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Protective Effect of Ginger (*Zingiber officinale*) Extract on Gasoline-induced Oxidative Stress in Albino Rats

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ABSTRACT

There were many attempts to find a way to protect humanity from leaded gasoline pollution which causing harmful effects to the human health, generating massive amount of reactive oxygen species in body organs including eyes. The present study mainly aimed to investigate whether ginger (*Zingiber officinale*) aqueous extract protects rat's eyes from oxidative stress induced by leaded gasoline. Sixty male adult albino rats (120-150 gm) were divided into 10 groups (n=6) inside inhalation chambers. Control group (G1) subsisted without any special treatment. Gasoline groups (G2-G5) inhaled leaded gasoline with nominal concentration of 18.18 ppm for exposure times 3, 6, 9 and 12 hrs/days for 14 days. Ginger group (G6) orally received 100 mg of ginger/kg once/day for 14 days. Protection groups (G7-G10) inhaled gasoline (like groups 2-5) and orally receiving 100 mg/kg ginger once/day for 14 days. After sacrificing animals, lead level, lipid peroxidation (MDA), protein carbonyl (PCC) and glutathione (GSH) content were determined in blood and eyes. The gasoline exposure groups revealed a significant increase in blood-lead level, blood and eye's contents of both MDA and PCC, and significant decrease in GSH content in blood and eyes. On the other hand, ginger reduced the gasoline effects in blood and eye's MDA, PCC and blood-lead contents, and elevated GSH level of eyes. Accordingly, ginger may have a remarkable protective role against oxidative stress induced by leaded gasoline exposure.
Keywords: leaded gasoline, ginger, inhalation, blood, eyes, oxidative stress, albino rat.

INTRODUCTION

Pollution from motor vehicles is one of the most ubiquitous environmental health problems of many communities (Cohen, *et al*., 2004). Petroleum derivatives are major environmental pollutants widespread in such a way that human exposure occurs. Gasoline is a complex mixture of relatively volatile hydrocarbon which contains over 500 saturated or unsaturated hydrocarbons having from 3 to 12 carbons (Sittig, 1984). The use of gasoline in the industries and homes has rapidly increased in the recent times. In the course of usage, individuals are frequently exposed to pollutants from gasoline fuel in both outdoor and indoor environments. Because of the predominant role of gasoline as a motor vehicle fuel, the effects of gasoline engine emissions are potentially greater problem. Millions of people are daily facing a major toxic risk by breathing exhaust fumes and evaporative emissions, and occasional skin contact from spills (Wixtrom and Brown, 1992).

Eyes are the most sensitive organs toward the oxidative damages caused by exhaust, toxics and smokes (Guthrie *et al*., 2014). Those oxidative damage in ocular tissues have been hypothesized to play a role in diseases such as cataract, glaucoma, uveitis, and age-related macular degeneration (Ohia *et al*., 2005), intraocular inflammatory diseases (Rose *et al*., 1998). Vision loss results from damage inflicted by the inflammatory cell infiltration into the retina (Rao and Wu, 2000). Retina is considered to be the tissue most susceptible to oxidative stress. This makes the mitochondria an excellent target for reactive oxygen species (ROS) (Forrest and Futterman, 1972).

Currently, various medicinal plants (including ginger; *Zingiber officinale*) are occasionally used for curing many diseases and health disorders instead of synthetic materials. The rhizomes of ginger contain over 20 phenolic compounds which have been reported to display diverse biological activities such as antioxidant, antiiflammatory, anticarcinogenic, antidiabetic, hypoglycemic, hypolipidmic and aldose reductase inhibitory properties (Weider and Sigwart, 2000; Ojewole, 2006, Al-Amin *et al*., 2006; White, 2007 and Islam and Choi, 2008). Moreover, several studies reported protective role of ginger against toxicity induced by xenobiotics (Khaki and Khaki, 2010; Haleagrahara *et al*., 2010). For its biological and therapeutic activities, ginger has been effective against development of diabetic cataract in rats (Saraswat *et al*., 2010).

In this regard, the present study was designed to examine oxidative stress elicited in rat eyes and blood after exposure to leaded gasoline vapour and to evaluate possible protective role of ginger.

MATERIAL AND METHODS

Animals and ethics

Male *albino* rats (120–150 g) were housed in polyethylene cages (65cm×25cm×15cm), under conditions of controlled humidity (22±2 °C) and on a 12 h-light/dark cycle, with free access to standard laboratory rat *ad libitum* and water. All procedures relating to care and maintenance of the animals were in accordance with International Guiding Principles for Animal Research and were overseen and approved by the Suez Canal University Bioethics and Animal Ethics Committee.

Gasoline and preparation of ginger extract

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Gasoline was supplied by Misr petroleum station octane 95 (lead content 0.037mg/l) according to the company manuscript.

Aqueous extract of *Zingiber officinale* rhizome was prepared according to Fatehi-Hassanabad, *et al*. 2005. The fresh ginger rhizomes were purchased from a local store and grounded in a grinder. 100 mg of ginger powder were dissolved in 5 ml boiled water and filtrates to be administrated by using oral intragastric syringe for each animal of experiment.

Experiment design

Each group of the experimental animals were housed individually in 10 L plastic cages (Fig. 1) with a stainless steel wire mesh at the bottom and the top of the cage (B). At the top of the cage covered with piece of glass contain fans (C). Beneath this cage, the gasoline container of 10 L was placed (A) with nominal concentration equals 18.18 ppm and actual concentration is from 0.1 ppm to 17.8 ppm after t99 time equals 30 sec. (the required time during which the vapours concentration would reach 99% inside a chamber). Both living cage and gasoline container were placed and operated dynamically in a laminar hood (D) with volume (60 x 90 x 45) cm3 at a calibrated airflow rate of approximately 550 L/min. Chamber size and airflow rates were adequate for an animal-loading factor below 5% and an oxygen level above 19%. Animal didn't receive food or water during the exposure time.

Rats were divided to ten groups (6 animals/group), four of them were exposed to gasoline vapours for different exposure times (3, 6, 9 and 12 hours/day) for 14 days. Another four groups with the same treatments were received oral dose equals 100 mg/kg of ginger extract solution daily just before applying the gasoline inhalation. A control group subsisted without any special treatment. Six rats of a positive control group were exposed to fresh air and orally received 100 mg/kg ginger extract solution for 14 days. After sacrificing animals, various biochemical parameters [(lipid peroxidation (MDA), protein carbonyl (PCC) and glutathione (GSH)] have been estimated in blood and dissected eyes

Figure (1): Dynamic inhalation chamber and laminar hood. (**a**) Gasoline container, (**b**) living cage, (**c**) fans of living cage and (**d**) laminar hood.

Cytotoxic assays

2.4.1. Blood collection and tissue homogenate

After collecting the blood samples using orbital sinus technique (Sanford, 1954), rats were sacrificed, and eyes were collected from each, washed by saline solution dried by blotting between filter papers, and weight. The eye tissue was washed with 0.15 M KCl, and homogenized in 2 ml of cold 0.15 M KCl using cooling homogenizer for 3 minutes.

Lipid peroxidation assay (MDA)

Lipid peroxidation was determined in blood plasma and eye homogenate by the method of Satoh (1978), using a spectrophotometer assay kit (Biodiagnostic, Cat no. MD2529, Egypt), by measuring a chromogenic reagent (Thiobarabituric acid) in acidic medium at 95oC for 30 min. with the breakdown products of peroxidized lipids (e.g. malonyldialdehyde) and measuring the absorbance of breakdown products at 532 nm. The results were expressed as nMol of malonyldialdehyde (MDA)/mL plasma. Malonaldehyde bis (dimethyl acetal) (Sigma) was used as an external standard

Protein carbonyl assay (PCC)

Plasma and eye protein carbonyl content, as a marker of protein oxidation, was measured according to Reznick and Packer (1994). Protein was precipitated with an equal volume of 1% trichloroacetic acid (TCA) and the pellet was resuspended in 1 mL of 2,4-dinitrophenylhydrazide (DNPH (Sigma), 10 mM, dissolved in 2 N HCl). Samples were left at room temperature for 1 h in the dark and vortexed every 15 min. An equal volume of 20% TCA was added and after centrifugation (12,000 g, 1 min, 4 °C), pellets were washed three times with 1 mL of an ethanol: ethyl acetate mixture (1:1) to remove the free DNPH. The final pellet was dissolved in 1 mL of 6M guanidine and kept at 37 °C for 1 h in a shaking water bath. The solution was centrifuged (12,000 g, 15 min) and the carbonyl content (nmol/mg) measured as protein phenylhydrazone derivatives, and was determined at 370 nm using an absorption coefficient of 22,000 M−1 Cm1.

Reduced glutathione content (GSH)

The reduced GSH content of blood and eye homogenate of control and treated rats was estimated according to the method of Beutler *et al*. (1963). Aliquots of 0.2 mL of blood or tissue homogenate were added to 1.8 mL distilled water and 3 mL of precipitating solution (1.67 g glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 mL distilled water). The mixture was then centrifuged (2200 g, 15 min, 4°C). One milliliter of supernatant was added to 4 mL Na_2HPO_4 (0.3 M) and 0.5 mL dithiobis-2-nitrobenzoic acid reagent (DTNB, Sigma-Aldrich) (40 mg DTNB in 100 mL 1% sodium citrate) and the absorbance was measured at 412 nm. Glutathione reduced form (Sigma) was used as a standard.

Statistical analysis

Results were represented as means ± standard error of mean. The data were statistically analyzed using SPSS software (Statistical Package for social Science, version 13.01) (Dancey and Reidy, 2002). Tabulation and graphics of data were done using Microsoft and Excel XP. Differences in the effects of gasoline and ginger between control and treated groups of rats were assessed using the Student's unpaired t-test (Snedecor, 1956). One-way analysis of variance (ANOVA) was performed to evaluate the eventual significant differences in the measurements between control and treated groups. The probability criterion for significance for each statistical test was P≤0.05

RESULTS

The effects of gasoline inhalation (18.18 ppm) and ginger oral administration (100 mg/kg B.wt) on the oxidative stress biomarkers (MDA and PCC) and reduced GSH in rat eyes and blood are presented in Figs. 2A, B; 3A, B and 4A, B respectively.

P<0.05) using one-way ANOVA

Results in Figure (2A) illustrate the content of plasma lipid peroxidation products in control, gasoline-inhaled, ginger-received and gasoline/ginger groups at different time intervals (3, 6, 9 and 12 hours). It was noticed that the levels of MDA were significantly increased (P<0.05) in all time intervals in gasoline groups, as well as gasoline/ginger groups but for less extent, comparing to control values. The one-way ANOVA analysis (Table 1) between gasoline groups revealed significant difference (F3,4=4.471, P<0.05), but no significant difference was found between gasoline/ginger groups (F3,4=1.280, P<0.05) in the level of plasma MDA products. In Figure (2B) which illustrates the lipid peroxidation content of eyes in control and treated groups at different time intervals, the results showed that the MDA level were significantly increased in gasoline groups after all time intervals, and were significantly increased in gasoline/ginger groups only after 6 h. treatment (P<0.05), with respect to control groups. Significant differences in eye MDA contents were observed between gasoline groups and between gasoline/ginger groups (F3,4=4.232, and F43,4=3.925 respectively, *P*<0.05) using one-way ANOVA.

Protein carbonyl contents in blood plasma and eye of rats for control and treated groups were illustrated in Figures (3(A and B), respectively. Plasma PCC in rats inhaled gasoline were significantly increased in all time intervals, and after 9 and 12h. of gasoline/ginger groups (P<0.05) as compared to control group. By using one-way ANOVA, highly significant difference was found in PCC of rat's plasma after inhaling gasoline (F3,4=25.124, P<0.05), and significance difference between gasoline/ginger groups (F3,4=5.552, P<0.05). Similarly, in Figure (3B), PCC in eyes of gasoline groups was significantly different after all time intervals, and after 9 and 12h. in gasoline/ginger groups (P<0.05), comparing to control group. One-way ANOVA revealed highly significant difference between gasoline groups (F3,4=17.719, P<0.05) and significant difference between gasoline/ginger groups (F3,4=6.964, P<0.05).

The concentrations of reduced GSH in blood were significantly decreased in gasoline groups after all time intervals except 3h. post treatment (P<0.05) as illustrated in Figure (4A); while in gasoline/ginger groups didn't show any significant difference (P<0.05) at any time interval and returned to control values. One-way ANOVA support this result, that gasoline groups revealed significant difference (F3,4=25.124, P<0.05), but gasoline/ginger groups showed none. Contrary, GSH levels in eyes of gasoline groups were increased significantly (P<0.05) after all time intervals, but significant increase appeared after 3 and 6h. post treatment with gasoline and ginger (P<0.05).

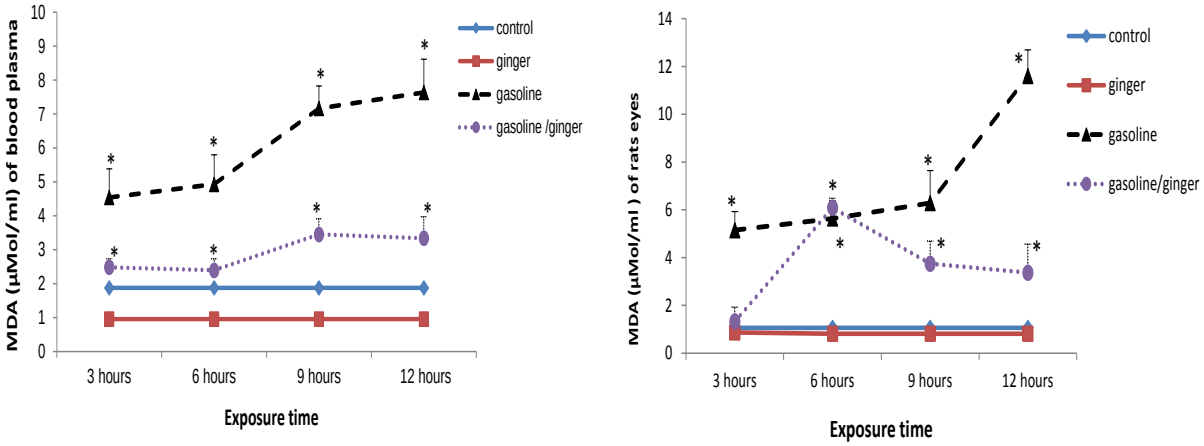

Figure (2): Effects of leaded gasoline inhalation (18.18 ppm) and orally receiving ginger rhizome extract (100 mg/Kg) on lipid peroxidation content (MDA µMol/ml) of rats at different time intervals. (A) on blood plasma, (B) on eyes. **(*)** represents a significant difference between control and treated groups using Student Unpaired t-test ($p < 0.05$).

Significant differences were detected in reduced GSH in eyes of both gasoline groups (F3,4=10.789, P<0.05) and gasoline/ginger groups (F3,4=10.228, P<0.05), using one-way ANOVA

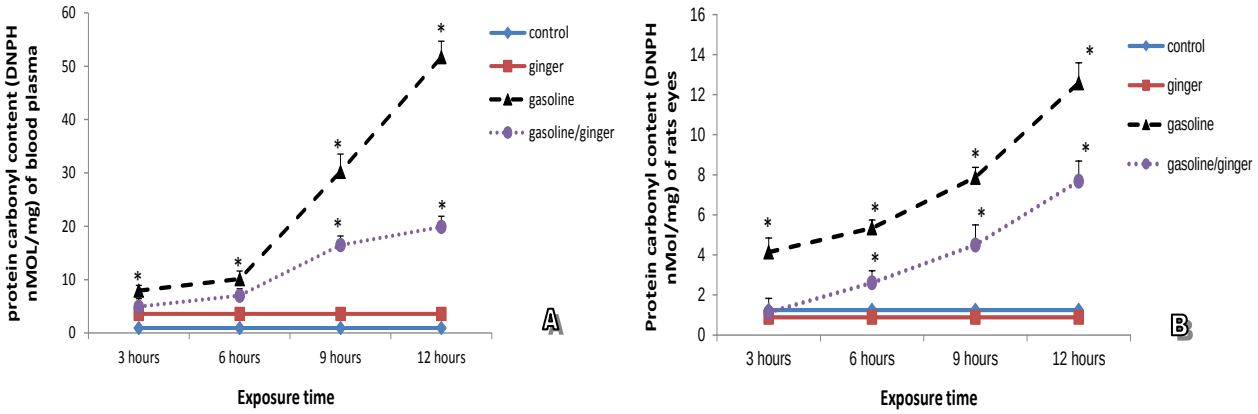

Figure (3): Effects of leaded gasoline inhalation (18.18 ppm) and orally receiving ginger rhizome extract (100 mg/kg) on Protein carbonyl content (DNPH nMol/mg) of rats at different time intervals. (A) on blood plasma, (B) on eyes. **(*)** represents a significant difference between control and treated groups using Student Unpaired t-test ($p < 0.05$)

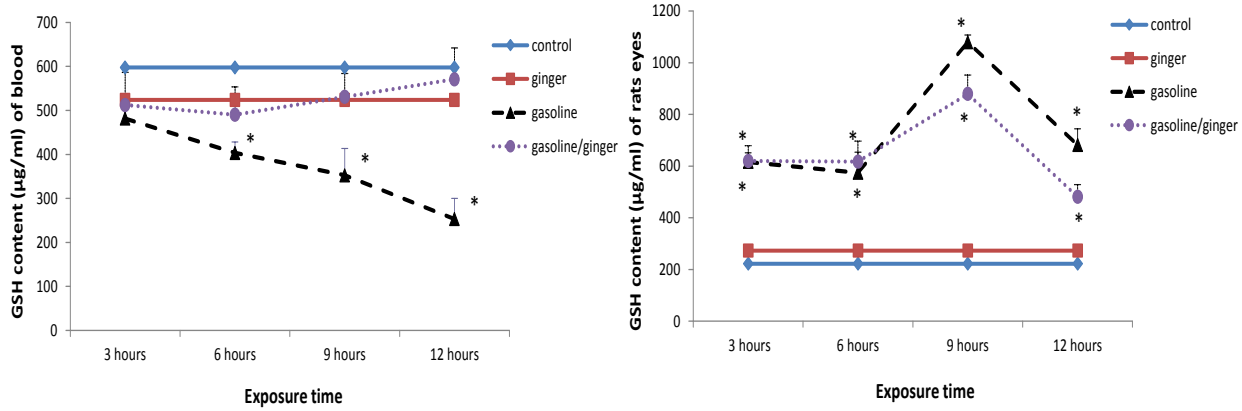

Figure (4): Effects of leaded gasoline inhalation (18.18 ppm) and orally receiving ginger rhizome extract (100 mg/kg) on Blood GSH content (µg/ml) of rats at different time intervals. (A) on blood, (B) on eyes. (*) represents a significant difference between control and treated groups using Student Unpaired t-test ($p < 0.05$).

Table (1): Change in the biomarkers, reduced glutathione and Lead levels after administration of gasoline (18.18 ppm) and ginger (100 mg/kg) using one-way ANOVA. (*) represents a significant difference between control and treated groups using Student Unpaired t-test ($p < 0.05$)

Parameter	Organ	Gasoline	Gasoline/Ginger
Lipid peroxidation content	Blood plasma	4.471 *	1.280
	Eye	4.232 *	3.925 *
Protein carbonyl content	Blood plasma	25.124 *	5.552 *
	Eye	17.719 *	6.964 *
Reduced Glutathione level	Blood	5.633 *	1.121
	Eye	10.789 *	10.228 *
Lead level	Blood	34.950 *	1.085

DISCUSSION

In the last decades, many attempts have been made aiming the assessment of health impact of gasoline vapours and other petroleum fumes in human and experimental animals (Uboh *et al*., 2005 and Akinosun *et al*., 2006). Pranjic *et al*., 2002; Lewne *et al*., 2006 and Azeez *et al*., 2012 suggested that, xenobiotics, like metals, quinones, nitroaromatic compounds, are subjected within the living organisms to many and divergent biotransformational reactions in order to be excreted easily from the body. One of these reactions is redox cycling reaction, and one of its unwanted byproducts is ROS or oxyradicals (Premereur *et al*., 1986). Some of the main manifestations of oxidative damage are lipid peroxidation and protein carbonyl contents. The potential subsequences of peroxidative process on lipids of cellular membrane include unsaturated fatty acids loss, modified membrane permeability by conformational changes in ion transport system and associated enzymes, liberating subcellular components and generation of lipid cytotoxic metabolites of lipid hydroperoxide (Rice-Evans, 1994). Similarly, protein oxidative changes lead to different functional consequences, such as inhibition of enzymatic activities, increased susceptibility to proteolysis and aggregation, and modified immunogenicity (Shacter, 2000). Stadtman and Levine (2000) reported that lipid peroxidation and protein oxidation occur too soon following cells exposure to oxidative stress, retiring consequent cellular death. Accordingly, aerobic organisms have evolved defense systems participate in the reactive radical removal, composed of antioxidant scavengers (glutathione, carotenoids and vitamin E and C) and specific antioxidant enzymes like catalase and superoxide dismutase (Di Giulio *et al*., 1989).

Oxidative stress may take place when the balance between the ROS synthesis rate and ROS inactivation rate by endogenous antioxidant scavenging system is disturbed by excess production of these ROS as a result of heavy existence of different xenobiotics (Gutteridge and Mitchell, 1999), causing detrimental cellular damage which lead to pathogenesis of major diseases such as atherosclerosis, cancer (Knight, 1995), and specific neurological disorders (Jenner, 1994). Fortunately, a plant-based diet with high intake of fruits, vegetables, and other nutrient-rich plant foods may repair this redox imbalance and reduces the risk of oxidative stress-related diseases (Joshipura *et al*., 1999 and 2001; Riboli et al., 2003; Johnson, 2004 and Stanner et al., 2004). One of these plant food is ginger (*Zingiber officinale*) rhizomes, which consumed worldwide as a spice and flavouring agent. These rhizomes contain many natural bioactive compounds with therapeutic potentiality, as 6-gingerol that has anti-angiogenic ability to resist many types of cancer especially the colonic one (Brown *et al*., 2009). The other medicinal properties attributed to ginger include anti-arthritic (Srivastava and Mustafa, 1992; Bliddal *et al*., 2000), anti-thrombotic (Thomson et al., 2002), anti-migraine (Cady et al., 2005), anti-inflammatory (Thomson et al., 2002; Penna *et al*., 2003), anti-nausea properties (Portnoi *et al.,* 2003) and hypolipidemic (Fuhrman *et al*., 2002 and Bhandari *et al*., 2005).

Our results revealed significant increase of lipid peroxidation and protein carbonyl contents in both blood and eyes after treatment with gasoline. This remarkable elevation in oxidative stress biomarkers could be an evidence of excessive production of ROS as a byproduct of redox cycling reaction of gasoline at the animal bodies. This may be due to reasons such as the presence of abundant mitochondria at ocular tissues (Adler, 1990), which consumes large amounts of oxygen resulting in production of free radicals and high amounts of polyunsaturated fatty acids. On the other hand, a remarkable elevation of GSH was noticed in eyes, and slight decreased in blood of gasoline-treated animals. This may be considered as a host defense response to antagonist the harmful effects of produced ROS. While the results of gasoline treatment after ginger oral administration showed a degree of increase in oxidative stress biomarkers in both blood and eyes less than the previous treatment, and significant increase in GSH, specially of blood, giving an initial impression that ginger administration can diminish the deleterious effects of ROS induced by gasoline inhalation, and protects the cells from oxidative stress damage.

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