, which in turn precludes the generation of hydroxyl radicals and the degradation of lipid hydroperoxides to reactive aldehydes [43]. Catechins prevent the consumption of the lipophilic main membrane antioxidative protector,  $\alpha$ -tocopherol, by repairing the tocopheryl radical [46]. The results of this study show that green tea has a preventive effect against vitamin E decrease.

The administration of ethanol together with green tea partly reduces the effect of ethanol on the rats; this could be seen in the markedly lower increase in phospholipid content and peroxidation and the consequent lower surface charge density. The lipid structure of liver cell membranes is not disturbed and thus cellular enzymes (ALT, AST) are not translocated into the blood.

In conclusion, long-term drinking of green tea partially prevents the decrease in the antioxidant abilities of the liver and the changes in the structure and function of membrane phospholipids caused by chronic ethanol intoxication. Evidence for the bioactivity of the phenolic compounds of green tea *in vivo* supports the notion that their antioxidant properties play an important role in health protection and disease prevention.

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## REFERENCES

1. Bunker, V.W. Free radicals, antioxidant and aging. **Med.Lab.Sci.** 49 (1992) 299-312.
2. Ames, B.N., Shigenaga, M.K. and Hagen T.M. Oxidants, antioxidants and the degenerative diseases of aging. **Proc. Natl. Acad. Sci. USA** 90 (1993) 7915-7922.
3. Lieber, C.S. Ethanol metabolism, cirrhosis and alcoholism. **Clin. Chim. Acta** 257 (1997) 59-84.
4. Kato, S., Kawase, T., Alderman, J., Inatori, N. and Lieber, C.S. Role of xanthine oxidase in ethanol-induced lipid peroxidation. **Gastroenterology** 98 (1990) 203-210.

5. Niemela, O., Parkkila, S., Pasanen, M., Iimuro, Y., Bradford, B. and Thurman, R.G. Early alcoholic liver injury: formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. **Alcohol Clin. Exp. Res.** 22 (1998) 2118-24.
6. Nagata, N., Nishizaki, Y., Watanabe, N., Tsuda, M. and Matsuzaki S. An enzyme immune assay for serum anti-acetaldehyde adduct antibody using low-density lipoprotein adduct and its significance in alcoholic liver injury. **Alcohol. Clin. Exp. Res.** 22 (1998) 150S-155S.
7. Gennis, R.B. **Biomembrane: Molecular structure and functions.** (Cantor C.R. Ed.), Springer-Verlang , New York 1989.
8. Zhao, B., Li, X., He, R., Cheng, S. and Wenjuan, X. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. **Cell. Biophys.** 14 (1989) 175-185.
9. Rice-Evans, C.A., Miller, N.J. and Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. **Free Radic. Biol. Med.** 20 (1996) 933-956.
10. Varga, M. and Buris, L. Some morphometric evidence of hepatoprotective effects of (+)-cyanidanol-3. **Pharmacol. Biochem. Behav.** 33 (1989) 523-526.
11. Varga, M. and Buris, L. Quantitative ultrastructural analysis of hepatoprotective effects of (+)-cyanidol-3 on alcoholic liver damage. **Exp. Mol. Pathol.** 52 (1990) 249-257.
12. Natsume, M., Osakabe, N., Oyama, M., Sasaki, M., Baba, S., Nakamura, Y., Osawa, T. and Terao, J. Structures of (-)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (-)-epicatechin: differences between human and rat. **Free Radic. Biol. Med.** 34 (2003) 840-849.
13. Okushio, K., Suzuki, M., Matsumoto, N., Nanjo, F. and Hara, Y. Identification of (-)-epicatechin metabolites and their metabolic fate in the rat. **Drug Metab. Dispos.** 27 (1999) 309-316.
14. Lieber, C.S. and DeCarli, L.M. Hepatic microsomal ethanol-oxidizing system. In vitro characteristics and adaptive properties in vitro. **J. Biol. Chem.** 245 (1970) 2505-2512.
15. Maiani, G., Serafini, M., Salucci, M., Azzini, E. and Ferro-Luzzi A. Application of a new high-performance liquid chromatographic method for measuring selected polyphenols in human plasma. **J. Chromatogr. B. Biomed. Sci. Appl.** 692 (1997) 311-317.
16. Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. and Milner, A. A novel method for measuring antioxidant capacity and its application to motoring the antioxidant status in premature neonates. **Clin. Sci.** 84 (1993) 407-412.
17. Salaris, S.C. and Babs, CF. A rapid, widely applicable screen for drugs that suppress free radical formation in ischemia and reperfusion. **J. Pharmacol. Meth.** 20 (1988) 325-346.

18. Krysiński, P. and Tien, H.Y. Membrane electrochemistry. **Prog. Surf. Sci.** 23 (1986) 317-412.
19. Barrow, G.M. **Physical Chemistry**, McGraw-Hill, Inc. New York, 1996.
20. Benga, G. and Holmes, R.P. Interactions between components in biological membranes and their implications for membrane function. **Prog. Biophys. Molec. Biol.** 43 (1984) 195-257.
21. Dołowy, K. Bioelectrochemistry of cell surface. **Prog. Surface Sci.** 15 (1984) 245-368.
22. Naeim, F. and Walford, R.L. Aging and cell membrane complexes: the lipid bilayer, integral proteins, and cytoskeleton. In: **Handbook of the Biology of Aging**. (Finch C.E. and Schneider E.L., Eds.) Von Nostrand Reinhold, 1985.
23. Slater, S.J., Ho C., Taddeo, F.J., Kelly, M.B. and Stubbs, Ch.D. Contribution of hydrogen bonding to lipid-lipid interactions in membranes and the role of lipid order: Effects of cholesterol, increased phospholipid unsaturation, and ethanol. **Biochemistry** 32 (1993) 3714-3721.
24. Perichon, R., Bourre, J.M., Kelly, J.F. and Roth, G.S. The role of peroxisomes in aging. **Cell Mol. Life Sci.** 54 (1998) 641-652.
25. Seppi, C., Castellana, M.A., Minetti, G., Piccinini, G., Balduini, C. and Brovelli, A. Evidence for membrane protein oxidation during in vivo aging of human erythrocytes. **Mech. Ageing Dev.** 57 (1991) 247-258.
26. Halliwell, B. and Gutteridge, J.M. **Free Radicals in Biology and Medicine**, Oxford University Press, 2001.
27. Skrzydlewska, E., Ostrowska, J., Stankiewicz A. and Farbiszewski, R. Green tea as a potent antioxidant in alcohol intoxication. **Addicton Biol.** 7 (2002) 307-314.
28. Łuczaj, W., Waszkiewicz, E., Skrzydlewska, E. and Roszkowska-Jakimiec W. Green tea protection against age-dependent ethanol-induced oxidative stress., **J. Toxicol. Environ. Health** 67 (2004) 595-606.
29. Ostrowska, J., Łuczaj, W., Kasacka, I., Różański, A. and Skrzydlewska, E. Green tea protects against ethanol-induced lipid peroxidation in rat organs. **Alcohol** 32 (2004) 25-32.
30. Rouach, H., Houze, P. and Orfanelli, M.T. Effect of acute ethanol administration on the subcellular distribution of iron in rat liver and cerebellum. **Biochem. Pharmacol.** 39 (1990) 1095-1100.
31. Dix, T. A. and Aikens, J. Mechanisms and biological relevance of lipid peroxidation initiation. **Chem. Res. Toxicol.** 6 (1993) 2-18.
32. Esterbauer, H., Schaur, R. J. and Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. **Free Radic. Biol. Med.** 11 (1991) 81-128.
33. Box, H.C., Freund, H.G., Budzinski, E.E., Wallace, J.C. and Maccubbin, A.E. Free radical-induced double base lesions. **Radiat. Res.** 141 (1995) 91-94.
34. Tuma, D.J., Donohue, T.M., Medina, V.A. and Sorrell M.F. Enhancement of acetaldehyde-protein adduct formation by L-ascorbate. **Arch. Biochem. Biophys.** 234 (1984) 377-381.

35. Tuma, D.J., Newman, M.R., Donohue, T.M. and Sorrel, M.F. Covalent binding of acetaldehyde to proteins: participation of lysine residues. **Alcohol. Clin. Exp. Res.** 11 (1987) 579-584.
36. Klemm, W.R. Dehydration: a new alcohol theory. **Alcohol** 7 (1990) 49-59.
37. Klemm, W.R. and Yuritas, L. The dehydration theory of alcohol intoxication. in: **Drug and Alcohol Abuse Reviews, vol. 3**, (Watson R.R. Ed.). The Humana Press Inc.1992, 169-185.
38. Scott, J.A. and Rabito C.A. Oxygen radicals and plasma membrane potential. **Free Radic. Biol. Med.** 5 (1988) 237-246.
39. Jain, S.K. *In vivo* externalization of phosphatidylserine and phosphatidylethanolamine in the membrane bilayer and hypercoagulability by the lipid peroxidation of erythrocytes in rats. **J. Clin. Invest.** 76 (1985) 281-286.
40. Chiu, D., Kuypers, F. and Lubin, B. Lipid peroxidation in human red cells. **Semin. Hematol.** 26 (1989) 257-276.
41. Lee, M.J., Prabhu, S., Meng, X., Li, C. and Yang, C.S. An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. **Anal. Bioch.** 279 (2000) 164-169.
42. Aucamp, J., Gaspar, A., Hara, Y. and Apostolides, Z. Inhibition of xanthine oxidase by catechins from tea (*Camelia Sinensis*). **Anticancer Res.** 17 (1997) 4381-4386.
43. Guo, Q., Zhao, B., Li, M., Shen, S. and Xin, W. Studies on protective mechanism of four components of green tea polyphenols against lipid peroxidation in synaptosomes. **Biochim. Biophys. Acta** 1304 (1996) 210-222.
44. Jovanovic, S.V., Hara, Y., Steenken, S. and Simic, M.G. Antioxidant potential of gallic catechins. A pulse radiolysis and laser photolysis study. **J Am. Chem. Soc.** 117 (1995) 9881-9888.
45. Khan, S.G., Katiyar, S.K., Agarwal, R. and Makhtar, H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. **Cancer Res.** 52 (1992) 4050-4052.
46. Zhu, Q.Y., Huang, Y., Tsang, D. and Chen Z.Y. Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. **J. Agric. Food Chem.** 47 (1999) 2020-2025.
47. Tsuchiya, H. Effects of green tea catechins on membrane fluidity. **Pharmacology** 59 (1999) 34-44.
48. Arora A., Byrem T.M., Nair M.G. and Strasburg G.M. Modulation of lysosomal membrane fluidity by flavonoids and isoflavonoids. **Arch. Biochem. Biophys.** 373 (2000) 102-109.
49. Chen, L., Yang, X., Jiao, H. and Zhao, B. Tea catechins protect against lead-induced cytotoxicity, lipid peroxidation, and membrane fluidity in HepG2 cells. **Toxicol. Sci.** 69 (2002) 149-156.