THE PROTECTIVE ROLE OF CURCUMIN AGAINST OXIDATIVE STRESS CAUSED BY GASOLINE

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ABSTRACT: 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 curcumin on these toxic effects of gasoline. Powdered curcumin was chosen as antioxidant and antitoxicity natural products. CD1 mice were taken as experimental model. Mice were exposed to gasoline vapor 2hours/day for 3 weeks in inhalation chamber. The concentration of gasoline is 9375 ppm and the concentration of benzene is 100 fold less than gasoline in equilibrium with pure benzene being 93.75 ppm. Powdered curcumin was added to the diet, these were taken before starting inhalation with one week and along the time of experiment till sacrificing the animals. Oxidative stress markers were measured in this study to determine the oxidative stress of gasoline and the protective and ameliorative role curcumin.

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 curcumin 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 curcumin.

3- Significant reduction in the activities of antioxidant enzymes which damaged by gasoline, some of them were improved with natural products and the others did not affect. Curcumin had improving effect on catalase and SOD.

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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 generate 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 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)

Curcumin is a dietary antioxidant derived from turmeric and the antiinflammatory, antimicrobial, antiviral, antifungal, antioxidant, chemosensitizing, radiosensitizing effects and wound healing activities have been proved (Okudan et al., 2013; Guzel et al., 2013; and Nazari et al., 2014)

Curcumin is a powerful scavenger of many free radicals such as anion, hydroxyl radical and nitric oxide (Elizabeth and Rao, 1990; Sreejayan and Rao, 1997 and Barzegar *et a*l., 2011). Jayaprakasha *et al*., (2006) demonstrated in vitro the antioxidant capacities and activities of curcumin, bisdemethoxycurcumin and demethoxycurcumin using the phosphomolybdenum method and linoleic acid peroxidation method. They reported that, by using phosphomolybdeum method curcumin, demethoxycurcumin and bisdemethoxycurcumin exhibited various degrees of antioxidant capacity. The antioxidant capacities of curcuminoids were found to decrease in the order: curcumin>demethoxycurcumin> bisdemethoxycurcumin. Also by using linoleic acid peroxidation method, they found the same orders of antioxidant activities of the three curcuminoid compounds.

Recent studies provide scientific evidence regarding the potential pharmacological, prophylactic or therapeutic use of Cur, as anti-inflammatory, anti-carcinogenic, anti-tumoral, anti-viral, antifungal, anti-parasitic, anti-mutagen, anti-infectious, anti-hepatotoxic and anti-oxidant compound (Chen et al., 2006; Aggarwal *et al*., 2007; Ciftci *et al*., 2010; 2011 and 2012; Shehzad *et al*., 2011 and Zhe *et al*.,2015).

In curcumin, the methoxy group seems to play a major role. The phenolic hydroxyl and the methoxyl group on the phenyl ring and the 1,3-diketone system seems to be important structural features that can contribute to these effects. The diketone system is a potent legand for metals such as iron. Another fact proposed is that the antioxidant activity increases when the phenolic hydroxyl group is at the ortho position with respect to methoxy group (Sreejayan and Rao, 1994). The photodynamic action of some drugs and pigments is also mediated through $_1O_2$. Light induced diseases including erythropoietic protoporphyria, pellagra and cataractogensis have been attributed in part to the toxicity of $_1O_2$. Thus, curcumin may be used in singlet oxygen-mediated diseases as a pharmacologic agent (Jayaprakasha *et al.,* 2005).

MATERIALS AND METHODS
Experimental animals
Sixty male mice (*Mus musculus*) weighting 20 – 25 g were 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 curcumin in the diet
The dried ground rhizomes of *Curcuma longa* were purchased from local market in Cairo, Egypt, grinded, powdered, and added to the diet of mice, 30gm to 1Kg of diet to form concentration of 3% (Conney et al., 1997).

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 10cm distance from the bottom of box, a wire mesh shelf 70x70 cm was fixed to put the mice on it. Under this shelf a 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 12.00 am 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 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 aromatics (5–55%), distilling in the approximate range of 30°C–220°C. Composition of gasoline varies with 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/m^3 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 aromatics, 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 follow:

1- ***Control group***, received only the ordinary mice diet and drinks water without any additions and kept two hours daily in the inhalation chamber without gasoline for three weeks.
2- ***Curcumin group***, these animals received powdered dried ground rhizomes of *Curcuma longa* (turmeric) in the diet (3%) and kept two hours daily in the inhalation chamber without gasoline for three weeks.
3- ***Gasoline inhalation group***, this is the intoxicated group with gasoline inhalation, these mice were kept 2 hours daily in inhalation chamber with gasoline for three weeks. This group drinks water and eat the ordinary diet.
4- ***Gasoline and curcumin group***, this group exposed to gasoline in the inhalation chamber, 2 hours daily for three weeks and received powdered dried ground rhizomes of *Curcuma longa* in their ordinary diet along the time of the experiment and drinks water.

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 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 6000 xg in cooling centrifuge (Hettich, Germany) at 4 °C for 15 min. The protein supernatant was separated in another clean and dry Eppendorf tubes 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 reaction mixture contained 50 ml phosphate buffer + 50µl H$_2$O$_2$ and the reaction were 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 H$_2$O$_2$ = 0.04 mM^{-1} cm^{-1} at 240 nm).

Glutathione peroxidase (GPX) Activity

In this method GPX catalyzes the reduction of H$_2$O$_2$ 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 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. H$_2$O$_2$ (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 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 inhibition of NADH oxidation by β-mercaptoethanol in the presence of EDTA and Mn. One unites 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 dinitrophenyl hydrazones.

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 of Sedlak and Lindsay (1968).

Estimation of non protein-pound thiol:

Non protein-pound thiols in the tissue homogenate were determined as the method of 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 Dunnett test to detect the significance of differences between each group and control. All analysis and graphics were performed by using, INSTAT and graph Pad 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 gasoline intoxicated group, but co-administration of curcumin with 3% of the diet before gasoline inhalation with one week and along the time of the experiment were decreased the protein carbonyl level significantly by -60.2% compared to gasoline alone group, there is no any significant differences on 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 curcumin administration. This means that curcumin 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 curcumin.

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 curcumin in the diet with gasoline inhalation resulted in levels of protection to lipid from oxidative stress of gasoline and eliminated this increase to reach only 43.93% for gasoline+curcumin group compared to control.

Consumption of curcumin 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 curcumin

Animal group	Control Mean ± SD	Curcumin Mean ± SD	Gasoline Mean ± SD	Gasoline + Curcumin Mean ±SD
Protein carbonyl group (nM/gm wwt)	185.2 ± 19.03	186.6 ± 71.29	523.4** ± 68.05	208.3 ± 51.77
Advanced oxidation protein products (µM/gm wwt)	54.57 ± 6.503	55.27 ± 7.747	70.76** ± 0.8577	58.84 ± 8.156
Thiobarbituric acid reactive substances (nM/gm wwt)	278.6 ± 44.01	275.2 ± 75.46	600.2** ± 124.7	367.4* ± 43.93

(*) 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 were studied as non-enzymatic antioxidants in brain tissue homogenate and showed different degrees of responses to gasoline intoxication and treatment by using green tea or curcumin.

Intoxicated group with gasoline inhalation showed a highly significant (P<0.01) reduction in total thiol level (-42.96) compared to control. Co-administration of curcumin in the diet with gasoline inhalation also reduced the change in total thiol to reach normal level. Total thiol concentration was elevated in response to administration of curcumin alone by 32.59% (P<0.05) (table 2).

On measuring nonprotein-pound thiol, was noticed that, by ingestion curcumin alone these were not showed any significant changes compared to control. Gasoline inhalation toxicity causes a highly significant decrease (P<0.01) in nonprotein-pound thiol concentration compared to control by -30.64% (Table 2). The protective role of curcumin mainly expressed on 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). Protein-pound thiol was protected and re-increased by curcumin to reach only 11.4% for gasoline+curcumin group compared to control. Curcumin alone increased significantly the concentration of protein-pound thiol.

Antioxidant enzymes

The influence of gasoline intoxication, and curcumin administration on antioxidant enzymes were illustrated in table 3. In table (3) the gasoline intoxication resulted in a state of oxidative stress on brain as manifested by the significant decline (P<0.01) in catalase enzyme activity by 43.91% compared to control, on the other hand curcumin exerted their protective effects on this enzyme and reduced this decline to -17.63 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 control. Curcumin failed to prevent this decrease of glutathione peroxidase activity (table 3).

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

Animal group	Control Mean ± SD	Curcumin Mean ± SD	Gasoline Mean ± SD	Gasoline + Curcumin Mean ±SD
Total thiol concentration (μM/gm wwt)	1.62 ± 0.112	2.148* ± 0.474	0.924** ± 0.0782	1.77 ± 0.29
Non protein-pound thiol (μM/gm wwt)	0.264 ± 0.0368	0.253 ± 0.0336	0.183** ± 0.0125	0.198* ± 0.0274
Protein-pound thiol (μM/gm wwt)	1.356 ± 0.075	1.895* ± 0.441	0.741** ± 0.065	1.51 ± 0.53

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

Curcumin 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 curcumin 3% in the diet simultaneously with gasoline intoxication, the level of glutathione-S-transferase activity reduced by -24.96% as illustrated in table (3).

Superoxide dismutase enzyme activity in brain tissue homogenate of CD1 mice intoxicated by gasoline inhalation and protected by using curcumin in diet was illustrated in table (3), SOD activity was affected significantly (P<0.05) by gasoline inhalation with or without addition of curcumin to the diet, in gasoline alone (-51.51), and gasoline plus curcumin (-38.66). Only by curcumin the oxidative stress of gasoline on SOD was reduced and the enzyme's activity re-increased by 26.51% in compared to gasoline alone group. Curcumin supplementation alone did not affect on the activities of all previous enzymes.

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

Animal group	Control Mean ± SD	Curcumin Mean ± SD	Gasoline Mean ± SD	Gasoline + Curcumin Mean ±SD
Catalase (μM/min/gm wwt)	999 ± 184.7	918.6 ± 101.8	560.3** ± 58.74	822.8 ± 131.4
Glutathion peroxidase (μM/min/gm wwt)	1.888 ± 0.404	1.737 ± 0.35	1.258** ± 0.151	1.291** ± 0.189
Glutathion-S-transferase (μM/min/gm wwt)	1.41 ± 0.107	1.36 ± 0.124	1.106** ± 0.11	1.058** ± 0.158
Superoxide dismutase (U/gm wwt)	139.5 ± 34.31	131.2 ± 32.54	67.63* ± 13.22	85.56* ± 1.533

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

DISCUSSION

The present study chooses brain tissues to assess the oxidative stress in CD1 mice exposed to gasoline 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 concentration 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 (tables 3) and total thiol and protein-pound thiol concentrations (tables 2). This provides more evidence for the involvement of oxidative stress in 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 to 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 (tables 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 P450 2E1 (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 include 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.

Co-administration of curcumin with gasoline fume inhalation resulted in decreased lipid peroxidation and improved antioxidant status. This may be due to the antioxidant sparing action of curcumin. The presence of π conjugation in curcumin makes it more hydrophobic. As a result curcumin get localized in the lipid bilayer membrane. Curcumin, being lipid soluble, reacts with the lipid peroxyl radicals and acts as a chain terminating antioxidant. It has also been known to inhibit radiation induced lipid peroxidation in rat liver microsomes (Khopde et al., 2000). Curcumin possesses distinct structural motifs that are responsible for its antioxidant activity. The presence of electron donating groups like phenolic hydroxyl groups and a β-diketone structure is responsible for the free radical scavenging activity and inhibiting lipid peroxidation (Srinivasan et al., 2006)

In the present study about the role of curcumin in reducing the level of oxidation biomarkers for lipid and proteins which elevated by gasoline intoxication we found the highly potential effect of curcumin to reduce these parameters which in agree with Wei et al. (2006a) who discussed that curcumin and many of its analogues could effectively inhibit the free radical induced lipid peroxidation and protein oxidative damage of rat liver mitochondria by H-atom abstraction from the phenolic groups. Also in agreement with the in vitro study of Jayaprakasha et al. (2006) which established the antioxidant potencies of individual curcuminoids by using the phosphomolybdenum method and linoleic acid peroxidation method, and also with the study of Chattopadhyay et al. (2006) on the gastroprotective effect of curcumin against indomethacin-induced gastric ulcer caused by reactive oxygen species by efficient removal of H_2O_2 and H_2O_2-derived OH by preventing peroxidase inactivation by indomethacin.

In the present study on curcumin's antioxidant properties against gasoline oxidative stress, was proved that curcumin has protective effect for total thiol, catalase and SOD, this team of antioxidant defense were improved by co-administration of curcumin with gasoline inhalation, these results are in agreement with the studies of Srinivasan et al. (2006) which established the protective effect of curcumin on SOD, catalase and GPX in lymphocytes exposed to γ ray in different concentrations. The study of Murugan and Pari (2006) also demonstrated the protective role of curcumin for SOD, catalase, GPX, GST and reduced glutathione from the oxidative stress caused in streptozotocin-nicotin amide-induced diabetes. The differences between these two studies and the present study in protecting GPX and GST may be due to species, toxin, or organ differences. Also the study of Farombi and Ekor (2006) had proved the protective role of curcumin on GPX, catalase and GSH against gentamicin-induced renal oxidative damage in rats. This study disagrees with the present study in SOD which did not protected by curcumin.

Studies have shown that curcumin significantly enhance the synthesis of antioxidant enzymes such as SOD, CAT and GPx in rat liver (Reddy et al., 1994). Dinkova-Kostava et al. (2001) have also reported that curcumin and several other structurally related polyphenolic compounds induce the activities of phase II detoxification enzymes, which appear to be crucial in protection against carcinogenesis and oxidative stress.

The specific chemical structure may play a crucial role in preferential affinity towards selective cysteine residues of targeted proteins that control the gene expression. Thus we suggest that the position of the hydroxyl groups in the curcumin may play an important role in the induction of antioxidant enzymes.

CONCLUSION

This study concluded that, addition of curcumin by 3% to the diet of CD-1 mice, ameliorated and protect the antioxidant status in brain tissue against oxidative stress which induced by gasoline fumes inhalation.

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