ANTIOXIDANT AND RADICAL SCAVENGING EFFECTS OF TEA AGAINST OXIDATIVE STATUS OF LIPOPROTEIN IN MALE MICE

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ABSTRACT

To investigate the effect of tea polyphenols against oxidative status of lipoprotein, thirty six male mice were used. Six groups of mice (5 each) were assigned. Groups 1 and 2 (GI and GII) were served as negative and positive control groups respectively. Groups 3 and 4 (GIII and GIV) drank black (12.5 gm/L) and green (12.5 gm/L) teas respectively, while groups 5 and 6 (GV and GVI) drank black and green teas respectively and both were injected with cyclophosphamide (20 mg/kg body weight, i.p) for three consecutive days, (used also with GII). The experimental period extends for 73 days.

Results indicated that the inactive ingredients of tea caused inhibition of urine nitrite and hydroxyamine formation in tea drinking groups (GIII and GIV), while liver cytochrome P450 decreased in green tea group (GIII). Also both teas lowered the concentration of plasma cholesterol, triglycerides (TG), low density lipoprotein cholesterol (LDL), and very low density lipoprotein cholesterol (VLDL) lipid fractions, while it increased the high density lipoprotein cholesterol (HDL), in group GV. Total antioxidant capacity (TAC) increased in all treated groups (GIII, GIV, GV and GVI), while it decreased in GII. Meanwhile Thiobarbituric acid reactive substances (TBARS) showed significant reduction in both green tea treated groups (GIV and GVI). In conclusion, both black and green teas polyphenols improved blood lipids profile, strengthens blood plasma antioxidant capacity leading to decrease in oxidation product levels TBARS, which demonstrated reduced oxidation reaction in the body. This work also showed that teas supplementation were capable of inhibiting the nitrosation of secondary amine.

Keywords: Green and black tea, nitrite, Lipid fractions, TBARS.

INTRODUCTION

Tea is particularly rich in polyphenols, including catechins, theaflavins and thearubignins, which are thought to contribute to the health benefits of tea. Tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions (Salz and Jane, 2003 and Peterson et al, 2005).

Green and black tea are both high in catechins. These compounds are powerful antioxidants, capable of rapid reduction of superoxide radical and alkyl proxy radicals. Catechins may also repair vitamin E radicals. Such potent antioxidant ability may be important in inhibiting the in-vivo oxidation of LDL and VLDL and the subsequent atherogenesis (Vinson and Dabbagh, 1998).

Oxidation of cholesterol fraction, in particular of low-density lipoprotein (LDL) cholesterol, has been accepted as playing an important role in atherosclerosis (Liu et al., 1992). Cholesterol, Cholesterol esters and
triglyceride components of the lipoprotein fractions can be oxidized by toxic radicals and can lose their chemical structure and cellular functions (Durak et al., 2004 and Gramza and korczak, 2005). Lipid peroxidation is accepted to be a free radical process implicated in the formation of athroesclerosis (Wen et al., 1998), and the aldehyde products of lipid hydroperoxide breakdown to be responsible for the modification of LDL apoprotein (Estrabaure et al., 1993).

Green tea extracts are described as preventive against induced cancers in animals (Wang et al., 1991). Epigallocatechin gallate (EGCG), a major constituent of green tea polyphenolics, has been shown to inhibit the promotion step of induced carcinogenesis in animals. Catechins are known to be reactive towards nitrite (Bartsch et al., 1993). Since a major source for the exposure of humans to carcinogenic nitrosoalkylamines is suspected to be nitroso compounds formed in the digestive tract from dialkyamines and nitrite, degradation of nitrite by dietary catechins may be regarded as a possible protective measure against human exposure to these carcinogenic compounds (Tanaka et al., 1998).

The present study was designed to ascertain the beneficial role of tea polyphenols against oxidative status of lipoprotein and nitrite-derived health hazards in male mice.

MATERIALS AND METHODS

1. Chemicals
   Cyclophosphamide, thiobarbituric acid and all other chemicals used were purchased from Sigma Chemical Company (Saint Louis, USA).

2. Animals
   Thirty six male mice with average body weight of 25-30 gm were obtained from Medical Research Institute, Alexandria University, Egypt, and acclimated for two weeks prior to the experiment. They were assigned to six groups and housed in Universal galvanized wire cages at room temperature (22-25 °C) and in a photoperiod of 12 hrs/day. Animals were provided with a commercial balanced diet.

3. Experimental design
   Six groups of mice (8 each) were randomly assigned as: Group I (G1) served as control and injected with physiological saline, group II (GII) was injected with cyclophosphamide (20 mg/kg body weight, i.p.) for three consecutive days to induce oxidative stress, group III (GIII) drank black tea (12.5 gm/L) and injected with physiological saline, group IV (GIV) drank green tea (12.5 gm/L) and injected with physiological saline, group V (GV) was injected with cyclophosphamide (20 mg/kg body weight, i.p.) and drank black tea, group VI (GVI) was injected with cyclophosphamide (20 mg/kg body weight, i.p.) and drank green tea. Urine was collected weekly (for nine weeks) after 7 days post cyclophosphamide treatment, using metabolic cages. Animals were decapitated at the end of the experimental period (73 days).
4. Tissue preparation
4.1. Preparation of liver microsomes

At the end of the treatment period, mice were fasted for 24 hrs prior to being sacrificed by cervical dislocation. The abdominal cavity was opened immediately and liver was removed, washed with cold 0.1 M phosphate buffer, pH 7.4, weighed and chilled on ice. All the following procedures were carried out in cold conditions. A 33% (W/V) crude homogenate was prepared in 0.1 M phosphate buffer, pH 7.4 by homogenization with a teflon pestle, using 5 strokes. The crude homogenate was then centrifuged at 11,000 xg for 20 min at 4°C to remove the intact cells, nuclei and mitochondria. The supernatant solution was subsequently centrifuged at 105,000 xg for 60 min at 4°C to sediment the microsomal pellet. The pellet was resuspended in 0.1 M phosphate buffer, pH 7.4, kept in ice bath and used as the enzyme source.

4.2. Separation of blood plasma

Blood samples were obtained by sacrificing the animals, and were placed immediately on ice. Heparin was used as anticoagulant. Plasma was obtained by centrifugation at 3,000 rpm for 20 min and then stored at −20°C until used for analysis.

5. Biochemical assays
5.1. Protein determination

Protein concentration of the hepatic microsomal fraction was determined by the method of Lowery et al. (1951).

5.2. Liver cytochrome P450

Liver microsomal cytochrome P450 was determined according to Omura and Sato (1964), using molar extinction coefficient 91 cm⁻¹ mM⁻¹.

5.3. Blood biochemical assays

Blood plasma cholesterol was determined using commercial kits obtained from Bio ADWIC, Egypt. Plasma triglyceride (TG) was determined by triglyceride-GPO kits obtained from Pasteur Lab, Egypt, according to McGowan et al. (1983). Plasma low-density lipoprotein (LDL) and serum high-density lipoprotein (HDL) were assayed using Biosystems reagents Kits, Spain, according to Assman et al. (1984) and Biosystems reagents Kits, Spain, according to Burstein et al. (1980), respectively. Plasma very low density lipoprotein (VLDL) was calculated from triacylglycerols according to Friedwald et al. (1972) who reported that VLDL is present in a concentration equal to one fifth of triacylglycerols concentration in blood plasma of less than 400 mg/dl. Thiobarbituric acid-reactive substances (TBARS), were measured in blood plasma as described by Tappe and Zalkin (1959). The color intensity of the TBARS reactants was measured at 532 nm and a molar extinction coefficient of 156,000 cm⁻¹ M⁻¹ was used for calculation of the concentration. Total antioxidant capacity was measured according to the method of Koracevic et al. (2001) using commercial kits obtained from Biodiagnostic Co., Egypt.

5.4. Urine biochemical assays

Hydroxylamine and nitrite in urine were determined using formation of Azo-dye compound according to Feigl and Anger (1968).
6. Statistical analyses

Statistical analyses were made to obtain the standard deviation and standard errors of mean. The data for the treated animals were compared with data for the control animals by using the Student's t-test SAS (2000).

RESULTS AND DISCUSSION

Nitrite represents a potential hazard because of its involvement in the nitrosation reaction. The present study showed that tea polyphenols, in particular green tea, were markedly reduced nitrite formation specially in the last four weeks in urine samples (Figure 1).

Fig 1: Effect of teas supplementation on nitrite (mg/l) formation in male mice urine
(NS = p > 0.05, * = p < 0.05, ** = p < 0.01, *** = p < 0.001)
It was noticed that cyclophosphamide (positive control) has no adverse effect in regard with nitrite. The same trend was observed for hydroxyl amine, where green tea polyphenols highly affected the inhibition of hydroxylamine formation, also cyclophosphamide has no adverse effect towards hydroxyamine (Figure 2). There are number of reports dealing with inhibition of nitrosodiakylamine formation by dietary components (Bartsch et al., 1993).

Fig 2: Effect of teas supplementation on hydroxylamine (mg/l) formation in male mice urine.
(NS = p > 0.05, * = p < 0.05, ** = p < 0.01, *** = p < 0.001)
Regarding the tea extracts, Nakamura and Kawabata (1981) briefly described the blocking ability of the extracts against N-nitrosodimethylamine (NDMA) formation in vitro from dimethylamine and nitrite. The present study revealed that tea polyphenols, in particular green tea, is a powerful antioxidant capable of rapid inhibiting formation of nitrite, which are involved in the nitrosation reaction with appropriate nitrosatable substrate (S) giving rise to the formation of a potent of chemical carcinogens, the N-nitroso compounds, most of which have induced tumors in many species of laboratory animals tested, and in virtually every tissue (Schlag et al., 1982).

The same observation was indicated for hydroxylamine, where tea polyphenones were capable of reducing the formation of this hazardous compound. Hydroxylamine derivatives are formed in the liver and then converted into glucuronide. The glucuronide conjugate is excreted in urine, where the acidic pH can convert it back to hydroxylamine which is rearranged to form nitrenium ion: by a loss of water.

The electrophilic nitrenium ion can then react with nucleophilic targets in urinary bladder epithelium (Kaldilbar et al., 1977).

The importance of nutrition in protecting the living organisms from toxic effects of environmental carcinogens has recently been realized. Table (1) revealed that the hepatic content of microsomal cytochrome P450 was significantly (p<0.05) decreased by 33% in the group treated with green tea. Meanwhile, cyclophosphamide significantly (p<0.05) increased this content by 67% than that of the control group.

The cytochrome P450 enzymes are responsible for the oxidation of xenobiotic chemicals including drugs, pesticides and carcinoogens. Inhibition of cytochrome P450 system was found to be effective in protecting the liver against the toxicity of a wide variety of toxic agents (Sheweita et al., 2001 and Jorduera et al., 1996). Treatment of male mice with black and green tea only was found to decrease the hepatic content of cytochrome P450. Inhibition of cytochrome P450 could protect the liver against the possible side effects of cyclophosphamide.

The effect of tea polyphenols on cholesterol, triglycerides (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL) were also investigated. Results presented in (Table 1) showed that black tea, green tea, black tea+cyclophosphamide and green tea+cyclophosphamide treated groups significantly (p<0.001) decreased the level of cholesterol by 29, 34, 32 and 36% respectively. Triglycerides were significantly (p<0.001) decreased in the same above mentioned groups by 25, 43, 28 and 35% respectively. HDL was significantly (p<0.001) decreased in cyclophosphamide treated group by 36%, meanwhile black tea + cyclophosphamide significantly (p<0.001) increased HDL by 27%. As shown in (Table 1), both teas were powerful inhibitors of LDL and VLDL.

Green tea was significantly better in vivo as antioxidant than black tea, where the total antioxidant capacity (TAC) increased significantly (p<0.001) in green and black tea treated groups compared with control by 78 and 41% respectively (Table 1).
Table 1: Effects of teas supplementation on liver microsomal cytochrome \( \text{P450} \), blood plasma lipid, total antioxidant capacity and lipid peroxidation in male mice (Mean ± SEM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (GII)</th>
<th>Positive (GIII)</th>
<th>Black T (GIV)</th>
<th>Green T (GVI)</th>
<th>Black (GV)</th>
<th>Green (GVI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyt. P450 (p mol/mg protein)</td>
<td>2.27 ± 0.08</td>
<td>3.79 ± 0.18**</td>
<td>2.04 ± 0.09NS</td>
<td>1.51 ± 0.01*</td>
<td>1.82 ± 0.14NS</td>
<td>2.06 ± 0.12NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>329 ± 19.09</td>
<td>326 ± 49.3NS</td>
<td>233 ± 12.2***</td>
<td>216 ± 9.2***</td>
<td>225 ± 11.0***</td>
<td>208 ± 7.1***</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>107 ± 13.7</td>
<td>105 ± 12.9**</td>
<td>140 ± 11.2***</td>
<td>106 ± 8.5***</td>
<td>134 ± 9.2***</td>
<td>122 ± 11.0***</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>36.8 ± 6.24</td>
<td>23.4 ± 3.35***</td>
<td>43.0 ± 6.10NS</td>
<td>40.0 ± 6.42NS</td>
<td>46.0 ± 5.28***</td>
<td>40.3 ± 6.14NS</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>111.3 ± 12.97</td>
<td>50.7 ± 5.80***</td>
<td>57.0 ± 3.94***</td>
<td>39.3 ± 5.90***</td>
<td>54.5 ± 6.60***</td>
<td>33.6 ± 4.39***</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>37.4 ± 2.73</td>
<td>33.0 ± 2.57**</td>
<td>28.0 ± 2.24***</td>
<td>21.3 ± 1.70***</td>
<td>26.7 ± 1.83***</td>
<td>24.4 ± 2.19***</td>
</tr>
<tr>
<td>TAC (mM/L)</td>
<td>1.06 ± 0.10</td>
<td>0.66 ± 0.13NS</td>
<td>1.49 ± 0.06***</td>
<td>1.89 ± 0.05***</td>
<td>1.32 ± 0.03**</td>
<td>1.71 ± 0.03***</td>
</tr>
<tr>
<td>TBARS (\· mole/g tissue)</td>
<td>2.07 ± 0.11</td>
<td>4.28 ± 0.18**</td>
<td>2.82 ± 0.04NS</td>
<td>1.62 ± 0.07***</td>
<td>2.93 ± 0.03NS</td>
<td>1.76 ± 0.06***</td>
</tr>
</tbody>
</table>

NS = \( p > 0.05 \)  * = \( p < 0.05 \)  ** = \( p < 0.01 \)  *** = \( p < 0.001 \)
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Thiobarbituric acid reactive substances (TBARS), which is an important indicator of lipid peroxidation increased significantly \((p<0.01)\) in cyclophosphamide treated group, meanwhile green tea decreased this level significantly \((p<0.001)\) and corrected the damage caused by cyclophosphamide as seen in Table (1).

As seen from the results, tea polyphenols, in particular green tea which represent the richest source of natural polyphenols, can lower blood cholesterol level and can improve blood lipid profile to a significant extent. It also increased blood plasma antioxidant potential and decreased TBARS level. All of these results showed that tea polyphenols exerted considerable antioxidant power in vivo as well, and protected cellular structures against peroxidation. This high antioxidant potential of tea may be a result of its high content of epicatechin and epigallocatechin gallate (Skrzydlewski et al., 2002). Oxidation of cholesterol fractions (in particular, LDL) has been accepted as playing an important role in atherosclerotic process (Liu et al., 1992), and because lipid peroxidation is a radical process implicated in this formation (Yen et al., 1996). It has been proposed that extracts such as teas, that are rich in antioxidant content may confer beneficial effects in this regard. HDL has a protective function in the prevention of oxidation reaction and the consumption of antioxidant potency (Curak et al., 2004). With respect to the cholesterol lowering property of tea polyphenols, it has been suggested that some constituents (as tea epicatechins) may act as inhibitors of some enzymes such as hydroxymethyl glutaryl CoA reductase, which participates in cholesterol synthesis (Chan et al., 1999).

In conclusion, the present work demonstrated that adding black and green tea in drinking water to mice for 73 days lowered the concentration of lipid peroxidation products and increased the total antioxidant potential of the liver and blood plasma and improving lipid profile, also these results may be useful in evaluating the role of teas in the inhibition of tumor initations.

REFERENCES


تأثير الشاي كمضادات للأكسدة وإمتلاك الفيتامينات في الغرب
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1- قسم الدراسات البيئية - معهد الدراسات العليا والبحوث - جامعة الإسكندريه
2- المركز المركزي للأغذية والأطعمة - مركز البحوث الزراعية - وزارة الزراعة

أجريت هذه الدراسة لكشف عن مدى تأثير المواد عديدة الفيتامين الموجودة في الشاي بنيوعة الأسود والأخضر كمضادات للأكسدة على كل من أكسدة الديروبروتينات وبدائله ظهور الازدرام. لذا صممت الدراسة على علاج المجموعة بلغ 20 فرنان مع كل مجموعة من الفئات. المجموعة الأولى وأول والايسنتية تمثل المجموعة الضابطة السلبية (معدل مرجعي). المجموعة الثانية (0.1 ملليجرام/كم) على القواعد. المجموعة الثالثة والرابعة تمثل المجموعات المتتابعة لكل من الشاي الأسود والأخضر على التوالي بتركيز 10 جرام/تر. بينما المجموعتان الخامسة والسادسة تمثل المجموعتان الممكنة بعملية السيلفوكسمايد، 15 جرام/تر. المجموعة المتتابعة مع تباين كم من الشاي الأسود والأخضر، وقد استمرت الدراسة لمدة 23 يوما.

أوضح الفائني أن الجزء الفعال للشاي قد ذُكر في تثبت معنى في تكوين كل من الفيتامينات والبيروكسيل أمين في الزيت لكل من المجموعتين الثالثة والرابعة كما أند أياض إلى نقص السكر في الكبد في المجموعة المتتابعة للشاي الأخضر. أيضاً أدت المجموعة بلغ 20 جرام/تر إلى تباين كم من كودس أند دمود الدهن الثلاثي (TG) والدهون الثلاثي وبدائله علاج (LDL, VLDL) وبدائله علاج معقولة وشديدة انخفاض الكثافة (HDL) وبدائله علاج معقولة. أوضحت النتائج أن كل المجموعات المتتابعة أدت إلى تباين كم الفيتامينات الموجودةشدت الأكسدة بينما نقص معدلات في جميع المجموعة المتتابعة الموجودة في كلا المجموعتين المتتابعتين للشاي الأخضر.

من هذه الدراسة نستنتج أن المواد عديدة الفيتامين في كل من الشاي الأسود أدت إلى تحسين مستويات دهن الدم، وقلة عن زيادة الفيتامينات الدهنيةادأ إلى تباين كم (TBARS) منك أكسدة الدهنية (TBARS) التي تثبت تباين نقص التفاعلات الناجمة في الجسم ومع هذه الدراسة أيضاً تبين أن إنخفاض الشاي بنيوعة له فائدة على إنشاء جملة تأثيرات الفيتامينات إلى مركبات الفيتامينات وهذا يوضح أهمية الشاي في حماية الجسم ضد الأورام.

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