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Effect of Green and Black Teas on Immobilization Induced Stress in Male Wistar Albino Rats

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Abstract: The present study was undertaken to investigate the potential of green and black teas to modulate restraint stress-induced oxidative changes in male Wistar albino rats. Repeated immobilization for 4 h daily for five consecutive days per week (for 2 and 4 weeks) was used as a test model. Repeated immobilization stress significantly decreased glutathione (GSH), RNA and total protein levels, while malondialdehyde (MDA) levels were elevated in brain and liver tissues. Daily drinking of green or black tea only attenuated the RNA decrease and the MDA increase in stressed groups in liver tissues. Green tea group attenuated the decrease in GSH and RNA and the increase in MDA induced by immobilization stress in brain tissues. However, black tea only attenuated the increase in brain MDA in stressed animals. The effect of green tea on restraint stress was higher in brain than liver. In conclusion, the present results revealed that the antioxidative effect of green tea during immobilization stress was higher, possibly attributed to the presence of relatively higher concentrations of flavonoids than in black tea.

Key words: Black tea, green tea, immobilization stress, GSH, MDA, nucleic acids

INTRODUCTION

Tea overall is one of the most consumed beverages worldwide. Epidemiological studies have reported that around three billion kilograms of tea are produced and consumed annually. Additionally, drinking tea is highly linked to protecting risk factors for cardiovascular diseases, cancer and stress (Khan and Mukhtar, 2007). In animal and cell culture models, studies have indicated a potentially beneficial effect of tea on hepatic and brain tissues, gene transcription and cell proliferation (Khan and Mukhtar, 2007). Tea is generally consumed in different ways as green, black or Oolong tea. About 78% of the populations use black tea, mostly consumed in the Western countries, while 20% uses green tea, commonly consumed in Asian countries. The consumption of tea or tea constituents for the potential prevention or reduction of chronic illnesses is the current subject for extensive scientific investigations (McKay and Blumberg, 2002). Using a variety of disease models, the potential antioxidant and free radical scavenging properties are widely reported as important contributors in teas beneficial effects (Mukhtar and Ahmad, 2000; Higdon and Frei, 2003). These epidemiological evaluations, therefore, provide strong circumstantial evidence of the potential health benefits from green and black teas.

The tea plant, *Cammelia sinensis* (L.), is a member of the family Theaceae (Harold and Graham, 1992). Fresh tea leaves are rich in catechins and flavonoids and well known to have an anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities (Harold and Graham, 1992; Yang *et al*., 1998; Middleton *et al*., 2000; Weisburger and Chung, 2002; Tedeschi *et al*., 2004). It has been demonstrated that tea catechins and polyphenols act as an effective antioxidants *in vitro* by directly scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions (Paquay *et al*., 2000; Nakagawa and Yokozawa, 2002; Frei and Higdon, 2003). In parallel, they may indirectly function as antioxidants through: (i) inhibiting the redox-sensitive transcription factors, nuclear factor-κB and activator protein-1; (ii) inhibiting the pro-oxidant enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase and (iii) induction of phase II as well as antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases (Frei and Higdon, 2003).

Stress is usually recognized as a state of altered physiological homeostasis (Şahin and Gümüşlü, 2007). The ability to cope with such stressful stimuli is a crucial determinant of health and disease. Chronic psychological stress is one of the major non-genomic factors that contribute to several pathological states such as psychiatric disorders, neurological impairments and immunosuppressant. For instance, liver organs are subject

to acute and potentially lethal injury and the antioxidant properties of flavonoids could have an impact on the hepatoprotective effects against free radical attacks (Middleton *et al*., 2000). In addition, the brain is particularly vulnerable to free radical attacks since brain tissue contains large amounts of polyunsaturated fatty acids (Olanow, 1993; Gutteridge, 1995; Reiter, 1995; Cui *et al*., 2004). Although the major factors involved in stress-related disorders remain to be specified, oxidative stress has been mainly implicated in hepatic and neuropathologic disorders. This implication has led to the notion that antioxidant defense mechanisms, particularly in the brain, are not sufficient enough to prevent the increase in oxidative damage and that dietary intake of a variety of antioxidants might be beneficial for preserving brain functions. Immobilization/restraint stress is an easy and convenient method to induce both psychological, an escape reaction and physical stress, a muscle work, which leads to restricted mobility and aggression (Ramanova *et al*., 1994; Singh *et al*., 1993). Thus, several studies examined the effects of stress on the antioxidant system and induction of lipid peroxidation using immobilization-induced stress as a test model (Madrigal *et al*., 2001; Kashif *et al*., 2004; Şahin and Gümüşlü, 2004). Using this model, the present study was designed to investigate the effects of drinking excess green or black teas on some of the biochemical changes in plasma, liver and brain using male Wistar albino rats as an animal model.

MATERIALS AND METHODS

The present study was designed and conducted in the Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia during 2008.

Animal: Seventy two male Wistar albino rats, roughly the same age and weighing 180-200 g were received from the Experimental Animal Care Center (King Saud University, Riyadh, Saudi Arabia). Animals were maintained under controlled conditions of temperature (22±1°C), humidity (50-55%) and light (12 h light/dark cycles) and were provided with Purina chow (Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia) and water *ad libitum* (unless otherwise indicted during the experiment). All procedures including euthanasia procedure were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications No. 80-23; 1996) and 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 and black teas were purchased locally (packed by Lipton®, Unilever Brand). The voucher and specimen of the green and black teas were kept in the herbarium at College of Pharmacy (King Saud University, Riyadh, Saudi Arabia) for the record. The tea was prepared by adding 10 g of either tea into 1 L of boiled drinking water (1% w/v). The preparation was allowed to simmer for few minutes and cool to room temperature and then was poured into animal's feeding bottles. Animals were supplied with freshly prepared tea every morning at the same time (Mohamadin *et al*., 2005).

Experimental procedure: The current project was designed to complete in two sets of experiments (2 and 4 weeks of treatments). For each set of experiment, animals were randomly divided into six groups by taking six rats for each group as follows: (1) control (vehicle; tap water) (2) green tea (3) black tea (4) vehicle (tap water) + immobilization stress (5) green tea + immobilization stress and (6) black tea + immobilization stress. The immobilization stress method used in the present study was modified from earlier reports (Nadeem *et al*., 2006; Zaidi *et al*., 2003). In a review by Pare and Glavin (1986), it was concluded that placing animals in their exact size tube was a good restraint procedure since it involves minimum pain with minimum movement including that of the tail.

Immobilization stress was accomplished by placing individual animals in plastic/well-ventilated tubes of their size. Animals in stress groups were exposed to immobilization stress procedure for 4 h daily for five consecutive days per week. Daily fresh tea solutions were continued even during the unstressed days. The rats were deprived of food and water during stress exposure (Liu *et al*., 1996). Body weights were recorded weekly for the entire study. At the end of each treatment period, animals were sacrificed immediately after the last stress session and blood samples were collected through cardiac puncture in heparin coated centrifuge tubes. Plasma was separated and kept in freezer at 20°C till analysis. Immediately brain and liver tissues were excised, washed with chilled normal saline, dipped in liquid nitrogen for one minute then preserved at -70°C till analysis.

Biochemical assays in plasma: The activity of aspartate transminase (AST), alanine transminase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) and the levels of glucose, albumin, total cholesterol and triglycerides were estimated in plasma by using commercially available diagnostic kits (Randox diagnostic reagents, Randox Laboratories, USA).

Estimation of GSH in tissues: Glutathione (GSH) in tissues was assayed according to the method described by Sedlak and Lindsay (1968). A cross section of liver or brain tissues (200 mg) were dissected and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). The aliquots of 0.5 mL of tissue homogenates were 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). Sample tubes were centrifuged at 3000 g at room temperature for 15 min. The absorbance of the clear supernatants was recorded 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 to estimate malondialdehyde (MDA) in tissues. In brief, liver or brain tissues (200 mg) were homogenized in aqueous 0.15 M KCl solution to give 10% homogenate. One milliliter of homogenate was mixed with 1 mL of ice-cold 10% trichloroacetic acid (TCA) and centrifuged at 3,000 rpm for 15 min. Then, 1 mL supernatant was suspended into 1 mL of 0.67% 2-thiobarbutaric acid. Samples were then placed in a boiling water bath for 15 min. Samples were allowed to cool down at room temperature and then centrifuged at 3000 rpm for 15 min. Optical density of the clear pink supernatants was measured at 532 nm.

Estimation of nucleic acids and total proteins in tissues: Total proteins in liver and brain tissues were estimated by using a 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 (DNA and RNA). Liver or brain tissues were homogenized in 4 mL ice-cold distilled water and 2 mL homogenate were suspended in 5 mL of 10% ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted twice with 95% ethanol. Finally the nucleic acids were extracted in 5% TCA. DNA was 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 read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Estimation of SOD activity in tissues: Superoxide dismutases (SOD) activity in liver and brain tissues was assayed spectrophotometerically (560 nm) using the method described by Kakkar *et al.* (1984). Briefly, the 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 mL phenazine methosulphate (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 mixture was then incubated for 90 min at 30°C. Four milliliter of n-butanol and 1 mL of acetic acid were then added. The mixture was shaken vigorously. Following centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 560 nm using a spectrophotometer (LKB-Pharmacia, Mark II, Ireland).

Statistical analysis: All data were presented as the Mean±Standard Deviation (SD). The data were evaluated by a one-way ANOVA using GraphPad InStat program (version 3.06) and the differences between means were assessed using Student Newman-Keuls. The differences were considered statistically significant at $p<0.05$.

RESULTS

Effect on body weight: Body weights were significantly decreased in restraint animals following 2 and 4 weeks ($p<0.001$; Table 1) compared to their respective unstressed groups. Green and black teas supplementation for 2 and 4 weeks to restraint rats did not alter the body weight significantly when compared to stress untreated group. However, weight of green tea treated stressed rats were significantly less decreased than the other 2 weeks stressed groups ($p<0.01$). Similar significant ($p<0.001$) effect was seen in green tea stressed group after 4 weeks.

Effect on plasma biomarkers: Immobilization stress up to 4 weeks did not alter plasma AST levels either in treated or untreated animals as compared to their respective unstressed rats (Table 2). Treatments with vehicle or green tea to animals exposed to immobilized stress for 4 weeks showed significant increase in plasma ALT ($p<0.05$) levels when compared to their respective

Table 1: Effect of green and black teas (1% w/v) on body weight increase (g) in normal and restraint rats

	Body weight increase (g)	
Treatments	After 2 weeks	After 4 weeks
Control (tap water)	43.3±8.4	73.00±13.4
Green tea	48.5±9.4	82.30±13.9
Black tea	32.7±5.9	75.70±15.5
Stress +Tap water	20.3±4.5^{a}***	41.20±11.9^{a}***
Stress + Green tea	22.8±4.6^{b}**	48.50±7.2^{b}***
Stress + Black tea	18.7±7.9^{c}***	50.50±12.4^{c}***

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *$p<0.05$, **$p<0.01$ and ***$p<0.001$. ^{a}Control vs control stress; ^{b}Green tea vs green tea stress; ^{c}Black tea vs black tea stress. Six rats were used in each group. Data are presented as Mean±SD

Table 2: Effect of green and black teas (1% w/v) on plasma AST and ALT concentrations in normal and restraint rats

	AST (U L^{-1})		ALT (U L^{-1})	
Treatments	2 weeks	4 weeks	2 weeks	4 weeks
Control (tap water)	45.1±7.0	43.7±5.8	26.1±5.9	28.7±4.7
Green tea	47.9±5.4	44.3±5.9	28.3±3.6	28.6±5.8
Black tea	49.2±6.8	48.1±8.6	30.8±8.4	31.6±8.6
Stress + Tap water	57.7±6.4	54.3±6.7	33.2±5.5	45.1±6.9[a]*
Stress + Green tea	56.4±8.7	47.5±9.5	37.5±10.5	45.5±7.9[b]*
Stress + Black tea	55.6±9.7	48.4±10.5	31.4±13.3	42.7±10.2

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *p<0.05. [a]Control vs control stress; [b]Green tea vs green tea stress; [c]Black tea vs black tea stress. Six rats were used in each group. Data are presented as Mean±SD

Table 3: Effect of green and black teas (1% w/v) on plasma ACP and ALP concentrations in normal and restraint rats

	ACP (U L^{-1})		ALP (U L^{-1})	
Treatments	2 weeks	4 weeks	2 weeks	4 weeks
Control (tap water)	123.3±8.9	112.8±12.1	336.7±67.1	308.6±31.5
Green tea	122.7±19.9	107.9±13.3	339.6±36.8	321.6±37.9
Black tea	128.9±9.5	115.5±11.7	346.9±59.6	315.5±47.8
Stress + Tap water	132.9±15.4	121.1±11.6	358.1±39.6	356.9±46.8
Stress + Green tea	125.4±12.7	116.9±14.8	367.9±36.2	348.6±22.9
Stress + Black tea	128.7±9.6	118.9±10.4	376.5±35.2	338.9±29.6

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group. Data are presented as Mean±SD

unstressed rats (Table 2). The enzymatic activities of ACP and ALP in plasma were not altered significantly in all restraint rats as compared to their respective controls (Table 3). Plasma glucose levels were significantly ($p<0.05$) elevated after 4 weeks immobilization stress sessions in vehicle treated rats as compared to controls (Table 4). However, albumin and the plasma lipids (total cholesterol and triglycerides) were not altered by the restrain stress up to 4 weeks (Table 4, 5).

Effects on nucleic acids and total proteins: DNA and total protein concentrations in liver and brain tissues were not significantly altered either by the immobilization stress or tea supplementation (Table 6, 8). However, hepatic RNA levels were significantly decreased in stressed animals after 2 and 4 weeks ($p<0.05$, $p<0.01$, respectively). Treatments with green or black tea for 2 weeks to the restraint animals prevented the decrease in liver RNA levels induced by immobilization stress (Table 7). In control stressed animals, brain RNA levels were significantly decreased after 2 weeks and 4 weeks ($p<0.05$ and $p<0.01$, respectively) compared to the respective unstressed group of animals. Black tea supplementation for 2 weeks and 4 weeks to restraint stress animals were significantly decreased the brain RNA ($p<0.01$ and $p<0.05$, respectively) levels compared to respective unstressed black tea fed animals (Table 7).

Table 4: Effect of green and black teas (1% w/v) on plasma glucose and albumin concentrations in normal and restraint rats

	Glucose (mg dL^{-1})		Albumin (mg L^{-1})	
Treatments	2 weeks	4 weeks	2 weeks	4 weeks
Control (tap water)	157.2±18.1	156.2±26.5	41.1±8.6	39.0±4.0
Green tea	159.9±11.6	169.1±18.9	37.8±5.7	36.9±4.9
Black tea	164.9±27.2	162.2±26.4	42.6±5.9	41.6±2.9
Stress + Tap water	180.8±23.3	198.4±34.4*	43.4±7.5	40.1±3.3
Stress + Green tea	174.3±10.2	184.2±11.7	43.6±8.4	43.9±5.6
Stress + Black tea	176.5±21.9	188.6±20.5	40.5±10.6	42.6±5.3

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *p<0.05; Control vs control stress. Six rats were used in each group. Data are presented as Mean±SD

Table 5: Effect of green and black teas (1% w/v) on plasma total cholesterol and triglycerides levels in normal and restraint rats

	Total cholesterol (mg dL^{-1})		Triglycerides (mg dL^{-1})	
Treatments	2 weeks	4 weeks	2 weeks	4 weeks
Control (tap water)	72.8±8.1	67.2±11.8	143.2±33.6	150.0±49.3
Green tea	74.3±6.0	68.1±11.9	153.7±28.2	163.2±22.1
Black tea	77.3±9.6	75.6±11.3	151.4±28.9	188.5±64.5
Stress + Tap water	85.6±5.4	81.7±12.5	155.6±20.7	181.3±19.4
Stress + Green tea	81.1±9.8	76.2±7.1	172.6±13.1	175.8±18.9
Stress + Black tea	83.1±8.4	77.1±9.9	167.4±19.7	168.3±40.1

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group. Data are presented as Mean±SD

Table 6: Effect of green and black teas (1% w/v) on DNA concentrations of liver and brain in normal and restraint rats

	Liver DNA		Brain DNA	
	2 weeks	4 weeks	2 weeks	4 weeks
Treatments	(μg/100 mg wet tissue)			
Control (tap water)	167.5±98.0	167.5±25.6	116.4±8.1	117.1±15.8
Green tea	175.2±28.6	163.1±17.4	124.8±7.1	119.9±16.8
Black tea	177.3±19.6	170.2±18.8	118.3±15.6	115.7±12.8
Stress + Tap water	179.6±6.8	151.1±19.8	107.6±17.2	98.6±20.4
Stress + Green tea	174.2±25.9	156.0±16.3	107.5±15.5	104.8±9.5
Stress + Black tea	184.3±14.8	157.3±5.9	109.7±13.9	106.3±20.6

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group. Data are presented as Mean±SD

Effects on GSH concentrations: Glutathione concentrations were significantly ($p<0.001$) decreased in liver tissues of stressed animals (2 and 4 weeks) compared to their respective control groups (Table 9). Supplementation with both green or black tea to restraint animals for 2 and 4 weeks also decreased hepatic GSH levels compared to their respective unstressed groups ($p<0.001$). Brain GSH concentrations were significantly ($p<0.01$) decreased in stressed animals after 4 weeks compared to respective controls (Table 9). Treatments with either green or black tea kept brain GSH levels steady in restraint rats similar to their respective unstressed controls.

Effects on MDA concentrations: Lipid peroxidation marker, MDA, in liver tissues was significantly increased

Table 7: Effect of green and black teas (1% w/v) on RNA concentrations of liver and brain in normal and restraint rat

	Liver RNA		Brain RNA	
	2 weeks	4 weeks	2 weeks	4 weeks
Treatments	(μg/100 mg wet tissue)			
Control (tap water)	643.5±15.9	612.4±6.3	266.5±11.9	264.3±6.9
Green tea	639.9±16.1	587.6±11.4	272.3±10.3	267.7±5.5
Black tea	652.2±8.1	599.2±15.5	284.1±8.6	269.9±7.4
Stress + Tap water	572.9±13.0**	533.6±19.0***	226.8±4.2[a]**	213.9±8.9[a]***
Stress + Green tea	587.6±13.8[b]**	570.5±17.6	238.2±17.5	249.3±7.4
Stress + Black tea	591.3±15.9[c]**	555.6±24.6	230.8±7.6[c]**	224.4±9.6[c]**

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *p<0.05 and **p<0.01. [a]Control vs control stress; [b]Green tea vs green tea stress; [c]Black tea vs black tea stress. Six rats were used in each group. Data are presented as Mean±SD

Table 8: Effect of green and black teas (1% w/v) on total protein concentrations of liver and brain in normal and restraint rats

	Liver total protein		Brain total protein	
	2 weeks	4 weeks	2 weeks	4 weeks
Treatments	(mg/100 mg wet tissue)			
Control (tap water)	15.3±1.0	15.3±1.2	5.4±0.6	5.4±0.7
Green tea	15.3±0.9	15.3±1.0	5.5±0.5	5.5±0.5
Black tea	15.6±0.5	15.7±0.8	5.8±0.5	5.9±0.8
Stress + Tap water	15.1±0.7	14.4±1.6	5.2±0.6	5.2±0.7
Stress + Green tea	14.9±0.7	14.6±0.6	5.4±0.5	5.5±0.7
Stress + Black tea	15.0±0.7	14.5±0.9	4.9±0.7	5.3±0.9

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group. Data are presented as Mean±SD

Table 9: Effect of green and black teas (1% w/v) on GSH concentrations of liver and brain in normal and restraint rats

	Liver GSH		Brain GSH	
	2 weeks	4 weeks	2 weeks	4 weeks
Treatments	(μg g^{-1} wet tissue)			
Control (tap water)	274.7±9.2	270.3±6.8	171.6±7.1	165.4±5.2
Green tea	282.5±8.5	266.0±4.7	166.6±7.0	170.9±8.6
Black tea	284.9±13.7	273.7±12.0	170.6±5.4	167.4±6.8
Stress + Tap water	204.9±4.7[a]**	192.7±8.8[a]**	146.6±10.0	127.4±8.3[a]**
Stress + Green tea	214.6±10.2[b]**	211.2±13.1[b]**	154.5±5.7	144.3±4.0
Stress + Black tea	209.5±10.5[c]**	206.1±7.9[c]**	145.6±4.7	146.0±4.5

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *p<0.01 and **p<0.001. [a]Control vs control stress; [b]Green tea vs green tea stress; [c]Black tea vs black tea stress. Six rats were used in each group. Data are presented as Mean±SD

Table 10: Effect of green and black teas (1% w/v) on MDA concentrations of liver and brain in normal and restraint rats

	Liver MDA		Brain MDA	
	2 weeks	4 weeks	2 weeks	4 weeks
Treatments	(nmol g^{-1} wet tissue)			
Control (tap water)	228.3±7.9	226.3±7.1	404.8±15.7	379.2±20.6
Green tea	226.4±9.6	222.1±9.5	399.2±14.6	387.1±10.2
Black tea	225.3±13.0	217.5±6.9	410.1±9.5	386.8±13.2
Stress + Tap water	282.9±12.3[a]***	320.3±10.3[a]***	467.2±19.5	461.1±25.6[a]**
Stress + Green tea	263.6±14.2	284.6±9.7[b]**	441.8±11.1	418.8±22.6
Stress + Black tea	270.8±15.3	280.5±12.2[**c]	432.9±6.9	430.7±12.8

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *p<0.05, **p<0.01 and ***p<0.001. [a]Control vs control stress; [b]Green tea vs green tea stress; [c]Black tea vs black tea stress. Six rats were used in each group. Data are presented as Mean±SD

after 2 weeks (p<0.01) and 4 weeks (p<0.001) of immobilization stress as compared to controls (Table 10). Green or black tea supplementation for 4 weeks to stressed animals caused significant (p<0.01) increase in hepatic MDA levels as compared to their respective controls. Brain MDA concentrations were significantly (p<0.05) elevated in the 4 weeks untreated stressed animals, when compared to control group (Table 10). Green or black tea supplementation in restraint animals for 2 and 4 weeks did not induce any significant changes in brain MDA levels compared to treated unstressed animals.

Effect on SOD activity: SOD levels in hepatic tissues were not altered by either green or black tea supplementation to stressed animals or control immobilization stressed

Table 11: Effect of green and black teas (1% w/v) on SOD activities of liver and brain in normal and restraint rats

	Liver SOD		Brain SOD	
	2 weeks	4 weeks	2 weeks	4 weeks
Treatments	($U\ g^{-1}$ of proten)			
Control (tap water)	5.2±0.2	5.4±0.3	8.6±0.4	8.4±0.2
Green tea	5.3±0.3	5.1±0.3	8.9±0.3	8.4±0.3
Black tea	5.5±0.2	5.4±0.2	8.9±0.3	8.8±0.3
Stress + Tap water	4.5±0.2	4.5±0.3	8.2±0.3	6.9±0.2*
Stress + Green tea	5.2±0.2	4.9±0.2	8.2±0.2	7.2±0.3
Stress + Black tea	4.8±0.2	4.5±0.3	8.2±0.5	7.5±0.4

Values were analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test where *$p<0.05$; Control vs control stress. Six rats were used in each group. Data are presented as Mean±SD

animals (Table 11). In brain SOD levels, a decrease was only observed in the control restraint animals compared to unstressed ($p<0.05$).

DISCUSSION

Studies using animal and cell culture models indicated a potentially beneficial effect of tea on hepatic and brain tissues in gene transcription, cell proliferation and other molecular functions (Khan and Mukhtar, 2007). The potential health benefits from green and black teas are partially attributed to their antioxidative properties of polyphenols (Mukhtar and Ahmad, 2000). It was reported that restraint/immobilization stress is a good model for investigating the alterations occurring in oxidant-antioxidant balances in animal tissues and specifically targets brain for lipid peroxidation and liver for protein oxidation (Singh *et al*., 1993; Ramanova *et al*., 1994). Therefore, the main point of this study was to examine the antioxidant property of green tea and black tea in animals exposed to immobilization stress in plasma, liver and brain tissues.

In this study, body weight response in rats subjected to immobilization stress was compared with rats that received green or black tea. Rats subjected to repeated immobilization stress for 4 h showed a significant reduction in body weight in vehicle, green and black teas treated groups. These results suggest that repeated immobilization stress may have induced long-term effects on body weight and possibly body growth. It was shown that food intake in rats exposed to repeated immobilization stress was transiently decreased after the stress termination (Harris *et al*., 2004). In addition, immobilization stress was reported to increase sympathetic activity (Dronjak *et al*., 2004). Therefore, the increased sympathetic activity could be involved in the effects of repeated immobilization stress on body weight changes in the present study (Yoshihara and Yawaka, 2008). Alternatively, repeated immobilization stress may have suppressed the levels of circulating growth hormones (Yoshihara and Yawaka, 2008). It is well established that any stress exposure would suppresses food intake (Bhatnagar *et al*., 2006; Dallman *et al*., 2004). Feeding and drinking are also suppressed by stress-induced increase in sympathetic activity (Taylor and Samson, 2005). These changes related to nutrition induced by repeated immobilization stress may have ultimately affected the development of body weight in the current study.

Considerable variations in plasma biomarkers by either green or black tea were not observed in present study at any duration (2 or 4 weeks) of immobilized stress. Few animal studies showed significant changes in plasma intracellular enzymatic activities. Some reports suggested that tea or tea polyphenols consumption are not effective on plasma antioxidative parameter (Frei and Higdon, 2003; Higdon and Frei, 2003). In addition, studies in healthy humans have not found tea or tea polyphenol consumption caused any significant changes in plasma antioxidant capacity (Higdon and Frei, 2003). Indeed, the consumption of tea or tea polyphenols frequently results in only modest transient increases in the total antioxidant capacity of plasma (Higdon and Frei, 2003). Thus, increasing the treatment duration of tea consumption in the present study, i.e., more than 4 week stress exposure, may results in the increase of plasma antioxidant capacity.

In liver and brain tissues, animals subjected to repeated immobilization stress for 4 h for five consecutive days showed a significant reduction in GSH and RNA while there was an increase in MDA levels. The decreased GSH level in liver following exposure to immobilization stress was in agreement with others (Zaidi *et al*., 2005; Şahin and Gümüşlü, 2007). It was suggested that free radicals were stimulated by immobilization stress in the liver of adult and old rats (Davydov *et al*., 2004). In addition, it was found that immobilization stress decreased the activity of antioxidant enzymes, such as catalase, in liver (Zaidi *et al*., 2005; Şahin and Gümüşlü, 2007). The inactivation of liver antioxidant enzymes may ultimately increase lipid peroxidation (Şahin and Gümüşlü, 2007) and that may play a potential role in aggravating liver diseases like hepatic inflammation through generation of ROS (Zaidi *et al*., 2005). In present study, stressed groups subjected to either green or black tea attenuated the decrease in hepatic or brain GSH and RNA and increase in MDA concentrations compared to untreated stressed animals.

Green tea treatment attenuated the oxidative stress induced by immobilization in brain tissue. Black tea, however, only attenuated the increase in brain MDA induced by stressed animals. Therefore, the possible protection against the restraint stress effect on brain was found higher in green tea compared to black tea.

Although green and black teas come from the same plant, both have different active constituents. When catechins come in contact with polyphenol oxidase, they will be oxidized and form a flavanol dimmers, known as the aflavins and polymers, known as thearubigins (Balentine and Paetau-Robinson, 2000; Frei and Higdon, 2003). 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. However, green tea is withered and then steamed resulting in the inactivation of polyphenol oxidase. Green tea, thus, contains relatively high concentrations of catechins and low concentrations of theaflavins and thearubigins. Therefore, the antioxidative effect of green tea in the present study was possibly attributed to the presence of relatively higher concentrations of catechins or other polyphenols than in black tea.

The low penetration of the blood brain barrier is another possibility of the minor effects observed in brain of animals drinking black tea. In contrast, it was reported that green tea and tea catechins penetrates the blood brain barrier and achieve effective concentration in the central nervous tissue (Skrzydlewska *et al*., 2002; Khan and Mukhtar, 2007). Thus, green tea constituents may possess inhibitory effects against lipid peroxidation in synaptosomes and neuro-degeneration induced by peroxyl radicals. These reported antioxidative constituents may, at least in part, be responsible for the observed protection by pretreatment with green tea.

In agreement with others, Madrigal *et al*. (2001) also found a decrease in the brain GSH in rats exposed to immobilization stress. The brain is thought to be the most vulnerable to oxidative damage in the body because of the need for high oxygen consumption and the presence of high levels of poly unsaturated fatly acids, which may ultimately lead to various neurodegenerative disorders (Gutteridge, 1995; Reiter, 1995; Cui *et al*., 2006). ROS may be enhancing the initial attack on lipid rich membranes of the brain neurons to cause lipid peroxidation (Das and Kanna, 1997). Depletion of brain GSH, as one of the guardian factors against oxidative stress and ROS, may also enhance lipid peroxidation (Lewine, 1982). It was reported that stress reduces GSH levels and increases levels of ROS (Liu *et al*., 1996), which may explain the vulnerability of brain by immobilization stress and the possible protection by green and black teas.

We concluded that green or black tea consumption possesses an antioxidative effect during immobilization stress. In view of these observations, these antioxidant effects are possibly attributed to the presence of relatively higher concentrations of catechins or other polyphenols. These reported antioxidative constituents may, in part, be responsible for the observed protection by pretreatment with green or black tea.

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