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Original Research Article

The protective and ameliorative effect of green tea extract on antioxidant status of brain tissue exposed to oxidative stress

Ata Sedik Ibrahim Elsayed[1*], Mohamed Abdulmonem Hegazi[2], Hala Abd-ElAzim Mostafa[2] and Mona Mohamed Hegazi[2]

[1]Department of Biomedical Sciences, Faculty of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia.
[2]Department of Zoology, Faculty of Science, Tanta University, Egypt.

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This study aimed to investigate the toxic effects of gasoline on antioxidant status of brain tissue of mice. On the other hand, studying the protective and the ameliorative role of green tea extract on these toxic effects of gasoline. Green tea extract was chosen as an antioxidant and antitoxicity natural products. CD1 mice were taken as an experimental model. Mice were exposed to gasoline vapor 2hours/day for 3 weeks in inhalation chamber. Green tea extract was provided to mice as their sole source of drinking water, these were taken before starting inhalation with one week and along the time of experiment till sacrificing the animals.

Oxidative stress markers were done in this study to determine the oxidative stress of gasoline, on the other hand to examine the protective and the ameliorative role of green tea.

The results of this study were concluded as:

1. **Marked increase in lipid and protein oxidation levels in brain tissue by gasoline inhalation with highly protective effects of green tea on this oxidative stress of gasoline.**
2. **Destruction for thiol compounds, which acted as non-enzymatic antioxidants, with gasoline, these returned to the normal levels with green tea.**
3. **Significant reduction in the activities of antioxidant enzymes, which damaged by gasoline, some of them were improved with green tea and the others did not affect. Catalase and GPX were improved with green tea, but G-S-T and SOD did not affect.**

Key words: Gasoline, green tea, oxidative stress, antioxidant.

INTRODUCTION

Reactive oxygen species (ROS) are molecules that include both free radical molecules, such as superoxide (O2-), hydroxyl radical, and NO, and nonradical molecules such as hydrogen peroxide (H2O2).These molecules are highly reactive (they can oxidize amino acids in proteins or nucleic acids in RNA or DNA) because they have an unpaired electron. ROS can be generated in response to environmental activators, such as pollutants in the air, smoke, smog, and exposure to radiation (e.g., ultraviolet light) (Rhoades and Bell, 2013). Under normal circumstances, oxidoreductases that are part of the mitochondrial electron transport system generates ROS, but there are other cellular sources, such as xanthine oxidoreductases, lipoxygenases, cyclooxygenases, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. NADPH oxidase is one of the major enzyme sources responsible for ROS generation and the source of ROS that are involved in signaling. The physiologic role of ROS generation by NADPH oxidases includes the respiratory burst produced by phagocytic cells such as neutrophils and macrophages that results in large amounts of ROS production.

The respiratory burst is a critical feature in the host response to infection and leads to the destruction of bacteria or fungi. A second physiologic role of NADPH oxidase–generated ROS arises from their ability to react with amino acid residues in proteins, leading to modifications in their activities, localization, and stability. In addition to direct modification of

Corresponding Author: ata4121967@hotmail.com Tel: +966594543240

proteins, ROS can also oxidize nucleic acids, such as RNA and DNA. Oxidative damage to DNA can result in mutations in genes or alter gene expression by the mispairing of the damaged bases. (Rhoades and Bell, 2013)

Tea is obtained mainly from the leaves and the terminal apical buds of the tropical shrub *Camellia sinensis*. The plant was originally discovered in South East Asia 1000 of years ago. It is now the most popular beverage, next to water, consumed by over two-thirds of the world's population. It is grown mainly in the subtropical zones. It is rich in substances with antioxidant properties and contains traces of proteins, carbohydrates, amino acids and lipids, as well as, more significant quantities of some vitamins and minerals (Gupta *et al.,* 2002). The tea leaves are distinguished by their content of methylxanthines, and polyphenols especially flavonols of the catechin type. The major green tea polyphenols are: (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (+)-gallocatechin (GC), (-)-epicatechin (EC), gallocatechin gallate (GCG) and catechin (C) which together may constitute 30% of the dry leaf weight, in addition to caffeine, theobromine, theophylline and phenolic acid, such as gallic acid are also present as minor constituents of green tea (Gupta *et al.,* 2002, Castro *et al.,* 2010, Mazzanti et al., 2015).

A major development over the past two decades has been the realization that free radical mediated peroxidation of membrane lipids and oxidative damage of DNA are associated with a variety of chronic health problems, such as cancer, atherosclerosis, neurodegenerative diseases and aging (Finkel and Holbrook, 2000; Perwez Hussain *et al.,* 2003; Barnham *et al.,* 2004). Therefore, inhibition of oxidative damage by supplementation of antioxidants becomes an attractive therapeutic strategy to reduce the risk of these diseases (Rice-Evans and Diplock, 1993; Brash and Harve, 2002 Vuong *et al.,* 2011 and Zhang *et al.,* 2012). Antioxidant enzymes in liver homogenate of rats as Cu,Zn-SOD, catalase, GPX, and GSSG-R all of these were improved and elevated significantly by adding green tea extract 7g/L to drinking water. Non-enzymatic antioxidant as GSH, vitamin C, vitamin E, vitamin A and β-carotene were decreased with age, and some of them re-elevated again by green tea extract as GSH, β-carotene, and vitamin E (Augustyniak *at al.,* 2005).In the same study intoxication of ethanol leads to decrease of antioxidant enzymes and most of them were returned to the normal range with green tea extract treatment. The activity of SOD is low in diabetes mellitus. The alloxan-induced diabetic rats when were treated with green tea polyphenols, they showed a decrease in lipid peroxidation associated with increased activity of SOD and GSH (Sabu *et al.,* 2002).

In a study performed by Yu et al. (2015) they have shown that EGCG ameliorates liver inflammation, necrosis and fibrosis and suppressed the expression of TNF-α, IL-1β, TGF-β, MMP9, α-SMA, and Col-1α1. Similar results were obtained in HSC cell line LX-2, where EGCG was capable of suppressing TGF-β1, Col-1α1, MMP2, MMP9, TIMP1, and α-SMA. Administration of tea and tea polyphenols has been reported to prevent or attenuate decreases in antioxidant enzyme activities in a number of animal models of oxidative stress. Providing hairless mice with green tea polyphenols in their drinking water significantly inhibited UVB-induced decreases in epidermal catalase and glutathione reductase activities (Agarwal *et al.,* 1993). Oral administration of green tea extract to mice infected with M. tuberculosis attenuated infection-associated decreases in erythrocyte superoxide dismutase (SOD) activity (Guleria *et al.,* 2002), while oral administration of either black or green tea extract resulted in increased serum SOD activity in mice exposed to the carcinogen, 3-methylcolanthrene (Das *et al.,* 2002). Providing rats with green tea extract in their drinking water attenuated ethanol-associated decreases in serum and liver SOD as well as liver glutathione peroxidase (GPX) and catalase activities (Skrzydlewska *et al.,* 2002b).

MATERIALS AND METHODS

Experimental animals

Sixty male mice (*Mus musculus*) weighting 20 – 25g was purchased from the Egyptian Organization for Serological and Vaccine Production, Egypt, were used as experimental animals throughout the present work. The animals were housed individually in plastic cages and acclimated for 1 week before gasoline-fume exposure. Food and water were offered ad libitum. Animals were maintained at 22± 2 °C at normal light/dark cycle.

Preparation of green tea extract

Green tea (*Camellia sinensis*) was purchased from Shanghai tea import & export Corporation, China. The green tea extract was made according to Maity et al. (1998), by soaking 15 gm of instant green tea powder in 1L of boiling water for 5 minutes. The solution was filtered to obtain 1.5% green tea extract; this solution was provided to mice as their sole source of drinking water

Inhalation of gasoline

A glass cubic box its length is 70cm, width is 70cm and high is 70cm, was manufactured to make as gasoline inhalation chamber, there are two orifices in both right and left sides of the box in the upper portion of the box to make aeration, each orifice 5cm in diameter covered with wire mesh to prevent mice escaping. At a 10cm distance from the bottom of the box, a wire mesh shelf 70x70 cm was fixed to put the mice on it. Under this shelf, 200 ml cans containing 150 ml of gasoline were placed in the exposure chamber and the animals were allowed to inhale the fumes evaporating from the cans. The gasoline, which evaporated during the time of inhalation, was about 80 ml/2hours.The time of exposure was 10.00 to 12.00am and the cans were withdrawn and the inhalation stopped. The experimental fume gasoline inhalation was exceeded for successive three weeks as 2hours/day/three weeks.

The gasoline

The Egyptian commercial unleaded gasoline (octane 90) was purchased from a filling station. Gasoline is a petroleum-derived liquid mixture consisting mostly of more than 300 individual hydrocarbons primarily (in volume) of paraffins (30–90%), cycloparaffins (1–35%), olefins (0–20%), and aromatic (5–55%), distilling in the approximate range of 30°C–220°C. Composition of gasoline varies with the source of the crude oil, refinery processes, conditions, and the blending of refinery streams in the gasoline boiling range to meet performance criteria as well as regulatory requirements (Roberts *et al.,* 2001). Volatile organic compound emissions from gasoline storage showed that total organic compounds per cubic meter gasoline loaded is 35 g/m3 saturated vapor at 25 °C.

Gasoline Dose

Based on analysis reported by Johnson *et al.,* (1990) the concentration in equilibrium with gasoline is 9375 ppm. Benzene is 100-fold less than in equilibrium with pure benzene being 93.75 ppm. This dose of benzene is in equilibrium with gasoline in the inhalant mice cages in the current study. However, gasoline fraction differs from whole gasoline by containing far less aromatic, longer chain and longer aliphatic hydrocarbons. Analysis of workplace exposure to gasoline vapors revealed that C4–C5 length hydrocarbons constitute from 67 to 74% by weight of the typical vapor (Halder *et al.,* 1986).

Animal Groups

After an acclimation period for 1 week, animals were classified into four groups; each group consists of fifteen mice as follows:

Control group: received only the ordinary mice diet and drink water without any additions and kept two hours daily in the inhalation chamber without gasoline for three weeks.

Green tea group: received ordinary diet, drink green tea extract (1.5%) as a sole source of drinking water and kept two hours daily in the inhalation chamber without gasoline for three weeks.

Gasoline inhalation group: this is the intoxicated group with gasoline inhalation; these mice were kept 2 hours daily in an inhalation chamber with gasoline for three weeks. This group drinks water and eat the ordinary diet.

Gasoline and green tea group: these animals exposed to gasoline 2 hours daily in an inhalation chamber for three weeks and received green tea extract (1.5%) eat the ordinary diet.

Tissue preparation for enzyme assays

The brain was removed immediately, washed in ice-cold isotonic saline and blotted between two filter papers, weighted, used directly for the determination of oxidation biomarkers, non-enzymatic antioxidants, and antioxidant enzymes. The brain was homogenized in about 10% w/v ice-cold phosphate buffer (50mM pH 7.4, 0.1% triton X and 0.5 mM EDTA) by using Omni international homogenizer (U.S.A). The homogenate was centrifuged at 6000xg in cooling centrifuge (Hettich, Germany) at 4 °C for 15 min. The protein supernatant was separated in another clean and dry Eppendorf tube for biochemical enzyme assays.

Determination of Antioxidant Enzymes Activity

Catalase (CAT) Activity

Catalase activity was estimated by measuring spectrophotometericaly the breakdown of hydrogen peroxide in the reaction mixture by using the method of Cohen *et al.,* (1970). The enzyme was kinetically assayed in a reaction mixture contained 50 ml phosphate buffer + 50µl H2O2 and the reaction was started by the addition suitable amount of protein supernatant (10-20µL).The enzyme activity was expressed as µM/minute/gm weight wet tissue. The extinction coefficient (Ex) of H2O2 = 0.04 mM−1 cm−1 at 240 nm).

Glutathione peroxidase (GPX) Activity

In this method GPX catalyzes the reduction of H2O2 in the presence of reduced glutathione. GPX activity was measured by using the method of Paglia and Valentine (1967). GPX activity was measured by a coupled assay with GR catalyzed oxidation of NADPH. The enzyme was kinetically assayed in a reaction mixture contained 100 mM Na+/K+-phosphate buffer (pH 7.4), 0.25 mM NADPH, 4 mM sodium azide, 1 U/ml yeast GR, 15 mM GSH, and 10-20 µl of protein supernatant.H2O2 (10 µl) was then added to a final concentration of 0.2 mM. Ex of NADPH = 6.22mM−1 cm−1 at 340 nm).

Glutathione-S-transferase (GST) Activity

GST activity was measured by using the method of Habig *et al.,* (1974).The enzyme activity was measured through the conjugation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (CDNB). The formation adduct of CDNB, S-2,4-dinitrophenyl glutathione, was monitored by measuring the rate of increase in absorbance. The enzyme was kinetically assayed in a reaction mixture contained 100 mM potassium phosphate buffer, pH 6.5, 1 mM CDNB in ethanol, and 1 mM GSH. The formation of the adduct of CDNB, S-2,4-dinitrophenyl glutathione, was monitored by measuring the rate of increase in absorbance. Ex) of CDNB = 9.600 mM−1 cm−1 at 340 nm.

Superoxide dismutase (SOD) Activity

SOD activity was determined according to the method Paoletti and Mocali (1990). Samples were assayed by measuring the inhibition of NADH oxidation by β-mercaptoethanol in the presence of EDTA and Mn. One unit of SOD activity was defined as the amount of enzyme inhibiting the rate of NADH oxidation by 50%. All the measurements were done using JENWAY (6505) Uv/Vis Spectrophotometer (U.K) at the constant temperature 25°C.

Determination of Oxidation Biomarkers (nM/gm weight wet tissue)

Protein carbonyl level

Protein carbonyls were measured spectrophotometrically by using the method of Reznick and Packer (1994). Carbonyl groups react with 2,4-dinitrophenylhydrazine (DNPH) to generate chromophoric dinitrophenylhydrazones.

Advanced Oxidation Protein Product (AOPP) level

Spectrophotometric determination of AOPP level was performed by modification Witko's method (Witko et al., 1992).

Lipid Peroxidation

Lipid peroxidation was measured using a thiobarbituric acid reactive substances (TBARS) assay as described by Uchiyama and Mihara (1978) and modified by Hermes-Lima et al., (1995). This method involves the reaction of a degradation product of lipid peroxidation, malondialdehyde (MDA), with Thiobarbituric acid (TBA) under conditions of high temperature and acidity to generate a colored adduct that is measured spectrophotometrically.

Determination of Non-enzymatic Antioxidants (µM/gm weight wet tissue)

Estimation of total thiol

Total thiol groups in the tissue homogenate were determined as the method by Sedlak and Lindsay (1968).

Estimation of non-protein-pound thiol

Non protein-pound thiols in the tissue homogenate were determined as the method by Sedlak and Lindsay (1968).

Estimation of protein-pound thiol

This was calculated by subtracting non protein-pound thiol from total thiol.

Statistical Analysis

Data are expressed as mean±SD. The level of statistical significance was taken at $P < 0.05$, using one way analysis of variance (ANOVA) test followed by a Dunnett test to detect the significance of differences between each group and control. All analysis and graphics were performed by using, INSTAT and graphPad Prism software version 4.

RESULTS

Oxidative stress biomarkers

Oxidative stress of gasoline on brain cells in this study was expressed by protein oxidation or lipid peroxidation. Protein oxidation was determined by measuring protein carbonyl and advanced oxidation protein products (AOPP) levels in brain tissue homogenate. The protein carbonyl level in brain tissue homogenate as illustrated in table (1) was about three folds more than normal in the gasoline intoxicated group, but co-administration of green tea extract as a sole source of drinking water before gasoline inhalation with one week and along the time of the experiment were decreased the protein carbonyl level significantly by -63.1%, compared to gasoline alone group, there is no any significant differences in comparing to control.

On measuring advanced oxidation protein products (AOPP) the level of them were elevated by gasoline inhalation by 29.66% compared to control ($P<0.01$) and improved by the green tea administration. This means that green tea provides some protection for proteins against oxidative modification caused by gasoline inhalation (table 1). Thiobarbituric acid reactive substances (TBRS) or malondialdehyde (MDA) is a marker of lipid peroxidation, was used in this study to monitor the degree of modification occurred for lipid in response to gasoline inhalation and the protective role of green tea. In table (1) TBRS level in brain tissue homogenate of CD1 mice inhaled gasoline 2 hours daily for three weeks, showed a highly significant increase (115.43%) compared to control group ($P<0.01$), on the other hand, co-administration of green tea extract with gasoline inhalation resulted in levels of protection of lipid from oxidative stress of gasoline and eliminated this increase to reach only 35.11% for gasoline + green tea group compared to control. Consumption of green tea extract alone did not show any significant changes in levels of TBRS compared to control ($P>0.05$).

Table 1: Oxidative stress biomarkers in whole brain tissue of CD1 mice exposed to gasoline inhalation and effect of green tea

Animal group	Control Mean ± SD	Green tea Mean ± SD	Gasoline Mean ± SD	Gasoline + green tea Mean ± SD
Protein carbonyl group level(nM/gm wwt)	185.2 ± 19.03	179.2 ± 23.54	523.4** ± 68.05	193.1 ± 63.52
Advanced oxidation protein products (µM/gm wwt)	54.57 ± 6.503	54.92 ± 6.557	70.76** ± 0.8577	61.73 ± 4.795
Thiobarbituric acid reactive substances (nM/gm wwt)	278.6 ± 44.01	247.3 ± 74.75	600.2** ± 124.7	401.1** ± 35.11

(*) significant difference compared to control group (P < 0.05)
(**) highly significant difference compared to control group (P < 0.01)

Table 2: Non-enzymatic antioxidants in whole brain tissue of CD1 mice exposed to gasoline inhalation and effect of green tea

Animal group	Control Mean ± SD	Green tea Mean ± SD	Gasoline Mean ± SD	Gasoline + green tea Mean ± SD
Total thiol concentration (µM/gm wwt)	1.62 ± 0.112	2.028 ± 0.483	0.924** ± 0.0782	1.216** ± 0.177
Non protein-pound thiol (µM/gm wwt)	0.264 ± 0.0368	0.245 ± 0.0366	0.183** ± 0.0125	0.21 ± 0.0378
Protein-pound thiol (µM/gm wwt)	1.356 ± 0.075	1.783* ± 0.446	0.741** ± 0.065	1.006 ± 0.14

(*) significant difference compared to control group (P < 0.05)
(**) highly significant difference compared to control group (P < 0.01)

Table 3: Antioxidant enzymes in whole brain tissue of CD1 mice exposed to gasoline inhalation and effect of green tea

Animal group	Control Mean ± SD	Green tea Mean ± SD	Gasoline Mean ± SD	Gasoline + green tea Mean ± SD
Catalase (µM/min/gm wwt)	999 ± 184.7	916 ± 114.9	560.3** ± 58.74	830 ± 90.23
Glutathion peroxidase (µM/min/gm wwt)	1.888 ± 0.404	1.758 ± 0.306	1.258** ± 0.151	1.496 ± 0.0668
Glutathion-S-transferase (µM/min/gm wwt)	1.41 ± 0.107	1.36 ± 0.124	1.106** ± 0.11	1.145** ± 0.067
Superoxide dismutase (U/gm wwt)	139.5 ± 34.31	124 ± 32.29	67.63* ± 13.22	72.49* ± 15.82

(*) significant difference compared to control group (P < 0.05)
(**) highly significant difference compared to control group (P < 0.01)

Non-enzymatic antioxidants

Total thiol, nonprotein-pound thiol and protein-pound thiol was studied as non-enzymatic antioxidants in brain tissue homogenate and showed different degrees of responses to gasoline intoxication and treatment by using green tea. An intoxicated group with gasoline inhalation showed a highly significant (P<0.01) reduction in total thiol level (-42.96) compared to control. A simultaneous treatment with green tea reduced these changes to reach -24.93% compared to control. Green tea alone did not cause any significant change compared to control (table 2). On measuring nonprotein-pound thiol, was noticed that, by ingestion green tea extracts alone, these were not shown any significant changes compared to control. The gasoline inhalation toxicity causes a highly significant decrease (P<0.01) in nonprotein-pound thiol concentration compared to control by -30.64%, on the other hand green tea when consumed with gasoline inhalation diminished the effect of gasoline on nonprotein-pound thiol to reach the normal level(table 2).

The protective role of green tea and curcumin mainly expressed on the protein-pound thiol as illustrated in table (2) which showed a highly significant reduction in protein-pound thiol by inhalation of gasoline (2 hours daily for three weeks) by about the half of control (-45.35%) (P<0.01).The protein-pound thiol was protected and re-increased by green tea extract each only -25.81% for gasoline + green tea group compared to control. Green tea alone increased significantly the concentration of the protein-pound thiol.

Antioxidant enzymes

The influence of gasoline intoxication, green tea extract administration of antioxidant enzymes were illustrated in table 3.The gasoline intoxication resulted in a state of oxidative stress on the brain as manifested by the significant decline (P<0.01) in catalase enzyme activity by 43.91% compared to control, on the other hand green tea exerted its protective effect on this enzyme and reduced this decline to -16.91, only compared to control. On measuring glutathione peroxidase enzyme activity was noticed that gasoline inhalation (2 hours daily for 3 weeks) caused a highly significant decline (P<0.01) in enzyme activity (-33.36%) compared to controls. Green tea succeeded to overlap this effect (only -20.67% compared to control) (table 3).

Green tea extract failed to protect glutathione-S-transferase in brain tissue from oxidative stress of gasoline, which caused a reduction of glutathione-S-transferase activity by -21.56% compared to control, also by using green tea extract as a sole source of drinking water simultaneously with gasoline intoxication, the level of glutathione-S-transferase activity reduced by -18.79%, illustrated in table (3). Superoxide dismutase enzyme activity in brain tissue homogenate of CD1 mice intoxicated by gasoline inhalation and protected by using green tea extract illustrated in table (3), SOD activity was affected significantly (P<0.05) by gasoline inhalation with or without addition of green tea, in gasoline alone (-51.51), gasoline plus green tea (-48.03). Green tea extract supplementation alone did not affect on the activities of all previous enzymes.

DISCUSSION

Gasoline

Motor fuels are complex organic mixtures comprised of hundreds of specific compounds. Indicator compounds are usually defined as those compounds which can be considered the most toxic and, the most mobile in soil and groundwater. For these reasons, many cleanup standards or guidelines focus on benzene, toluene, ethylbenzene, and xylenes, commonly known as “BTEX.” The BTEX chemicals are volatile monoaromatic hydrocarbons which are commonly found together in crude petroleum and petroleum products such as gasoline. The BTEX compounds represent some of the most hazardous components of gasoline (MacDonald, 2000). They are also produced on the scale of megatons per year as bulk chemicals for industrial use as solvents and starting materials for the manufacture of pesticides, plastics, and synthetic fibers (Chen *et al.,* 2007).

One of the most common sources for BTEX contamination of soil and groundwater are spills involving the release of petroleum products such as oxygenated gasoline from leaking oil tanks. Because of the relatively high water solubility, these compounds will tend to be dissolved in the water phase or evaporated into the air spaces of the soil. Because of their relative hydrophilic nature, they are not attenuated very much by the soil particles or constituents and can be transported rather long distances if the right conditions are there. In some sites, some BTEX are found several kilometres downstream the source. The reason why the BTEX entering our soil and groundwater system are considered such a serious problem is that they all have some acute and long-term toxic effects. The major aromatic constituents of gasoline, collectively known as BTEX compounds, have high water solubility relative to the aliphatic constituents of gasoline.

Gasoline and Oxidative Stress in Brain and protective effect of green tea

The present study chooses brain tissues to assess the oxidative stress in CD1 mice exposed to benzene fumes. This is due to that the nervous system cells of both humans and animals are especially vulnerable to oxidative damage caused by free radicals for a number of reasons. These include high concentrations of readily oxidazible substrate, in particular membrane lipid polyunsaturated fatty acid, low level of protective antioxidant enzymes (catalase and glutathione peroxidase), high ratio of membrane surface area to cytoplasmic volume, and extended axonal morphology prone to peripheral injury (Daniel et al., 2004).In addition, some regions have high non-heme iron concentrations. Thus, antioxidative defense is critically important in nervous tissue protection. Growing fundamental and clinical data indicate that the redox state in neural structures plays a significant role in the pathogenesis of age-associated disorder observed in humans (Skrzydlewska *et al.,* 2005).

The brain contains particularly large amounts of polyunsaturated fatty acids and a high content of catalytically active metal ions, especially in the striatum and hippocampus (Rafalowska *et al.,* 1989). Thus, the brain tissue is particularly vulnerable to membrane lipid peroxidation that disturbs fundamental functions of the brain. Findings from numerous studies have shown that lipid peroxidation (an outcome of free radical generation) may be implicated in the irreversible loss of neuronal tissue after brain or spinal cord injury as well as in degenerative neurologic disorders (Siems *et al.,* 1996). The damage of nerve endings by peroxidation products may lead to large changes in neurotransmitter transport, resulting in an alteration of the function of the CNS (Ostrowska *et al.,* 2004). This process is regulated as a passive event ultimately because of ATP depletion, leading to failure of Na+/K+ ion pumps, secondary cell swelling, as well as lysis of intracellular components into surrounding tissue and a low concentration of ATP (below 15%), which can cause cell death (Siems *et al.,* 1996 and Ostrowska *et al.,* 2004).A relationship between oxidative stress and brain toxicity has been speculated in many experimental animal models (Daniel *et al.,* 2004; Ostrowska *et al.,* 2004; Skrzydlewska *et al.,* 2005). The implication of ROS in benzene toxicity was strengthened by the fact that many free radical scavengers provide marked functional and histopathological protection against benzene toxicity. Antioxidant defenses consist of three general classes, including water soluble reductants such as glutathione, fat soluble vitamins such as α-tocopherol and SOD, CAT, glutathione related enzymes (GST, GPx and GR) (Zhang et al., 2004). One of the important features of these latter enzymes is their inducibility under conditions of oxidative stress, and such induction can be an important adaptation to pollutant-induced stress.

In the present study, gasoline inhalation significantly decreased the activities of antioxidant enzymes SOD, CAT, GPx, and GST (table 3) and total thiol and protein-pound thiol concentrations (tables 2). This provides more evidence for the involvement of oxidative stress in a gasoline fume exposure. These changes lead to an enhanced lipid peroxidation and protein oxidation. It is well known that endogenous antioxidant enzymes and non-enzymatic antioxidants are responsible for preventing and neutralizing the free radicals-induced oxidative damage. These antioxidant enzymes, thiol groups and reduced glutathione are the major supportive team of defense against free radicals (Mohamadin *et al.,* 2005). In biological systems, antioxidant defense mechanisms are carried out by agents that prevent the noxious action of free radicals or other reactive oxygen species. These antioxidant enzymes are inducible enzymes. They can be induced by a slight oxidative stress due to compensatory response; however, a severe oxidative stress suppresses the activities of these enzymes due to oxidative damage and a loss in compensatory mechanisms (Halliwell and Gutteridge, 1986). Protein-pound thiol and nonprotein-thiol are the major cytosolic low molecular weight sulfhydryl compound that acts as a cellular reducing and a protective reagent against numerous toxic substances, including most inorganic pollutants, through the –SH group (Mosialou et al., 1993).Hence, thiol is often the first line of defense against oxidative stress. Thiol levels can be increased due to an adaptive mechanism by slight oxidative stress through an increase in its synthesis; however, a severe oxidative stress may decrease thiol levels due to loss of adaptive mechanisms.

In the present study the protein carbonyl, advanced oxidation protein products, and lipid peroxidation concentration were significantly increased in mice exposed to gasoline fumes (table 1). Protein carbonyl content is actually the most general indicator and by far the most commonly used marker of protein oxidation, and accumulation of protein carbonyls has been observed in several human diseases, including Alzheimer's disease, diabetes, inflammatory bowel disease, and arthritis (Dalle-Donne *et al.,* 2003; Almorth *et al.,* 2005). Lipid peroxidation products are formed with the abstraction of a hydrogen atom from an unsaturated fatty acid (Halliwell and Gutteridge, 1991).The lipid peroxidation process influences membrane fluidity as well as the integrity of biomolecules associated with the membrane (membrane bound proteins or cholesterol). These highly oxidizable lipids may, then, in turn, attack nearby proteins causing the formation of an excess of protein carbonyls (Almorth *et al.,* 2005).A major development over the past two decades has been the realization that free radical mediated peroxidation of membrane lipids and oxidative damage of DNA are associated with a variety of chronic health problems, such as cancer (Mukhtar and Ahmad, 2000), atherosclerosis (Tijburg *et al.,* 1997; Miura *et al.,* 2001), neurodegenerative diseases (Rafalowska *et al.,* 1988; 1989) and aging (Finkel and Holbrook, 2000). Therefore, inhibition of oxidative damage by supplementation of antioxidants becomes an attractive therapeutic strategy to reduce the risk of these diseases (Dai *et al.,* 2006).

A common occupational source of exposure to benzene is handling of gasoline. The oxidation of benzene in the liver by Cytochrome P4502E1 (CYP2E1) to form reactive intermediates such as benzene oxide, phenol, and hydroquinone is an initial step in the bioactivation of benzene and is a prerequisite for cellular toxicity (Snyder, 2002; 2004; Wan *et al.,* 2005). Benzene oxide can be hydrolyzed by microsomal epoxide hydrolase to benzene dihydrodiol that is then converted to catechol or can undergo ring opening to produce trans–trans-muconaldehyde or can spontaneously rearrange to form phenol, which is then hydroxylated in the liver to form hydroquinone. Evidence supporting ROS in benzene-initiated toxicity includes studies showing that mice treated with benzene, phenol, catechol or hydroquinone have elevated levels of oxidized DNA (Kolachana *et al.,* 1993; Faiola *et al.,* 2004).Furthermore, studies have shown that in vitro exposure to hydroquinone or benzoquinone causes a significant increase in ROS (Wan *et al.,* 2005; Badham and Winn, 2007). These data support a role for ROS in mediating benzene-initiated toxicity. In the present experiment the co-supplementation of green tea extract umin (tables 2, 3) were significantly reduced the damaging effect of benzene on enzymatic and nonenzymatic antioxidant in the brain tissue. The administration of green tea to mice in gasoline fume exposure resulted in normalization of lipid peroxidation process by reducing the TBARS level as a marker of lipid peroxidation in brain tissue. This might indicate the usefulness of green tea extract as excellent sources of antioxidants in modulating benzene-induced toxicity.

Some in vitro studies suggest that tea catechins function as powerful antioxidants, but their efficacy in altering in vivo antioxidant capacity is related to the amount ingested. The protective effect of antioxidant-rich diets in diseases involving oxidative damage has been reported. As a very rich source of polyphenols the strong antioxidant and oxygen radicals scavenging effects of tea have been documented (Camargo *et al.,* 2006; Farhoosh *et al.,* 2007; Jung et al., 2007).Green tea extract attenuates the oxidative stress of cyclosporine A on the kidney (Mohamadin *et al.,* 2005), alcohol on the liver (Ostrowska *et al.,* 2004), tamoxifen on liver (El-Beshbishy, 2005), and 4-Nitroquinoline 1-oxide-induced in vitro lipid peroxidation on liver homogenate (Srinivasan *et al.,* 2007). Arteel *et al.,* (2002) reported that simple dietary antioxidants, such as those found in green tea prevent early alcohol-induced liver injury by preventing oxidative stress. The work of Mohamadin *et al.,* (2005) proved the renoprotective potential of green tea extract in cyclosporine A-induced nephrotoxicity. Compounds of green tea scavenge a wide range of free radicals, including the most active hydroxyl radicals, which may initiate lipid peroxidation. Therefore, catechins may decrease the concentration of lipid free radicals and terminate initiation and propagation of lipid peroxidation. Catechins may chelate metal ions, especially iron and copper, which, in turn, inhibit the generation of hydroxyl radicals and degradation of lipid hydroperoxides, which causes the reactive aldehyde formation. Furthermore, the green tea polyphenols have been demonstrated to inhibit iron-induced oxidation of synaptosomes by scavenging hydroxyl radicals generated in the lecithin/lipoxidase system. The chelating effect of green tea results in a reduction of the free form of iron. Catechins, which are water-soluble antioxidants, could reduce the mobility of the free radicals into the lipid bilayer as well. Flavonoids preferentially enter the hydrophobic core of the membrane where they exert a membrane-stabilizing effect by modifying the lipid packing order (Arora *et al.,* 2000). They can penetrate the lipid bilayer, decreasing free radical concentration or influencing antioxidant capability in biomembranes (Saija *et al.,* 1995). Moreover, catechins can also interact with phospholipid head groups, particularly with those containing hydroxyl groups, so they could decrease the fluidity in the polar surface of phospholipid bilayer (Chen *et al.,* 2002).In addition, catechins prevent the loss of the lipophilic antioxidant α-tocopherol, by repairing tocopheryl radicals, and protection of the hydrophilic antioxidant ascorbate, which also repairs this radical (Skrzydlewska *et al.,* 2002a).

In such a way, they decrease the lipid peroxidation when membrane phospholipids are exposed to oxygen radicals from the aqueous phase. The oxidative attack from the aqueous phase seems to be an important reaction for initiating membrane lipid peroxidation. Perhydroxyl radicals, which are produced by the promotion of superoxide and whose concentration is enhanced during benzene intoxication, are regarded as one of the most likely radicals for initiating lipid peroxidation in vivo. Consequently, the slower pace of free radical reactions leads to inhibition of lipid peroxidation and, as a consequence, to a decrease in membrane fluidity (Tsuchiya, 1999). Augustyniak *et al.,* (2005) concluded that the use of green tea appears to be beneficial to rat liver by decreasing oxidative stress caused by ethanol and/or aging. Also the results are in agreement with Yamamoto et al. (2006) who indicated the protective effect of green tea catechins on mucosal oxidative stress and iron-induced lipid peroxidation, also with Farhoosh *et al.,* (2007) who detected the antioxidant activities of both green tea and black tea extracts and also with Jung *et al.,* (2007) who proved the neuroprotective effect of EGCG against nitric oxide oxidative stress in vitro.

Catalase, GPX, GST, and SOD activities were also inhibited by gasoline oxidative stress, but only catalase and GPX were improved by the antioxidant potential effect of green tea. These results are in agreement with Augustyniak *et al.,* (2005) in the ability of green tea to reduce the oxidative stress caused by ethanol and/or aging and elevated the activities of catalase and GPX, on the other hand, in the same study green tea could also to protect SOD and GST from oxidative stress caused by ethanol and/or aging this difference may be due to dose, species or toxin differences. Green tea also in the study of El-Beshbishi (2005) who established the role of green tea extract in facing the toxic effect of tamoxifen on catalase and GPX which showed a highly significant improvement with green tea compared to tamoxifen alone. The destructive effects of benzene on GST and GPX were reported by Jr *et al.,* (2005) study on rats exposed to a mixture of benzene, cyclohexanone and cyclohexan, the results of this study are in agreement with the present study.

CONCLUSION

This study concluded that, addition of green tea extract as a sole source of drinking to CD-1 mice, ameliorated and protect the antioxidant status in brain tissue against oxidative stress which induced by gasoline fume inhalation.

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