Anti-stress Effects of *Camellia Sinensis* in Rats Subjected to Restraint Stress

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Abstract: This investigation aimed to evaluate the effect of *Camellia sinensis* (green tea) on immobilization stress-induced oxidative damage in male Sprague – Dawley rats. Twenty four rats were divided into four groups each of six rats. **Group (I):** served as control group, **Group (II):** rats were given green tea extract alone in drinking bottles (1% w/v)/ day for 2 weeks, **Group (III):** restraint stress group, animals were subjected to immobilization/ stress 4 h/day for 2 weeks, **Group (IV):** in this group, stressed rats were treated with green tea (1% w/v)/ day for 2 weeks. At the end of the experiment the animals were sacrificed and three different brain parts of animals (cerebrum, cerebellum and brain stem) were taken for biochemical studies. Malondialdehyde (MDA) a marker of lipid peroxidation, nucleic acids and total protein were estimated in three different brain parts of animals. Obtained results revealed that restraint stress caused a significant elevation in the rate of lipid peroxidation, reduction in nucleic acids and protein as compared to control in all three parts of brain of rats. Treatment with green tea extract counteracted the restraint stress-induced changes in these biochemical parameters. In conclusion; this study indicate the protective nature of green tea (GT) extract on different brain parts against the detrimental effect of restraint stress.
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Introduction:

Stress can be defined as physical and psychological modifications that disrupt the homeostasis and the balance of organisms. It can be induced in experimental animals in various forms e.g. Immobilization, forced swim, exposure to cold environment, starvation etc. The mechanism underlying stress-induced tissue damages are not yet fully understood, however, accumulating evidence has implied that the production of free radicals plays a critical role in these processes **(Olivenza et al., 2000; Zaidi et al., 2003; Ahmad et al., 2010).** Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity **(Zahir et al.,2006).** Brain is the target for different stressors because of its high sensitivity to stress induced degenerative conditions. Restraint stress resulted in elevated levels of malondialdehyde (MDA), an index of free radical generation and lipid peroxidation in brain **(Pal et al., 2006; Chakraborti et al., 2007).** Less well understood is the contribution of stress to oxidant production, especially in the brain. This is important because of considerable evidence that the formation of oxidants, damaging cellular molecules such as DNA, is a major contributor to ageing and the degenerative diseases of ageing such as brain dysfunction, cancer, cardiovascular diseases, and immune system decline **(Guedj et al., 2009).** The study of RNA is very helpful in knowing the rate of protein synthesis, and also to understand the functional status of the nervous tissue **(Bergen et. al., 1974).** Protein is one of the important biochemical components of the brain in vertebrates. Cells generally contain thousands of different proteins each with a biological activity **(Bock, 1978).** This has wide implications as restraint stress damages biomolecules-DNA (nucleolar and mitochondrial), RNA and protein.

Medicinal plants are considered as abundant source of natural and biologically active 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).**

Green tea contain more of the simple flavonoids called catechins, These active constituent are known to have an anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities **(Middleton et al., 2000; Weisburger and Chung, 2002; Tedeschi et al., 2004; Khan and Mukhtar, 2007) .** 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 **(Nakagawa and Yokozawa, 2002; Frei and Higdon, 2003; Chan et al., 2011).** 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 **(Reiter, 1995; Muller et al., 1996; Cui et al., 2004).** This implication has led to the notion that brain tissues is vulnerable to oxidative damage and that the antioxidant defense mechanisms, particularly in the brain, may not be sufficient enough to prevent these harmful effects. 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).**

Therefore, the aim of the present study was to investigate the effects of drinking green tea on some of the antioxidant biomarkers in brain after immobilization induced stress using male rats as an animal model.

2. Materials and Methods:

Preparation of green tea

Green tea (GT) was purchased from local market (Cairo – Egypt).Green tea was prepared by adding (10 gm) of green 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 drinking bottles. Green tea extract was freshly prepared every morning at the same time **(Al-Rejaie, 2009).**

Experimental animals

Twenty-four adult male Sprague-Dawley rats about 200-250 g body weight,bred in the Animal House Colony of The National Research Centre, Dokki, Cairo, Egypt. Animals were allowed 7 days for acclimatization at 24ºC with 12 hr light – dark cycle and fed standared laboratory diet and water *ad libitum* before the experiment. Animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

Experimental design

After one week of acclimation, animals were randomly divided into four equal groups each of six. **Group 1** (Control Group): rats fed a plain chow diet **Group 2:** rats were given GT extract alone (1% w/v)/per day for 2 weeks. **Group 3:** rats of this group were subjected to immobilization/restraint stress 4h/day for 2 weeks.

Group 4: this group treated with GT extract (1% w/v) / day for 2 weeks after restraint stress

Stress Protocol

The model for immobilization/restraint stress used in the current investigation was applied from previous reports **(Zaidi and Banu, 2004; Nadeem et al., 2006)** 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. 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 2 weeks. During stress procedure, animals were deprived of food and water **(Zaidi et al., 2003).** Weekly body weight of each animal was recorded.

Isolation of brain parts

At the end of the experiment animals were sacrificed by cervical dislocation. Dissection for separating the cerebrum, cerebellum and brain stem was carried out. The tissues of different brain parts were used for the assay of malondialdehyde (MDA), nucleic acids (DNA, RNA) and total protein.

Extraction and estimation of MDA

The method described by **(Okhawa et al., 1979)** was used for MDA analysis. Briefly, different brain parts (200 mg) were homogenized in aqueous 0.15 M KCl solutions and 1ml of homogenate was mixed with 1 ml of 10% TCA and centrifuged at 3,000 rpm for 15 min. One milliliter supernatant was mixed with 1 ml of 0.67% 2-thiobarbutaric acid then placed the tubes at boiling water bath for 15 min. Optical density of the clear pink supernatants was measured at 532 nm.The level of lipid peroxidation was expressed as nmol/mg.tissue

Estimation of nucleic acids in tissues

The method described by **(Schneider,1945; Sambrook et al., 1989)** was used to determine the levels of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) of different brain parts. 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 (Shimadzu- Model UV-2401- Japan). The levels of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were expressed as mg/g tissue.

Protein measurement

Total protein content in different parts of brain were estimated by the modified Lowry method of **(Schacterle and Pollack, 1973)**. Bovine plasma

albumin was used as standard. The level was expressed as mg/g tissue.

Statistical analysis:

Data were expressed as mean ±S.E. The data were analyzed by an analysis of variance (ANOVA) and the level of significance was determined by **Ducan's** multiple range tests (**Ducan's, 1955).** The significant between the individual groups. P values less than 0.05 were considered significant. Results were processed by the computer programs.

3. Results:

Body weight:

Two 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 **(Fig. 1).**

Malondialdehyde (MDA):

Malondialdehyde (MDA) level, a marker of lipid peroxidation (LPO) increased significantly (P <0.001, 0.01) in cerebrum, cerebellum and brain stem by (38, 35 and 31%) respectively, after immobilization stress for 4h /day for 2 weeks in comparison to control rats. Treatment of stressed rats with GT extract (1% W/V) /day for 2 weeks, resulted in a significant decrease (P <0.01) in the lipid peroxide levels of cerebrum, cerebellum and stem brain by (24, 33 and 28%) respectively ,as compared to stressed rats, while treated with GT extract alone resulted in no change in the levels of MDA as compared to control. **(Table 1, Fig.2).**

Deoxyribonucleic acid:

Immobilization stress caused significant (P <0.001, P <0.01) decrease in the levels of DNA of cereblum, cerebellum and brain stem by (29, 34 and 27%) respectively, compared to control group. Meanwhile, oral administration of GT extract to stressed animals significantly (P <0.01) recovered DNA levels in cerebrum, cerebellum and brain stem by (28, 35 and 25%) respectively, as compared to restraint stress group. **(Table 2, Fig.3).**

Ribonucleic acid:

RNA level was decreased significantly (P <0.001, P <0.01) in various parts of brain after restraint stress by (24, 27 and 29%) respectively, as compared to control group. The decreased level of RNA in cerebrum, cerebellum and brain stem was significantly (P < 0.01, 0.05) brought back to normal level by (23, 28 and 29%) respectively, after treatment of stressed animals with GT extract. **(Table 2, Fig. 4).**

Protein:

Total protein content of different brain parts of stressed rats was significantly (P <0.001, P <0.01) inhibited by (22, 25 and 18%) respectively, as compared to control group. Green tea extract supplementation induced significant (P<0.001 , P <0.01) increment of protein content in cerebrum, cerebellum and brain stem of stressed rats by (26, 31 and 16%) respectively, compared to untreated stressed group. GT extract alone showed no change in protein content compared to control group. **(Table 1 Fig.5).**

Table (1): Effect of (GT) extract on (MDA) level and protein content on different brain parts of rats subjected to restraint stress.

Groups / Brain parts	Cerebrum		Cerebellum		Brain Stem	
	Protein (mg/g tissue)	MDA (nmol/mg tissue)	Protein (mg/g tissue)	MDA (nmol/mg tissue)	Protein (mg/g tissue)	MDA (nmol/mg tissue)
Control	112.21±1.121^{a}	4.01±0.182^{a}	117.51±1.43^{a}	3.72±0.031^{a}	89.66±1.15^{a}	2.98±0.062^{a}
GT	109.01±0.141^{a}	3.78±0.031^{a}	120.62±0.21^{a}	3.51±0.024^{a}	87.22±0.26^{a}	3.01±0.019^{a}
Restraint stress	87.58±1.260^{b**}	5.55±0.031^{b***}	88.44±1.09^{b***}	5.02±0.101^{b***}	73.55±1.01^{b**}	3.90±0.033^{b**}
Restraint stress + GT	110.35±0.102^{c***}	4.22±0.027^{c**}	115.86±0.24^{c***}	3.37±0.031^{c**}	85.32±0.35^{c**}	2.82±0.029^{c**}

Each value represent the mean ± S.E (n = 6).

Within each column, means superscript with the same letter are not significantly different. *P < 0.05, **P < 0.01 and ***P < 0.001 (b) Significantly different from control group, (c) Significantly different from restraint stress group.

Table (2): Effect of (GT) extract on (DNA) and (RNA) levels on different brain parts of rats subjected to restraint stress.

Groups / Brain parts	Cerebrum		Cerebellum		Brain Stem	
	DNA (mg/g tissue)	RNA (mg/g tissue)	DNA (mg/g tissue)	RNA (mg/g tissue)	DNA (mg/g tissue)	RNA (mg/g tissue)
Control	**5.84±0.113^{a}**	**6.11±0.051^{a}**	**7.25±0.160^{a}**	**7.03±0.031^{a}**	**5.02±0.146^{a}**	**5.28±0.037^{a}**
GT	**6.03±0.021^{a}**	**6.43±0.041^{a}**	**7.39±0.019^{a}**	**7.00±0.055^{a}**	**5.15±0.014^{a}**	**5.43±0.061^{a}**
Restraint stress	**4.13±0.101^{b**}**	**4.62±0.077^{b**}**	**4.75±0.123^{b***}**	**5.15±0.023^{b***}**	**3.66±0.061^{b**}**	**3.76±0.033^{b**}**
Restraint stress + GT	**5.30±0.003^{c**}**	**5.68±0.021^{c*}**	**6.41±0.015^{c**}**	**6.61±0.062^{c**}**	**4.58±0.012^{c**}**	**4.85±0.027^{c*}**

Each value represent the mean ± S.E (n = 6).
Within each column, means superscript with the same letter are not significantly different. *P < 0.05, **P < 0.01 and ***P < 0.001
(b) Significantly different from control group, (c) Significantly different from restraint stress group.

Discussion

In recent years, there has been considerable interest in investigating the neuroprotective effects of phenolic compounds from different botanical sources. Green tea is currently considered a source of dietary constituents endowed with biological and pharmacological activities relevant to human health (**Chen et al., 2010**)

Green tea supplementation to the stressed rats significantly increased the body weight 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 and Yawaka, 2008**). However, it is well established that food intake would be suppressed following any stress exposure (**Dallman et al., 2004; Bhatnagar et al., 2006**) 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.

Also restraint stress resulted in an increase in the level of lipid peroxidation (LPO) while, the levels of nucleic acids (DNA, RNA) and protein decreased in cerebrum, cerebellum and brain stem as compared to control group. The observed increase in MDA level is in agreement with previous studies (**Yargicoglu et al., 2003; Sahin and Gumuslu, 2007; Ahmad et al., 2010**). Restraint stress resulted in the generation of oxidative stress / reactive oxygen species (ROS). These ROS may propagate the initial attack on lipid rich membranes of the brain to cause LPO (**Sahin and Gumuslu, 2004**). Also,the decrease in DNA, RNA and protein are in accordance with earlier studies (**Zahir et al., 2006; Ramtej and Devjani,2008**) they reported that, decline in nucleic acids and protein may be due to DNA damage caused by the free radicals and inhibition of RNA by direct interaction of ROS. Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity. Our results provide strong evidence that H_2O_2 and O_2 cause DNA damage because LPO products were increased with the passage of time as well as restraint stress. (**Gupta et al., 1991; Grillo et al., 2003**). The predominant radicals encountered in higher organisms are superoxide (O_2), peroxyl (ROO•), nitroxy (NO•) and hydroxyl (HO•) radicals. Hydroxyl radical (HO•) is more reactive and is capable of causing damage to biomolecules such as lipids, proteins and DNA (**Chakraborti et al., 2007**). It is generally recognized that in physiological system HO• is produced under aerobic condition by Fenton's reaction (**Chen and Schopfer,1999**) and its interaction with DNA causes oxidative damage. Oxidative RNA damage is also a feature in vulnerable neurons at the earliest stages of these diseases suggesting that RNA oxidation may actively contribute to the onset or to the development of disease (**Nunomura et al., 2006**). In our study; a decrease in protein level of rats with restraint stress which may be attributed to accumulations of constituents like phospholipids and cholesterol in the brain. Decrease in protein level also suggests high rate of utilization of protein in restraint stress (**Afadlal et al., 2010**).

Treatment with GT extract significantly prevented the rise in MDA levels suggesting that it attenuates the excessive formation of ROS secondary to restraint stress. This is in agreement with the observation that GT possesses significant antioxidant activity (**Khan, 2006; Jain et al., 2011; Jówko et al., 2011**). Protective effect of GT extract against MDA has been reported by (**Geetha,et al., 2004;**

Alshatwi et al., 2011) Since the antioxidants interrupt the free-radical chain of oxidation by donating hydrogen from phenol's hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids (**Frei and Higdon, 2003**).

. Therefore, it can be assumed that GT may also be acting on similar lines. Treatment with GT extract significantly increased the levels of DNA, RNA and protein in different parts of brain. Our results are strongly in favour of **(Ramtej and Devjani, 2008; Rajavelu et al., 2011)**in which DNA, RNA and protein contents increased by Emblica officinalis aqueous extract induced by ochratoxin. Although the protective effect of GT on brain against stressors had been documented, the mechanism of action of GT extract has to be elucidated. Green tea contain more of the simple flavonoids called catechins and the potential beneficial effects of GT 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 (**Nakagawa and Yokozawa, 2002;Frei and Higdon, 2003.**

Green tea and tea catechins, however, were reported to penetrates the blood brain barrier, and achieve effective concentration in the central nervous tissue (**Skrzydlewska et al., 2002;Khan and Mukhtar, 2007**) and that green tea constituents may possess inhibitory effects against lipid peroxidation in synaptosomes and neuro-degeneration 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 **(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

Thus it could be said that the protective action of GT might be through the suppression of free radicals. Also, the increased nucleic acids and protein is therefore, an indication that the brain's antioxidant machinery is activated to excessive generation of free radicals (**chen et al., 2007).** Presence of flavonoids in GT may be held responsible for its attenuating activity because flavonoids have been reported as potentially useful exogenous agents in protecting the aging brain, other organs and tissues of the body against free radical induced damage (**Guedj et al., 2009; Dragicevic et al., 2011**) **.** So it is evident now that GT prevents the stress-induced changes in brain.

Conclusion

Our study indicated that restraint stress significantly induced alterations in lipid peroxidation, nucleic acids and proteins. GT could be used for the prevention of stress-induced elevation of MDA and reduction of nucleic acids and protein in cerebrum, cerebellum and brain stem. Thus, GT could be used as a potentially effective therapeutic agent in clinical conditions associated with free radical damage in central nervous system.

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References:

Afadlal, S.; Polaboon, N.; Surakul, P.; Govitrapong, P.and Jutapakdeegul, N. (2010): Prenatal stress alters presynaptic marker proteins in the hippocampus of rat pups. Neurosci, Lett. 5;470(1):24-7.

Ahmad, A.; Rasheed, N.; Banu, N. and Palit, G. (2010):Alterations in monoamine levels and oxidative systems in frontal cortex, striatum, and hippocampus of the rat brain during chronic unpredictable stress. Stress.,13(4):355-64.

Al-Rejaie, S.S.(2009): Effect of Green and Black Teas on Immobilization Induced Stress in Male Wistar Albino Rats. Int. J. Pharmacol, 5: 137-145.

Alshatwi, A.A.; Al Obaaid, M.A.; Al Sedairy, S.A.; Ramesh, E.aand Lei, K.Y.(2011):. Black and green tea improves lipid profile and lipid peroxidation parameters in Wistar rats fed a high-cholesterol diet. J Physiol Biochem., 67(1):95-104.

Bergen, W.G.; Czajka-Norins, O.M.; Fink, E.L.and Rimpau, E.S.(1974): Liver and brain nucleic acids and brain RNA synthesis in sucking rats fed dieldrin (38082). Proceedings of Society for Experimental Biology and Medicine, 146: 259-262.

Bhatnagar, S.; Vining, C.; Iyer, V.and Kinni, V.(2006): Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. J. Neuroendocrinol., 18: 13-24.

Bock, E.(1978): Nervous system specific proteins. Journal of Neurochemistry, 30(1): 7-14.

Chakraborti, A.; Gulati, K.; Banerjii, B.D.and Ray, A.(2007): Possible involvement of free radicals in the differential neurobehavioral responses to stress in male and female rats. Behavioural Brain Research, 179: 321-325 .

Chan, E.W.; Soh, E.Y.; Tie, P.P.and Law, Y.P.(2011): Antioxidant and antibacterial

properties of green, black, and herbal teas of Camellia sinensis. Pharmacognosy Res, Oct;3(4):266-72.

Chen, S.X.and Schopfer, P.(1999): Hydroxyl-radical production in physiological reactions. A novel functions of peroxidase. FEBS Journal, 260(3): 726-735.

Chen, W.; Cheng, Y.; Zhao, X. and Li, S. (2007): Effect of green tea polyphenols on behavioral performances in psychological stress rats. Wei Sheng Yan Jiu., 36(5):555-8.

Chen, WQ.; Zhao, XL.; Wang, DL.; Li, ST.; Hou, Y.; Hong, Y.and Cheng, Y.Y. (2010): Effects of epigallocatechin-3-gallate on behavioral impairments induced by psychological stress in rats. Exp Biol Med (Maywood). 235(5):577-83.

Cui, K.; Luo, X.; Xu. K.and Ven Murthy, M.R.(2004): Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. Prog. Neuropsychopharmacol. Biol. Psychiatry, 28: 771-799.

Dallman, M.F.; la Fleur, S.E.; Pecoraro, N.C.; Gomez, F.; Houshyar, H.and Akana, S.F.(2004): Minireview: glucocorticoids--food intake, abdominal obesity, and wealthy nations in Endocrinolog, 145: 2633-2638.

Dragicevic, N.; Smith, A.; Lin, X.; Yuan, F.; Copes, N.; Delic, V.; Tan, J.; Cao, C.; Shytle, RD. and Bradshaw, P.C. (2011): Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. J Alzheimers Dis ,26(3):507-21.

Dronjak, S.; Jezova, D.and Kvetnansky, R.(2004): Different effects of novel stressors on sympathoadrenal system activation in rats exposed to long-term immobilization. Ann. NY Acad. Sci., 1018: 113-123.

Duncan, D.B.(1955): Multiple Ranges and Multiple F – test. Biometrics, 11: 1-42.

Frei, B.and Higdon, J.(2003): Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J. Nutr, 133: 3275S-3284S.

Fujimura, Y.; Kurihara. K.; Ida, M.; Kosaka, R.; Miura, D.; Wariishi, H.; Maeda-Yamamoto, M.; Nesumi, A.; Saito, T.; Kanda. T.; Yamada, K.and Tachibna,H.(2011):Metabolomics-driven nutraceutical evaluation of diverse green tea cultivars. PLoS One, 6: e23426.

Geetha Kedlaya, R.and Vasudevan, D.M.(2004): Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum*: In vivo and in vitro studies. Life Science, 76: 21-28.

Grillo, C.A.; Piroli, G.G.; Rosell, D.R.; Hoskin, E.K.; Mcewen, B.S.and Reagan, L.P.(2003):. Region specific increases in oxidative stress and superoxide dismutase in the hippocampus of diabetic rats subjected to stress. Neuroscience. 121(1):133-40.

Guedj, F.; Sébrié, C.; Rivals, I.; Ledru, A.; Paly, E.; Bizot, J.C.; Smith, D.; Rubin, E.; Gillet, B.; Arbones, M.; Delabar, J.M..(2009): Green tea polyphenols rescue of brain defects induced by overexpression of DYRK1A. PLoS One, 4(2):e4606.

Gupta, A.; Hasan, M.; Chander, R.and Kapoor, N.K.(1991): Age related elevation of lipid peroxidation products: diminution of superoxide dismutase. activity in the CNS of rats. Gerontology, 37(6): 305-309.

Harris, R.B.; Gu, H.; Mitchell, T.D.; Endale, L.; Russo, M.and Ryan, D.H.(2004): Increased glucocorticoid response to a novel stress in rats that have been restrained. Physiol. Behav., 81: 557-568.

Jain, D.P.; Pancholi, S.S.and Patel, R. J.(2011): Synergistic antioxidant activity of green tea with some herbs. Adv pharm Technol Res., 2(3):177-83.

Jówko, E.; Sacharuk, J.; Balasińska, B.; Ostaszewski, P.; Charmas, M.and Charmas, R.(2011): Green tea extract supplementation gives protection against exercise-induced oxidative damage in healthy men. Nutr Res., 31(11):813-21.

Khan, N.and Mukhtar, H.(2007): Tea polyphenols for health promotion. Life Sci., 81: 519-533.

Khan, S.M.(2006): Protective effect of black tea extract on the levels of lipid peroxidation and antioxidant enzymes in liver of mice with pesticide-induced liver injury. Cell Biochem Funct., Jul-Aug;24(4):327-32.

Middleton, E. Jr.; Kandaswami, C.and Theoharides, T.C.(2000): The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev., 52: 673-751.

Muller, W.E.; Pergande, G.; Ushijima, H.; Schleger, C.; Kelve, M.and Perovic, S.(1996): Neurotoxicity in rat cortical cells caused by N-methyl-D- aspartate (NMDA) and gp120 of HIV-1: induction and pharmacological intervention. Prog. Mol. Subcell. Biol, 16: 44-57.

Nadeem, A.; Masood, A.; Masood, N.; Gilani, R.A.and Shah, Z.A.(2006): Immobilization stress causes extra-cellular oxidant-antioxidant imbalance in rats: restoration by L-NAME and vitamin E. Eur. Neuropsychopharmacol, 16: 260-267.

Nakagawa, T. and Yokozawa, T. (2002): Direct scavenging of nitric oxide and superoxide by green tea. Food Chem. Toxicol, 40: 1745-1750.

Nunomura, A.; Honda, K.; Takeda, A.; Hirai, K.; Zhu, X.; Smith, M.A.and Perry, G.(2006): Oxidative damage to RNA in neurodegenerative diseases. Journal of Biomedicine and Biotechnology, 1-6.

Okhawa, H.; Ohishi, N.and Yagi, K.(1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358.

Olivenza, R.; Moro, M.A.; Lizasoain, I.; Lorenzo, P.; Fernadez, A.P.; Rodrigo, J.; Bosca, L. and Leza, J.C. (2000): Chronic stress induces the expression of inducible nitric oxide synthase in rat brain cortex. Journal of. Neurochemistry, 74: 785-791.

Pal, R.; Gulati, K.; Banerjii, B.D.and Ray, A.(2006): Role of free radicals in stress induced neurobehavioral changes in rats. Indian Journal of Experimental Biology, 44: 816-820.

Rajavelu, A.; Tulyasheva, Z.; Jaiswal, R.; Jeltsch, A.and Kuhnert, N. (2011): The inhibition of the mammalian DNA methyltransferase 3a (Dnmt3a) by dietary black tea and coffee polyphenols. BMC Biochem, 21;12:16.

Ramtej, V.and Devjani, C.(2008): Alteration in DNA, RNA and Protein contents in liver and kidney of mice treated with ochratoxin and their amelioration by Emblica officinalis aqueous extract. Acta Poloniae-Drug Research, 65 (1): 3-9.

Reiter, R.J.(1995): Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J, 9: 526-533.

Sahin, E.and Gumuslu, S.(2004): Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. Behav. Brain Res., 155: 241-248.

Sahin, E.and Gumuslu, S.(2007): Immobilization stress in rat tissues: alterations in protein oxidation, lipid peroxidation and antioxidant defense system. Comp. Biochem. Physiol. Toxicol. Pharmacol., 144: 342-347.

Sambrook, J., Fritach, E.F. and Maniatis, T. (1989): Extraction and isolation of nucleic acids (DNA and RNAs) from mammalian cells. In: Molecular Cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbar Laboratory Press, Cold Spring Harbor, New York. pp:15-45.

Schacterle, G.R.and Pollack, R.L.(1973): A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal. Biochem, 51: 654-655.

Schneider,W.D. (1945): Phosphorus compounds in animal tissues. 1 extraction and estimation of deoxypentose nucleic acid and pentose nucleic acid. J. Biol. Chem., 161: 293-303.

Skrzydlewska, E.; Ostrowska, J.; Farbiszewski, R.and Michalak, K.(2002): Protective effect of green tea against lipid peroxidation in the rat liver, blood serum and the brain. Phytomedicine, 9: 232-238.

Taylor, M.M.and Samson, W.K.(2005): Stress hormone secretion is altered by central administration of intermedin/adrenomedullin-2. Brain Res.1045: 199-205.

Tedeschi, E.; Menegazzi, M.; Yao, Y.; Suzuki, H.; Forstermann, U.and Kleinert, H.(2004): Green tea inhibits human inducible nitric-oxide synthase expression by down-regulating signal transducer and activator of transcription-1alpha activation. Mol. Pharmacol. 65: 111-120.

Weisburger, J.H.and Chung, F.L.(2002): Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. Food Chem. Toxicol, 40: 1145-1154.

Yargicoglu, P.; Yaras, N.; Agar, A.; Gumuslu, S.; Bilmen, S.and Ozkaya, G.(2003): The effect of vitamin E on stress-induced changes in visual evoked potentials (VEPs) in rats exposed to different experimental stress models. Acta Ophthalmologica Scandinavica, 81: 181-187.

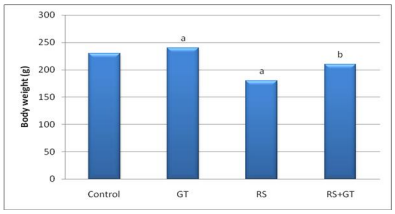
Yoshihara, T.and Yawaka, Y.(2008): Repeated immobilization stress in the early postnatal period increases stress response in adult rats. Physiol. Behav., 93: 322-326.

Zahir, F.; Rizvi, S.J.; Haq, S.K. and Khan, R.H.(2006): Effect of methyl mercury induced free radical stress on nucleic acids and protein: implications on cognitive and motor functions. Indian Journal of Clinical Biochemistry, 21: 149-152 .

Zaidi, S.M.; Al-Qirim. T.M.; Honda. N. and Banu, N.(2003): Modulation of restraint stress induced oxidative changes in rats by antioxidant vitamins. Journal of Nutritional Biochemistry, 14: 633-636.

Zaidi, S.M.and Banu, N.(2004): Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. Clin. Chim. Acta, 340: 229-233.

Zhao, H.; Zhang, M.; Zhao, L.; Ge, Y.K.; Sheng, J.and Shi W.(2011): Changes of constituents and activity to apoptosis and cell cycle during fermentation of tea. Int. J. Mol. Sci., 12: 1862-1875.



a: As compared to Control group

p: As compared to restraint stress group

Fig (1): Mean values of body weight of rats of different groups

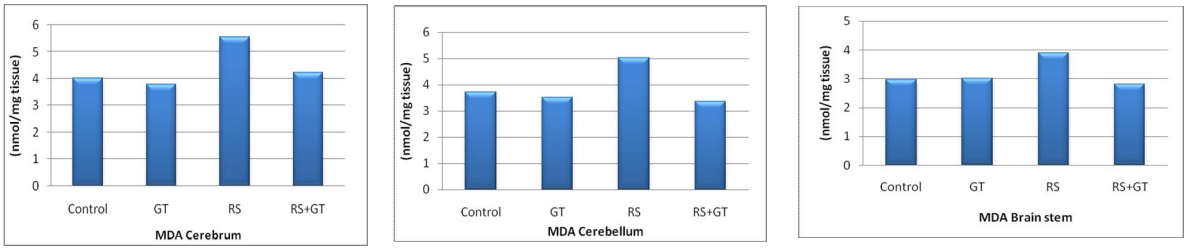


Fig (2): Effect of (GT) extract on **(MDA)** level of different brain parts of rats subjected to restraint stress.

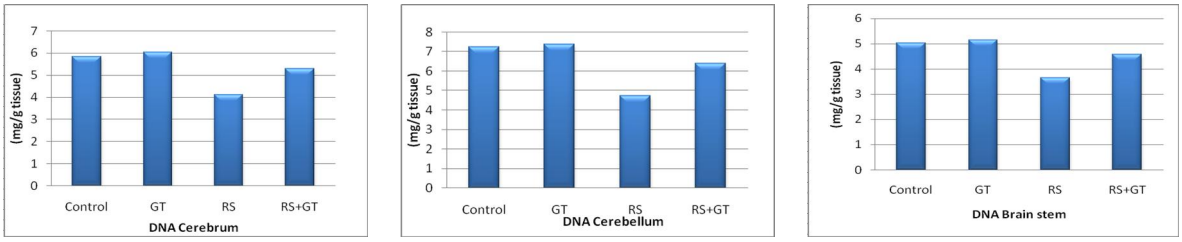


Fig (3): Effect of (GT) extract on **(DNA)** level of different brain parts of rats subjected to restraint stress.

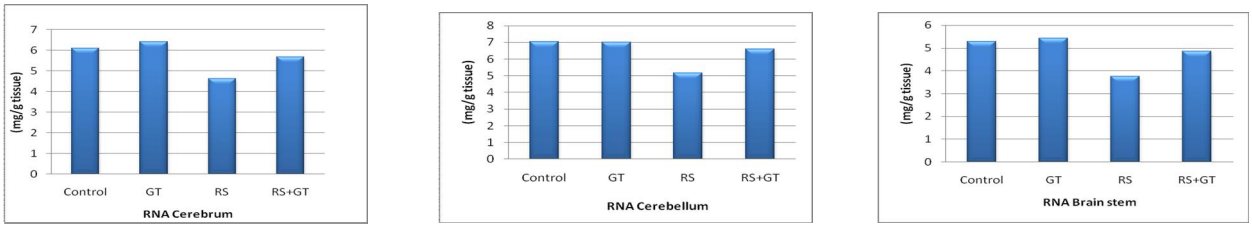


Fig (4): Effect of (GT) extract on **(RNA)** level of different brain parts of rats subjected to restraint stress.

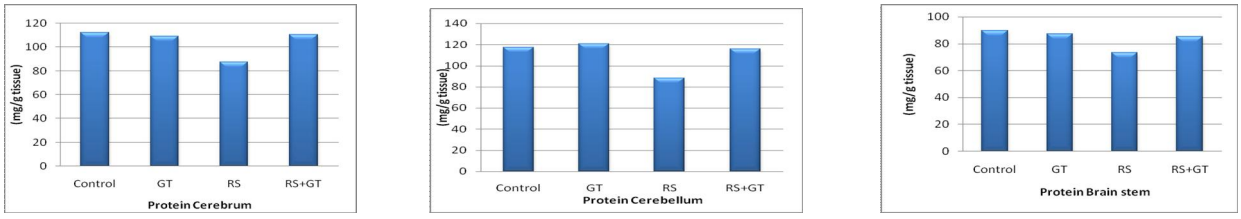


Fig (5): Effect of (GT) extract on **(Protein)** content level of different brain parts of rats subjected to restraint stress.

11/4/2012