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Immobilization stress-induced oxidative damage and its amelioration with green and black teas

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Stress is a state of vulnerable cellular homeostasis, which results in free radical generations and subsequent oxidative damage. The aim of this study is to evaluate the effect of green and black teas on immobilization stress-induced oxidative damage in male Wistar albino rats. Six weeks of repeated immobilization for 4 h daily for five consecutive working days per week was applied. Green or black teas were administrated continuously for six weeks in drinking bottles. Enzymatic activities and lipid profile were measured in serum. Liver enzymes and glucose levels were significantly increased by the green tea showed hypoglycemic and antilipidemic effect against stress induced changes. In liver and brain tissues glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), nucleic acids and total protein levels were estimated. Repeated immobilization stress significantly enhanced the lipid peroxidation in both the tissues. The present results revealed that, the green tea have more protective effect than black tea against stress particularly in brain as compared to liver. It concludes that the antioxidative affect of green tea over the immobilization-induced stress may attribute the rich flavonoids presence in green tea than black tea.

Key words: Restraint stress, glutathione, oxidative stress, malondialdehyde, superoxide dismutase.

INTRODUCTION

Medicinal plants are considered as abundant source of natural and biologically active compounds. The basis for development of numerous nutraceuticals and pharmaceuticals is heavily relay on many of these compounds. Tea is considered as one of the most popular beverage worldwide. It has received considerable attention as a medicinal herb because of its possible health effects (Fujimura et al., 2011). Around three billion kilograms of tea are produced and consumed annually. Drinking tea is highly linked to alleviating possible risk factors for cardiovascular diseases, cancer and stress (Khan and Mukhtar, 2007). Animal and cell culture models have indicated a potentially beneficial effect of tea on hepatic and brain tissues, gene transcription and cell proliferation (Khan and Mukhtar, 2007). According to the fermentation process, tea is classified into three main types; green (unfermented), oolong (semi-fermented), and black (fermented) (Fujimura et al., 2011). Black tea is the most consumed type of tea. Around 78% of the world population uses black tea while 20% uses green tea. Currently, extensive scientific investigations were made to study the potential benefits of consumption of tea or tea constituents in the prevention or reduction of chronic illnesses, such as heart disease, stroke and some type of cancers (McKay and Blumberg, 2002).

The potential antioxidant and free radical scavenging properties using a variety of disease models were reported as important contributors in teas beneficial effects (Mukhtar and Ahmad, 2000, Higdon and Frei, 2003). Therefore, these epidemiological studies provided a strong circumstantial evidence to further evaluate the...
potential health benefits of green and black tea. The beneficial biological activities and functions of tea on human and animal models are defined by their specific compositions (Fujimura et al., 2011). Green tea and black tea come from the leaves of the plant Camellia sinensis, which is a member of the family Theaceae and their chemical components vary according to species/cultivar, environment, growth, storage conditions, and leaf quality. Both green and black teas contain flavonoids. However, green tea contains more of the simple flavonoids called catechins, while flavonoids in black tea are converted to the more complex varieties called theaflavins and thearubigins. The chief catechins found in tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechingallate (EGCG), which is the most abundant catechin in tea (Mukhtar and Ahmad, 2000; Frei and Higdon, 2003).

These active constituents are known to have an anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities (Graham, 1992; Yang et al., 1998; Middleton et al., 2000; Weisburger and Chung, 2002; Tedeschi et al., 2004). The potential beneficial effects are through the antioxidant properties of tea catechins and polyphenols via directly scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions (Scott et al., 1993; Haenen et al., 1997; Guo et al., 1999; Paquay et al., 2000; Nakagawa and Yokozawa, 2002; Frei and Higdon, 2003). Animal immobilization or restraint is known to be an applicable, easy and convenient method to induce both psychological and physical stress. Immobilization or restraint can induce psychological escape reaction and physical muscle work, which results in restricted mobility and aggression (Romanova et al., 1994). Numerous studies have examined the effect of immobilization-induced stress on the antioxidant system and induction of lipid peroxidation (Madrigal et al., 2001; Zaidi et al., 2003; Sahin and Gumsulu; 2004). It was shown that chronic psychological stress is one of the major non-genomic factors contributing to several pathological states such as psychiatric disorders, neurological impairments, and immunosuppressant.

In addition, oxidative stress has been mainly implicated in hepatic and neuropathologic disorders. Stress can induce acute and lethal injury due to free radical attacks in both hepatic and brain tissues (Middleton et al., 2000). This effect is clearly harmful particularly on brain since it contains large amounts of polyunsaturated fatty acids (Olanow, 1993; Gutteridge, 1995; Reiter, 1995; Muller et al., 1996; Cui et al., 2004). This implication has led to the notion that both liver and brain tissues are vulnerable to oxidative damage and that the antioxidant defense mechanisms, particularly in the brain, may not be sufficient enough to prevent these harmful effects. Therefore, dietary intake or daily consumption of a variety of antioxidants might be beneficial for preserving the normal functions of these tissues. The effects of tea and/or tea constituents on stress have been investigated in several tissues, plasma and erythrocytes (Tijburg et al., 1997; Langley-Evans, 2000; Skrzydlewksa et al., 2002). However, there are no detailed studies, to the best of our knowledge, investigating the effects of green and black tea on the antioxidant biomarkers following immobilization stress. Therefore, the aim of the present study was to investigate the effects of drinking green or black tea on some of the antioxidant biomarkers in plasma, liver and brain after immobilization induced stress using male Wistar albino rats as an animal model.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (total animals = 36 rats) were received by the Experimental Animal Care Center (King Saud University, Riyadh, Saudi Arabia). Animals were roughly the same age; weighing 180 to 200 g. Animals were maintained under controlled conditions of temperature (22±1ºC), humidity (50 to 55%), and light (12 h light/dark cycles). Rats were provided with Purina chow (Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia) and water ad libitum. All methods including euthanasia procedure were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Institute of Health (NIH Publications No. 80 to 23; 1996) and approved by the Ethical Guidelines of the Experimental Animal Care Center (College of Pharmacy, King Saud University, Riyadh, Saudi Arabia).

Preparation of green and black teas

Green tea (GT) and black tea (BT) were purchased from local market (packed by Lipton®, Unilever Brand). The voucher specimen of both the teas was kept in the herbarium at College of Pharmacy, King Saud University, Riyadh, Saudi Arabiass record. Tea decoctions were prepared by adding 10 g of either tea into one liter of boiled drinking water (1% w/v) and allowed to simmer for few minutes. The preparation was kept to cool down to room temperature, filtered and then poured into animal’s feeding bottles. Both teas were freshly prepared every morning at the same time (Al-Rejaie, 2009; Mohamadin et al., 2005).

Experimental procedure

Animals were randomly divided into six groups by taking six rats in each: (i) Control (tap water), (ii) green tea, (iii) black tea, (iv) Stress (tap water), (v) green tea + stress and (vi) black tea + stress. The model for immobilization/restraint stress used in the current investigation was applied from previous reports (Nadeem et al., 2006; Zaidi et al., 2003; Zaidi and Banu, 2004) with slight modifications. Placing animals in the exact size tube was reported to be a good restraint procedure since it involves minimum pain with minimum movements including that of the tail (Pare and Glavin, 1986). Therefore, immobilization stress was induced by placing each animal in a plastic/well-ventilated tube of the same size. Immobilization stress exposure was carried out for 4 h per day for five consecutive days continued for 6 weeks. During stress procedure, animals were deprived of food and water (Liu et al., 1996). Weekly body weight of each animal was recorded. Animals were euthanized immediately after the last stress session by the
end of the treatment period. Blood samples were withdrawn through cardiac puncture into heparin coated centrifuge tubes. Samples were centrifuged; plasma was separated and kept at -20°C till analysis. Brain and liver tissues were excised after washing them with chilled normal saline, dipped in liquid nitrogen for 1 min and preserved at -70°C till analysis.

Biochemical assays in plasma

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, total cholesterol (TC) and triglycerides (TG) levels were estimated in plasma by using commercially available diagnostic kits (Randox diagnostic reagents, Randox Laboratories, USA).

Estimation of nucleic acids and total proteins in tissues

Total proteins were estimated by the modified Lowry method of Schacterle and Pollack (1973). Bovine plasma albumin was used as standard. The method described by Bregman (1983) was used to determine the levels of nucleic acids deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Liver and brain tissues (200 mg) were homogenized in ice-cold distilled water and the homogenates were suspended in 10% ice-cold trichloroacetic acid (TCA). Pellets were extracted with 95% ethanol twice. DNA levels were determined by treating the nucleic acid extract with diphenylamine reagent and measuring the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol reagent and the green color was recorded at 660 nm on spectrophotometer (LKB-Pharmacia, Mark II, Ireland).

Estimation of GSH in tissues

The concentration of GSH was measured using the method described by Sedlak and Lindsay (1968). Briefly, cross sections of liver and brain tissues (200 mg) were homogenized in ice-cold 0.02 M ethylene diamine tetraacetic acid (EDTA). An aliquot of 0.5 ml of tissue homogenates was mixed with 0.2 M Tris buffer, pH 8.2 and 0.1 ml of 0.01 M Ellman's reagent, [5,5'-dithiobis-(2-nitro-benzoic acid)] (DTNB). Each sample tube was centrifuged at 3000 g at room temperature for 15 min. The absorbance of the clear supernatants was measured using spectrophotometer at 412 nm in one centimeter quarts cells.

Estimation of MDA in tissues

The method described by Ohkawa et al. (1979) was used for MDA analysis. Briefly, tissues (200 mg) were homogenized in aqueous 0.15 M KCl solutions and 1 ml of homogenate was mixed with 1 ml of 10% TCA and centrifuged at 3000 rpm for 15 min. One milliliter supernatant was mixed with 1 ml of 0.67% 2-thiobarbituric acid then placed the tubes at boiling water bath for 15 min. Optical density of the clear pink supernatants was measured at 532 nm.

Estimation of SOD activity in tissues

Activity of SOD in liver and brain tissues was assayed using the method described by Kakkar et al. (1984) with the aid of nitroblue tetrazolium as the indicator. Liver or brain tissues (200 mg) were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents: sodium pyrophosphate buffer 1.2 ml (0.052 M) pH 8.3, 0.1 mephazinemethosulfate (186 µM), 0.3 ml nitro blue tetrazolium (300 µM) and 0.2 ml NADH (780 µM) were added to 0.1 ml of processed tissue sample. The sample mixture was incubated for 90 min at 30°C. 4 ml of n-butanol and 1 ml of acetic acid were then added to each sample and the mixture was shaken vigorously. Samples were centrifuged at 4000 rpm for 10 min and the organic layer was withdrawn and absorbance was measured at 560 nm using a spectrophotometer (LKB-Pharmacia, Mark II, Ireland).

Histopathological evaluations

Cross-sections of liver sample from each group were examined for histopathological changes. Brain was excluded for its histopathological screening because of improper dissection. The liver tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 3 µm, stained with Hematoxylin and Eosin stain and placed in slides for light microscopic examination. To avoid any type of bias, the slides were coded and examined by a histopathologist who was blinded to the treatment groups.

Statistical analysis

The data were expressed as mean±Standard Deviation (SD). The data were statistically evaluated by one-way ANOVA using Graph Pad in Statssoftware (version 3.06) and the differences between the means were recorded using Student Newman-Keuls. The differences were considered statistically significant at P<0.05.

RESULTS

Six-weeks of immobilization stress, significantly (P<0.01) decreased the body weight of rats. Green tea supplementation to stressed rats significantly (P<0.05) increased the body weights compared to untreated stressed group while the black tea also enhanced body weight but the values are not statistically significant (Figure 1). Enzymatic activity was significantly increased in immobilized rats as seen in AST and ALT levels while compared to control group. Teas supplementations reduced the enzymatic activity but not statistically significant compared to untreated stressed group. Immobilization stress caused significant increase in fasting glucose levels. Green tea showed significant hypoglycemic effect. Lipid levels (TC and TG) increased by the immobilization stress. Treatment with green and black teas to stressed rats significantly decreased the TG levels as compared to untreated stressed group (Table 1). Levels of DNA and total proteins in liver and brain tissues were not significantly altered by the immobilization stress. On the other hand, RNA levels significantly decreased in liver (P<0.01) and brain (P<0.001) tissues, respectively (Table 2).

GSH concentrations were significantly decreased in liver (P<0.001) and brain (P<0.05) of stressed group of rats. Green tea supplementation to stressed rats significantly enhanced the GSH levels compared to stress vehicle group. Immobilization stress significantly (P<0.01) increased MDA levels in hepatic cells, similar increase was also seen in brain tissue but values were not statistically significant, while compared to the control.
Figure 1. Effect GT and BT teas (1% w/v) for 6 weeks on body weight gain of normal and restraint male Wistar albino rats. Values (Mean±S.D.) were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. aControl group compared with GT, BT and Stress. bStress group compared with GT+Stress and BT+Stress black tea stress. *P<0.05, **P<0.01 and ***P<0.001. Six rats were used in each group.

Table 1. Effect of drinking teas on plasma biochemical parameters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>Glucose (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.2±9.6</td>
<td>27.5±8.7</td>
<td>173.7±27.9</td>
<td>57.3±8.2</td>
<td>141.2±55.9</td>
</tr>
<tr>
<td>GT</td>
<td>58.7±5.8</td>
<td>32.9±3.9</td>
<td>159.3±9.1</td>
<td>54.9±8.8</td>
<td>178.6±32.6</td>
</tr>
<tr>
<td>BT</td>
<td>63.2±14.9</td>
<td>39.4±4.9</td>
<td>168.5±23.4</td>
<td>60.5±9.8</td>
<td>172.3±75.9</td>
</tr>
<tr>
<td>Stress</td>
<td>67.0±7.4*</td>
<td>47.7±9.5*</td>
<td>212.4±23.4</td>
<td>70.3±6.6</td>
<td>163.0±24.1</td>
</tr>
<tr>
<td>GT+stress</td>
<td>66.5±10.1</td>
<td>45.5±7.9</td>
<td>174.2±11.6</td>
<td>68.9±14.2</td>
<td>135.4±5.6b</td>
</tr>
<tr>
<td>BT+stress</td>
<td>58.0±8.4</td>
<td>42.7±10.2</td>
<td>199.8±24.2</td>
<td>71.7±6.1</td>
<td>111.9±22.4**</td>
</tr>
</tbody>
</table>

Values (Mean±S.D.) were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. aControl group compared with GT, BT and Stress. bStress group compared with GT+stress and BT+stress black tea stress. *P<0.05, **P<0.01 and ***P<0.001. Six rats were used in each group.

Table 2. Effect of drinking teas on nucleic acids and total protein concentrations in liver and brain tissues.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA (µg/100 mg wet tissue)</th>
<th>RNA (µg/100 mg wet tissue)</th>
<th>Total protein (mg/100 mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Brain</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>166.8±17.6</td>
<td>115.1±23.9</td>
<td>607.4±21.7</td>
</tr>
<tr>
<td>GT</td>
<td>165.3±16.6</td>
<td>110.4±15.1</td>
<td>566.9±28.9</td>
</tr>
<tr>
<td>BT</td>
<td>170.0±10.6</td>
<td>109.9±15.6</td>
<td>572.7±39.7</td>
</tr>
<tr>
<td>Stress</td>
<td>148.3±15.1</td>
<td>101.7±17.9</td>
<td>527.5±49.9**a</td>
</tr>
<tr>
<td>GT+stress</td>
<td>154.6±11.2</td>
<td>106.1±16.1</td>
<td>621.4±26.1**b</td>
</tr>
<tr>
<td>BT+stress</td>
<td>154.2±9.1</td>
<td>103.6±15.0</td>
<td>588.3±57.2</td>
</tr>
</tbody>
</table>

Values (Mean±S.D.) were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. aControl group compared with GT, BT and Stress. bStress group compared with GT+Stress and BT+stress black tea stress. *P<0.05, **P<0.01 and ***P<0.001. Six rats were used in each group.
In stressed animals, SOD activity decreased significantly (P<0.05) in hepatic tissue where this finding was more significant (P<0.001) in brain tissue compared to controls. Green tea supplementation brings back the SOD activity to normal in both normal and restraint rats treated with green or black teas. Mild congestion in hepatocytes was observed in vehicle-treated animals exposed to immobilization stress for 6 weeks. Normal appearance of hepatocytes was seen in animals treated with green tea and exposed to immobilization stress while black tea treatment to stressed rats showed mild inflammation (Figure 2).

### DISCUSSION

Results in the present study, demonstrated that, drinking tea either green or black protects against oxidative affects induced by immobilization stress in rats. The present results showed that the effect of green tea on restraint animals was more prominent than black tea and also showed its effect on brain is better than liver. In vitro and in vivo studies were indicated that tea intake is potentially beneficial on hepatic and brain tissues, gene transcription, cell proliferation and other molecular functions (Khan and Mukhtar, 2007). Although these potential health benefits of green and black teas are partially attributed to their antioxidative properties particularly with the presence of rich polyphenols in green tea (Mukhtar and Ahmad, 2000). Immobilization stress was reported to be a good model for investigating the alterations occurring in oxidant–antioxidant balances in animal tissues specific the vulnerable organs are brain and liver (Singh et al., 1999; Romanova et al., 1994; Sahin and Gumuslu, 2007; Gumuslu et al., 2002). In present study, body growth of rats was significantly affected following the immobilization stress.

Green tea supplementation to the stressed rats significantly increased the body weights as compared to untreated stressed animals. It was reported that food intake in rats exposed to repeated immobilization stress was transiently decreased after the stress termination and was suggested to increase sympathetic activity by suppressing the levels of circulating growth hormones (Dronjak et al., 2004; Harris et al., 2004; Yoshihara andYawaka, 2008). However, it is well established that food intake would be suppressed following any stress exposure (Bhatnagar et al., 2006, Dallman et al., 2004) and that stress-induced increase in the sympathetic activity decreases feeding and drinking (Taylor and Samson, 2005). These changes related to nutrition induced by repeated immobilization stress may have ultimately affected the decrease of body weight by immobilization stress in the current study. Considerable variations in plasma biomarkers by either green or black tea were not observed in our study following 6 weeks of immobilized stress. These results are not surprising since other reports have shown that tea or tea polyphenol consumption were not as effective on plasma antioxidant parameter in some animal studies (Frei and Higdon, 2003; Higdon and Frei, 2003).

**Table 3. Effect of drinking teas on GSH, MDA levels and SOD activity in liver and brain tissues.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (µg/g wet tissue)</th>
<th>MDA (nmol/g wet tissue)</th>
<th>SOD (U/g of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Brain</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>262.0±20.1</td>
<td>160.7±14.6</td>
<td>256.4±23.2</td>
</tr>
<tr>
<td>GT</td>
<td>263.6±17.1</td>
<td>153.0±20.5</td>
<td>252.8±29.1</td>
</tr>
<tr>
<td>BT</td>
<td>266.3±21.4</td>
<td>161.2±21.5</td>
<td>258.6±20.8</td>
</tr>
<tr>
<td>Stress</td>
<td>197.5±15.3 ***a</td>
<td>128.4±9.0 *a</td>
<td>311.2±28.9 *a</td>
</tr>
<tr>
<td>GT+stress</td>
<td>207.8±23.4</td>
<td>152.7±15.7*ab</td>
<td>301.0±24.6</td>
</tr>
<tr>
<td>BT+stress</td>
<td>202.7±24.1</td>
<td>138.1±12.3</td>
<td>300.0±30.2</td>
</tr>
</tbody>
</table>

Values (Mean±S.D.) were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. *Control group compared with GT, BT and Stress. †Stress group compared with GT+Stress and BT+stress black tea stress. *P<0.05, **P<0.01 and ***P<0.001. Six rats were used in each group.
Figure 2. A. In control rat, showing normal liver portal triad and hepatocytes; B. In untreated stressed rat, showing mild congestion in hepatocytes; C. Green tea supplemented stressed rat, showing normal hepatocytes and; D. In untreated stressed rat, showing mild congestion in hepatocytes; C. Black tea supplemented stressed rat, showing normal hepatocytes.

2007). This inactivation of liver antioxidant enzymes may ultimately increased lipid peroxidation (Sahin and Gumuslu, 2007).

Finally, stress may have played a potential role in aggravating liver diseases like hepatic inflammation via generation of reactive oxygen species (ROS) (Zaidi et al., 2005). Our data have shown that stressed groups subjected to either green or black tea attenuated the decrease in hepatic RNA as well as the increase in MDA concentrations compared to untreated stressed animals. Moreover, results from histopathological assessment confirmed the protective effects of both treatments, especially green tea, on hepatic tissue. Immobilization stress for 6 weeks caused a mild congestion in hepatocytes. An effect that was prevented totally by green tea and partially by black tea supplementations for immobilized stressed animals. In brain tissues, animals subjected to 6 weeks of repeated immobilization stress for 4 h for five consecutive days showed a significant reduction in brain GSH and RNA levels as well as SOD activity. Green tea supplementation to stressed rats showed significant protection against the stress-induced reduction in brain of these parameters, whereas black tea treatment failed to increase these values in brain. Therefore, the possible protection against the restraint stress effect on brain was found higher in green tea compared to black tea.

Both green and black teas come from the same plant. However, both have different active constituents based on the method of fermentation. Green tea is withered and then steamed to avoid in the inactivation of polyphenol oxidase. Green tea, thus, contains relatively high concentrations of catechins. When polyphenol oxidase come in contact with catechins, they will be oxidized and form a flavanol dimmers, known as theaflavins, and polymers, known as thearubigins (Balentine, 2000, Frei and Higdon, 2003). In contrast to green tea, black tea is tea leaves rolled and allowed to oxidize (ferment) and
thus forming a relatively high concentrations of theaflavins and thearubigins and relatively low concentrations of catechins (Zhao et al., 2011). Therefore, the antioxidative effect of green tea in the current investigation was possibly attributed to the presence of relatively higher concentrations of catechins or other polyphenols than in black tea. Another possibility of the minor antioxidant effects of black tea in brain tissues in stressed animals is its low blood brain barrier penetration.

Green tea and tea catechins, however, were reported to penetrates the blood brain barrier, and achieve effective concentration in the central nervous tissue (Skrzyplewksa et al., 2002; Khan and Mukhtar, 2007) and that green tea constituents may possess inhibitory effects against lipid peroxidation in synaptosomes and neurodegeneration induced by peroxyl radicals. Therefore, the observed protection in stressed animals may, in part, be due to the easy penetration of green tea or its constituents through the blood brain barrier. Indeed, a decrease in the brain GSH was observed in rats following stress (Liu et al., 1996) and immobilization stress (Madrigal et al., 2001). Brain tissues are thought to be the most vulnerable to oxidative damage because of the need for high oxygen consumption and the presence of high levels of poly unsaturated fatsy acids, which may ultimately lead to various neurodegenerative disorders (Gutteridge, 1995; Reiter, 1995; Muller et al., 1996; Cui et al., 2004).

The oxidative damage may be trigger by ROS, which may enhances the initial attack on lipid rich membranes of the brain neurons to cause lipid peroxidation (Das and Khanna, 1997). Depletion of one of the guardian factors against oxidative stress, brain GSH, and the accumulation of ROS may also enhance lipid peroxidation (Levine, 1982). Taken together, stress was reported to reduce GSH levels and increases levels of ROS which may explain the vulnerability of brain by immobilization stress and the possible protection by green tea.

In conclusion, the green tea consumption showed significantly higher level of protection against the stress-induced changes than the black tea. This may be because of rich content of polyphenols in green tea compared to the oxidized tea (black tea). Further investigations are required to evaluate their potentials against neurotoxicity and hepatotoxicity that induces by physical and metal stressors.

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