



Gasoline inhalation induces perturbation in the rat lung antioxidant defense system and tissue structure

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ABSTRACT

Emissions of gasoline and its combustion products are considered major air pollutants with adverse effects on the respiratory system. The effects of exposure to the vapors of two kinds of motor gasoline (vehicle fuel in Egypt), one leaded (G_1) and the other unleaded (G_2), on rat lungs were investigated. The studies involved the ultrastructural alterations as well as the oxidative stress burden. Long term inhalation of $1/5$ LC_{50} of either G_1 or G_2 for 30 minutes daily along six consecutive weeks caused intensive histological alterations in the lining epithelial cells of the bronchioles. These changes included detachment and necrosis of the epithelial cells. Some bronchioles were even clogged with neoplastic cells. The electron micrographs of the bronchioles epithelial cells revealed dilatation of the smooth endoplasmic reticulum, loss of the secretory granules in the Clara cells and loss of cilia in the ciliated cells that exhibited bleb formation. In addition, prominent nuclear alterations were also seen in both types of cells. Necrotic type II pneumocytes, exhibited vacuolation and fragmentation of the rough endoplasmic reticulum and degeneration of mitochondria. Nuclear alterations and degeneration of lamellar bodies, along with microvillar atrophy were also observed in type II pneumocytes. A significant increase in the lung total (TGSH) and oxidized (GSSG) glutathione content as well as in the level of lipid peroxidation (LP) were found. The activities of the antioxidant enzymes glutathione- S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) were also elevated. On the other hand, a decline in the superoxide dismutase (SOD) activity and reduced glutathione (GSH) content were found. In conclusion, gasoline vapour inhalation induced lung tissue injury and cellular damage concomitant with impairment of the lung antioxidant defense system. These effects were more pronounced with the unleaded than with the leaded gasoline. This was probably attributed to the chemical additives that substitute lead to raise the octane number of the unleaded car fuel.

1. INTRODUCTION

Gasoline, a main vehicle fuel, is a multicomponent compound which contains a complex combination of paraffin and hydrocarbons such as benzene, hexane, toluene, xylene, ethyl alcohol and ethyl benzene. Hydrocarbons present in gasoline include a variety of branched and unsaturated aliphatic as

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well as aromatic compounds (Hartley *et al.*, 1992; Liu *et al.*, 2000; Brcic, 2004; Baroja *et al.*, 2005).

The characteristics of gasoline depend upon the origin of crude oil, differences in processing techniques and the blending scheme. The present trend of increasing the aromatic content of gasoline, in order to decrease the amount of lead in the environment, may represent a hazard that might be difficult to observe in the exposed population. Atmospheric concentration of gasoline of approximately 2000 ppm is considered unsafe and exposure to extremely high level may result in dizziness, coma and death (Scala, 1988; Vyskocil *et al.*, 1988).

Several of gasoline volatile components such as benzene, toluene, and hexane are used extensively as industrial solvents. Benzene is ubiquitous in the environment and the exposure of humans to trace levels "or more" of this chemical is highly conceivable. Estimates of the daily amounts of benzene consumed in drinking water and footstuffs vary considerably and are in the order of micrograms/day. Depending upon the assumptions made with respect to levels of benzene in tobacco products and food stuff, estimates for the exposure of the general smoking population in industrial countries range from 2000 to 3500 $\mu\text{g}/\text{kg}$ benzene / day. Adult (70kg) non-smokers are considered to be exposed to about 200 to 1700 $\mu\text{g}/\text{kg}$ benzene/day (about 3 to 25 $\mu\text{g}/\text{kg}$) body weight per day (Mc Connell, 1993).

Furthermore, several additives are added to the gasoline such as antiknocks and octane enhancer. In unleaded gasoline, these include methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), and tertiary butyl alcohol (TBA). In leaded gasoline, alkyl lead compounds such as tetramethyl lead (TML) and tetraethyl lead (TEL) are used. Because of the highly volatile nature of gasoline and its components, inhalation is considered a major possible route of environmental exposure to humans and the respiratory system would be the front target

(Nihlen *et al.*, 1998; Dakhel *et al.*, 2003; Williams *et al.*, 2003; Tardif *et al.*, 2004).

The geneotoxicity tests indicated that both types of gasoline, leaded and unleaded, could enhance the number of histidine independent colonies, cause DNA damage and increase the frequency of induced micronucleus in hamster lung cells (Yuan *et al.*, 2000).

Moreover, cancer risk from exposure to motor fuel containing the MTBE octane enhancer is now reasonably accepted (Buckley *et al.*, 1997; Belpoggi *et al.*, 1997). This effect is likely the result of glutamate-induced apoptotic cell death and neurotoxicity (Jiang *et al.*, 2000; Ezzat *et al.*, 2001).

Brain tissue injury and cellular damage were correlated with perturbation in cerebral cortex content of several free amino acids in male rats exposed to leaded and unleaded gasoline (Ezzat *et al.*, 2001; Fares *et al.*, 2001; Rouina *et al.*, 2002). Liver disorders, including lipid degeneration and cirrhosis were also noticed in workers at fuel stations (Pranjic *et al.*, 2003). Lung adenoma, interstitial fibrosis, alveolar destruction with air space enlargement and infiltration of inflammatory cells in the airways of rats were also reported (Lall *et al.*, 1998; Kato *et al.*, 2000).

In order to find out if the hazards produced by the unleaded gasoline and its combustion products and emissions are less than those produced by the leaded type, the two types of gasoline were tested in this study.

2. MATERIAL AND METHODS

Adult male albino rats weighing 130-160g were maintained in plastic-cages and housed for ten days, for adaptation to laboratory conditions, prior to the initiation of the experiments. Animals were fed a standard commercial pellet and tap water was provided *ad libitum*. Handling and use of animals agreed strictly with the regulations and guidelines set by the Research Ethics Committee of the Faculty of Science, Ain Shams University.

Ninety animals were divided into 2 groups each of 45; the first was subjected to the acute gasoline exposure and the other to the long term exposure as follows:

2.1 Acute Exposure

The 45 rats were divided into 3 subgroups each of 15. The first (AG₁) received single exposure to the vapors produced by evaporization of ½ LC₅₀ (18787.5 ppm) of leaded gasoline in a dynamic flow system (Ezzat *et al.*, 2001). The second (AG₂) was exposed to ½ LC₅₀ (19964 ppm) of the unleaded gasoline and the third to gasoline-free air flow (control). The three groups were exposed once for 30 minutes under the same conditions.

2.2 Long Term Exposure

In this experiment the second group of animals was similarly divided into 3 subgroups each of 15. The first (LG₁) was exposed to 1/5 LC₅₀ of the leaded gasoline (7495 ppm). The second (LG₂) was exposed to 1/5 LC₅₀ of the unleaded gasoline (7985.6 ppm). The third was exposed to a gasoline-free air flow (control). All subgroups were exposed to the different treatments for 30 minutes /day for six consecutive weeks. Body weight was recorded for each animal just before the start and at the end of the exposure regimen and differences in the body weights were recorded.

2.3 Tissue Preparation

At the end of the designated duration, animals were removed for autopsy. The chest was rapidly opened and the two lungs of each animal, in the exposed and control groups were carefully dissected out and weighed for the calculation of relative lung/body weight.

In all groups, the left lung of each animal was divided into 2 parts; the first was cut into small pieces (0.1mm in thickness) and fixed in 3% glutaraldehyde fixative for light and electron microscopy processing. The second part of the left lung was homogenized in 0.1M potassium phosphate buffer (pH 6.5), centrifuged at 1000 X for one hour and the supernatant was harvested and Kept at -

20C pending biochemical determinations of lung TGSH, GST, GPx and GR. The right lung was homogenized in cold 1.2% potassium chloride for estimation of TGSH and GSH (reduced) LP and SOD.

2.4 Preparation of lung tissue for microscopic examination

For the light and electron microscope examinations, small parts of the left lung were dissected in sufficient amount of 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 5m. These parts were further cut into small pieces (0.5-0.1mm in thickness), fixed in 3% glutaraldehyde, for 24 hours, and post-fixed in 1% osmium tetroxide. Semithin sections (1µm) were stained with toluidine blue. Thin sections (80-90 nm) were stained with uranyl acetate and lead citrate (Venable and Coggeshal, 1965) and examined on a Joel JJM-1200 EX II electron microscope (Tokyo-Japan).

2.5 Biochemical Analyses:

Lipid peroxides were measured by a colorimetric reaction with thiobarbituric acid - positive reactant substances (TBARS) according to the method of Stroev and Makarova (1988). Superoxide dismutase activity was assayed by the method of Nishikawa *et al.* (1999). TGHS content was determined colorimetrically according to the method of Saville (1958). GSH level was assayed by the method of Prins and Loose (1969). GSSG content was calculated by subtracting the amount of reduced glutathione from that of total glutathione for each sample individually. GST activity was determined spectrophotometrically according to the method described by Habig *et al.* (1974). GPx and GR activities were assayed spectrophotometrically according to the methods described by Pagalla and Valentine (1967) and Zanetti (1979), respectively.

2.6 Statistical Analysis

All data were analyzed by one way analysis of variance (ANOVA) using SPSS for Windows software, release 11.0 (SPSS Chicago, IL). The least significant difference

(LSD) test was used to distinguish between means (Tallarida and Murray, 1987).

3. RESULTS

3.1 Physiological Investigations

a- Acute exposure

TGSH content was significantly increased in the leaded (AG₁) and unleaded (AG₂) gasoline treated groups as compared with the corresponding control values ($P < 0.01$), but no statistical difference was found between the G₁ and G₂ treated groups. The GSH content was not significantly changed with AG₁ and AG₂ treatments in comparison with the control value. Moreover, no significant difference was found between the AG₁ and AG₂ treated groups. The level of GSSG was significantly elevated with AG₁ and AG₂ ($P < 0.01$) above the control value. No significant difference was found between AG₁ and AG₂ treated groups.

The activities of GST and GR increased significantly with AG₁ and AG₂ ($P < 0.05$) in comparison with the corresponding control values. Moreover, no significant differences between the AG₁ and AG₂ groups were detected in these enzymes activities. No significant difference was found in the GPx activity in either the AG₁ or the AG₂ treated groups as compared with the control and no significant difference was found between the G₁ and G₂ groups (table 1).

No significant difference was observed in SOD activity in rats treated with AG₁, while there was a significant decrease with AG₂ ($P < 0.001$) in comparison with the control. Moreover, the SOD activity of AG₂ group was significantly less ($P < 0.05$) than that of the AG₁ group. The level of LP was not significantly altered by either AG₁ or AG₂ treatment in comparison with the control value. Moreover, no significant difference was detected between AG₁ and AG₂ groups (table2).

Table1: Effect of acute exposure to two type of gasoline vapours on lung total glutathione (TGSH), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) in rats.

Parameter	TGSH (nmol/mg tissue)	GSH (nmol/mg tissue)	GSSG (nmol/mg tissue)	GST (nmol/mg tissue/min)	GPx (nmol/mg tissue/min)	GR (nmol/mg tissue/min)
Control	17.76 ± 0.30	6.86 ± 0.100	10.9 ± 0.28	3.49 ± 0.107	9.51 ± 0.37	4.55 ± 0.159
AG ₁	20.82 ± 0.305** (17.22)	6.77 ± 0.07 (-1.31)	14.05 ± 0.33** (28.89)	3.84 ± 0.095* (10.02)	10.28 ± 0.177 (8.09)	4.93 ± 0.070* (8.35)
AG ₂	20.55 ± 0.16** (15.70)	6.65 ± 0.143 (-3.06)	13.9 ± 0.15** (27.52)	3.85 ± 0.059* (10.31)	10.18 ± 0.165 (7.04)	4.96 ± 0.084* (9.010)

- n=8
- AG₁ leaded gasoline & AG₂ unleaded gasoline
- Values are expressed as means ± S.E.
- * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ indicate the level of significance in comparison with the corresponding control value.
- Values between parentheses indicate the percentage of change from the corresponding control value.

No significant difference was observed in SOD activity in rats treated with AG₁, while there was a significant decrease with AG₂ ($P < 0.001$) in comparison with the control. Moreover, the SOD activity of AG₂ group was significantly less ($P < 0.05$) than that of the AG₁ group. The level of LP was not significantly altered by either AG₁ or AG₂ treatment in comparison with the control value. Moreover, no significant difference was detected between AG₁ and AG₂ groups (table2).

b- Long term exposure

A significant increase in TGSH and GSSG was found in the groups exposed to

LG₁ ($P < 0.01$) and LG₂ ($P < 0.001$) as compared with the corresponding control values, but there was a significant decrease in the GSH level in LG₁ and LG₂ groups ($P < 0.05$). No significant difference was detected in GSH when the two groups LG₁ and LG₂ were compared together, but TGSH and GSSG levels were significantly higher ($P < 0.01$) in LG₂ group than in LG₁ group.

The GST activity increased significantly in LG₁ and LG₂-treated groups as compared with the control value ($P < 0.01$). No significant difference, however, was found between the LG₁ and LG₂ groups. The GPx activity increased significantly in LG₁

($P < 0.01$) and LG₂-treated group ($P < 0.001$) and the activity of GR increased significantly in LG₁ and LG₂ groups ($P < 0.01$) in comparison with the corresponding control values. On the other hand, GPx activity was significantly higher in LG₂-treated group than that of the LG₁ treated group ($P < 0.05$). Moreover, no significant difference was detected in GR activity when LG₁ and LG₂ groups were compared with each other (table 3).

The SOD activity was significantly decreased in LG₁ group ($P < 0.01$) and LG₂ group ($P < 0.001$) as compared with the control. No significant difference was found between LG₁ and LG₂ groups. The LP content was significantly higher in LG₁ ($P < 0.01$) and LG₂ ($P < 0.001$) than that of the control. Meanwhile, LP content was significantly higher in LG₂ group than that in LG₁ group ($P < 0.05$) (table 4).

3.2 The Structural Investigations of Rat Lungs

a- Light microscopy

Compared with the controls (Figs. 1 & 2), the light microscopic examination revealed that the single acute exposure to either AG1 or AG2 types of gasoline did not induce significant structural changes in the exposed rat lungs. On the contrary, the histological examination of the lungs taken out from the long term exposed rats revealed wide spread pin-point hemorrhage. In the LG1 animals, Clara cells were remarkably shrunk and contained few granules and their apical parts were decapitated (Fig. 3). Some of these cells possessed highly pyknotic nuclei, but other cells exhibited karyorrhexic and karyolytic nuclei. The columnar ciliated cells lining the bronchioles were highly degenerated, possessed karyolytic nuclei and lost most of their cilia. The bronchiole lumen was filled with amorphous materials along with red blood cells and active macrophages (Fig. 3). In the LG2 group, the bronchioles showed

marked changes in the epithelial layer. These changes comprised local destruction of the bronchiolar basal lamina and abnormal outgrowth of the inner bronchiolar wall into the bronchiolar lumen (Fig. 4). Most of these bronchioles were clogged with proliferated cells. In addition, in the LG1 animals, the alveolar tissue showed severe alveolar destruction with marked loss of alveolar septa. Necrotic type II pneumocytes which have lost their secretory granules were also observed. These cells exhibited damaged nuclei with distinct features of pyknosis. The air blood barrier appeared thickened (Fig. 5). Furthermore, some specimens showed a large multicellular area with central highly congested blood vessels and ruptured interalveolar septa to form large alveolar sacs (Fig. 6).

b) Electron microscopy

The electron micrographs revealed that the alveolar wall of the control lung consists of type I and type II pneumocytes (Fig. 7). The alveolar wall appears largely occupied with tiny blood capillaries lined by endothelial cells and containing red blood cells (Fig. 7). In addition, type II pneumocytes possessed large heterochromatic nuclei with prominent nucleoli (Fig. 8). The nucleus is surrounded by a considerable amount of cytoplasm rich in organelles including dense matrix mitochondria, small profiles of rough endoplasmic reticulum, Golgi complex, multivesicular bodies and multiple large spherical intracellular lamellar bodies (Fig. 8).

In LG1 group, large masses of collagen and elastic fibers were accumulated in the alveolar septa (Fig. 9). Furthermore, type II pneumocytes in this group appeared with few microvilli and highly indented nuclei (Fig. 10). Their cytoplasm contained fragmented rough endoplasmic reticulum, hypertrophied Golgi complex and

Table 2: Effect of acute exposure to two type of gasoline vapours on lung superoxide dismutase (SOD) activity and lipid peroxidation (LP) in rats.

Treatment	Parameter	SOD (U/g wet tissue)	LP (nmol/g wet tissue)
Control		130.16 ± 2.072	0.60 ± 0.108
AG ₁		122±3.356 (-6.24)	0.69 ± 0.0573 (15.00)
AG ₂		96±5.802*** ^a (-26.24)	0.71 ± 0.079 (-3.06)

- n=8
- AG₁ leaded gasoline & AG₂ unleaded gasoline
- Values are expressed as means ± S.E.
- *P< 0.05; **P< 0.01; *P< 0.001 indicate the level of significance in comparison with the corresponding control value.
- ^a indicate significance between G₂ and G₁; ^aP< 0.05.
- Values between parentheses indicate the percentage of change from the corresponding control value.

Table 3: Effect of long term exposure to two type of gasoline vapours on lung total glutathione (TGSH), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) in rats.

Treatment	Parameter	TGSH (nmol/mg tissue)	GSH (nmol/mg tissue)	GSSG (nmol/mg tissue)	GST (nmol/mg tissue/min)	GPx (nmol/mg tissue/min)	GR (nmol/mg tissue/min)
Control		17.03 ± 0.308	7.50±0.077	10.33±0.299	2.72±0.103	8.74±0.378	3.79±0.164
LG ₁		21.82±0.227** (26.36)	6.86±0.132* (-8.53)	14.55±0.234** (40.85)	4.07±0.092** (49.63)	10.28±0.22** (17.04)	4.94±0.097** (30.34)
LG ₂		26.41±0.240*** ^b (15.70)	6.69±0.121 (-10.80)	19.68±0.179*** ^b (90.54)	4.083±0.116** (50.11)	11.37±0.237*** ^a (30.09)	5.01±0.108** (32.18)

- n=8
- LG₁ leaded gasoline & LG₂ unleaded gasoline
- Values are expressed as means ± S.E.
- *P< 0.05; **P< 0.01; *P< 0.001 indicate the level of significance in comparison with the corresponding control value.
- ^{a,b} indicate significance between G₂ and the corresponding G₁ value; ^aP< 0.05; ^bP<0.01.
- Values between parentheses indicate the percentage of change from the corresponding control value.

Table 4: Effect of long term exposure to two type of gasoline vapours on lung superoxide dismutase (SOD) activity and lipid peroxidation (LP) in rats.

Treatment	Parameter	SOD (U/g wet tissue)	LP (nmol/g wet tissue)
Control		107 ± 2.503	0.77 ± 0.0319
LG ₁		81.83±1.94** (-23.52)	1.48 ± 0.079** (92.20)
LG ₂		77.0±2.408*** (-28.03)	2.45 ± 0.123*** ^a (-218.18)

- n=8
- LG₁ leaded gasoline & LG₂ unleaded gasoline.
- Values are expressed as means ± S.E.
- *P< 0.05; **P< 0.01; *P< 0.001 indicate the level of significance in comparison with the corresponding control value.
- ^a indicate significance between G₂ and G₁; ^aP< 0.05.
- Values between parentheses indicate the percentage of change from the corresponding control value.

Table 5: Effect of long term exposure to two types of gasoline vapours on the mean lung weight and on the relative lung / body weight (%) in rats.

	Mean lung weight (g)	Relative lung / body weight (%)
Control	1.041 ± 0.061	0.468 ± 0.046
LG ₁	1.483 ± 0.087* (42.45)	0.701 ± 0.061* (49.73)
LG ₂	1.52 ± 0.034** (46.01)	0.711 ± 0.039* (51.92)

- n=8
- G₁ leaded gasoline & O₂ unleaded gasoline.
- Values are expressed as means ± S.E.
- *P < 0.05; **P < 0.01; ***P < 0.001 indicate the level of significance in comparison with the corresponding control value.
- Values between parentheses indicate the percentage of change from the corresponding control value.

cytoplasmic vacuoles (Fig.11). The mitochondrial cristae in these cells exhibited obvious signs of degeneration (Fig.9).

In LG₂ group, the alveolar blood capillaries appeared dilated and congested with many red blood cells, and a number of neutrophils, some of which were adhered to the vascular endothelial cells (Fig.12). The endothelial cells of these capillaries were remarkably shrunk and many blood platelets were seen in the same capillaries (Fig.12). The masses of the lining epithelium of respiratory bronchioles were crammed with neutrophils, fibroblasts and large masses of collagen fibers (Fig.13). The proliferated cells were recognized by highly indented euchromatic nuclei with peripheral condensation of their heterochromatin and lack of structural organization in their cytoplasm. The alveolar space was highly reduced in these areas (Fig.13).

4. DISCUSSION

The present work revealed a significant increase in the relative lung / body weight ratio in both LG₁ and LG₂ groups. The increase might have been the result of the gasoline-induced histopathological changes manifested as accumulation of inflammatory cells, fibrosis and congestion of vessels and blood capillaries. In addition, the accumulation of appreciable amounts of amorphous materials, necrotic cells debris and neoplastic cells in the bronchioles and alveolar regions, as revealed by the

ultrastructural examination, must have added to the tissue weight.

The results of the present study demonstrated that the acute and chronic exposure to the two types of gasoline vapours produced significant changes in the glutathione antioxidant system. When the lung was exposed to various inhaled toxic products, the toxicity was mediated, at least in part, through the generation of free radicals (Housset, 1994; Bowler and Crapo, 2002; Pagano and Barazzone, 2003). The antioxidant system is the primary defense line against reactive oxygen species and lung tissues are protected against these oxidants by a variety of antioxidant mechanisms (Kinnula and Crapo, 2003).

GSH plays a vital role in counteracting the oxidative stress-induced injury in lung epithelial cells and abates the proinflammatory processes (Rahman *et al.*, 1999). Alteration in lung glutathione metabolism has been associated with a number of inflammatory lung diseases including idiopathic pulmonary fibrosis, cystic fibrosis, and chronic obstructive pulmonary disease (COPD), each of which has been suggested to be associated with increased oxidative tissue assault (Kelly, 1999; Rahman and Mac Nee, 1999; Boots *et al.*, 2003; Day *et al.*, 2004).

The increase in the TGSH observed in this study might have resulted from one or more of the following reasons. First, an increase in δ - Glutamyl transferase (δ GT)

activity (Murray *et al.* 1988), which eventually leads to an increase in the intracellular cysteine, glycine and glutamate contents and subsequently GSH level (Griffith and Meister, 1979). The second reason is the gasoline-induced stimulation of GST activity (Travis *et al.*, 1990; Henderson *et al.*, 2005) which is in agreement with the present results that revealed a significant increase in GST activity. This enzyme is required to catalyze the conjugation of GSH with aromatic or aliphatic hydrocarbons to produce mercapturic acid. In this reaction, glutamate and glycine residues are removed by hydrolysis and made available for recycling to regenerate new GSH. The third possible reason is attributed to increased GR activity which regenerates glutathione from its oxidized form. Therefore, the significant increase in GR, GST and δ -GT activities could have worked jointly in order to increase synthesis and regeneration of GSH as a compensatory adjustment to encounter the tissue assault.

On the other hand, there were no significant changes in GSH in acutely exposed group. This may be explained by the increased activity of GR paralleled with GSH consumption to produce GST-conjugates (a process catalyzed by GST). The activity of GST was also increased under the experiment conditions leading to equilibrium between the GSH regenerated and that consumed which might have lead to insignificant alterations in the amount of the GSH (Tatrai *et al.*, 2001).

In the chronically exposed group, there was a significant increase in GPx activity accompanied with a decrease in the GSH level. Also, the increase in GSSG in this group coincides with the increase in GPx activity observed herein. Another possible reason for the decrease in GSH is its use in detoxifying reactions catalyzed by GST to produce conjugates with the gasoline chemical constituents (Travis *et al.*, 1990).

Furthermore, the depletion of lung GSH was probably attributed to impaired NADPH production as a result of inhibition of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities which makes it impossible to restore GSH from GSSG even in the presence of increased

GR activity (Jaeschke, 1992; Hammerschmidt *et al.*, 2002).

On the other hand, the present results indicate that G₁ and G₂ caused a decline in the SOD lung activity paralleled with an elevation in lipid peroxidation (LP) content. SOD catalyzes the conversion of superoxide anions into H₂O₂ which represents a critical step in the antioxidant system. Inhibition of SOD activity involves a direct effect caused by the gasoline constituents and /or indirect inhibition by the free radicals which oxidize the enzyme itself. In this regard, it was reported that the interaction of hydroquinone, one of the benzene metabolites, with copper and zinc components of SOD causes degeneration of the enzyme activity and release of copper from the enzyme. The further reaction between the released Cu and H₂O₂ generates reactive oxygen species and initiates lipid oxidation chain reactions (Yunbo *et al.*, 1996).

On the ultrastructural level, G₁ and G₂ inhalation caused blebbing of the alveolar cell membranes and degeneration of their cytoplasm and organelles. Moreover, the damaging effects reached the nuclei which appeared pyknotic. The type II pneumocytes, responsible for the secretion of the surfactants which protect the lungs from collapsing during exhalation, were also severely affected. This wide spread cellular damage is likely related to the gasoline-induced impairment of the lung antioxidant defense system and to the imbalance of GSH metabolism in the tissue. Furthermore, thiol oxidation was found to be a step in the formation of the cell surface belbs that precedes cell death. Loss of GSH itself is a critical step that leads to bleb formation and subsequent cell death as a result of the oxidation of the SH groups (Plopper *et al.*, 2001). Accordingly, Tatrai *et al.* (2001) proposed that sufficient intracellular GSH content concomitant with efficient GSH-restoring mechanisms are prerequisites for maintaining cell integrity.

The ultrastructural results obtained, in the study revealed degeneration of mitochondria, broken cristae and deformed inner membrane structure which provide evidence of a poor mitochondrial ability to produce ATP needed by lung cells. A

previous study revealed that during hyperoxic conditions, there is a parallel change in the cellular contents of ATP and GSH concomitant with impaired cellular capacity to maintain all biological membranes (Aerts *et al.*, 1992).

Furthermore, the present results indicate that G₁ and G₂ caused a decline in the lung SOD activity concomitant with an elevation in the level of LP.

The decrease in the SOD enzyme activity, which represents the first line of defense against super anions, might have resulted from inhibited parenchymal cell production of SOD as a result of cellular damage or due to the excessive hydrogen peroxide generated by SOD activity which might have inactivated the enzyme via the feedback inhibition mechanism (Shinazaki *et al.*, 1992).

A correlation between elevated LP content and the morphological changes in lung tissues was detected in this study. The examination of lung sections from the long-term exposed group revealed severe injury in the architecture pattern manifested as alveolar damage, shedding of bronchiolar epithelia and loss of cell membrane integrity, concomitant with intensive interstitial and alveolar infiltration with neutrophils. These results are most likely ascribed to excessive membrane lipid peroxidation and gasoline induced inflammatory reactions (Sprong *et al.*, 1998).

5. REFERENCES

- Aerts, C.; Wallaert, B. and Voisio, C. (1992). In vitro effect of hyperoxia on alveolar type 11 pneumocytes: Inhibition of glutathione synthesis increases hyperoxic cell injury. *Exp. Lung Res.*, 18: 845-861.
- Baroja, O.; Rodriguez, E.; De Balugera, Z. G.; Goicolea, A.; Unceta, N.; Sampedro, C.; Alonso, A.; and Barrio, R. J. (2005). Speciation of volatile aromatic and chlorinated hydrocarbons in an urban atmosphere using TCT-GC/MS. *J Environ. Sci Health, A Tox. Hazard Subst. Environ. Eng.*, 40(2): 343-367.
- Belpoggi, F.; Soffritti, M.; Filippini, F. and Maltoni, C. (1997). Results of long-term experimental studies on the carcinogenicity of methyl tertiary-butyl ether. *Ann. Ny. Acad. Sci.*, 26: 837-877.
- Boots, A. W.; Haenen, G. R. and Best, A. (2003). Oxidant metabolism in chronic obstructive pulmonary disease. *Eur. Respir. J.*, 46: 14-27.
- Bowler, R.P. and Crapo, J.D. (2002). Oxidative stress in air ways. *Am. J. Respir. Crit. Care Med.*, 166:38-43.
- Brcic, I. (2004). General population exposure to volatile aromatic hydrocarbons. *Arh. Hig. Rada. Toksikol.*, 55(4): 291-300.
- Buckley, T. J.; Prah, J. D.; Ashley, D.; Zweidinger, R. A. and Watlace, L. A. (1997). Body burden measurements and models to assess inhalation exposure to methyl tertiary butyl ether (MTBE). *Air West Manag. Assoc.*, 47(7): 739-752.
- Dakheel, N.; Pasteris, G.; Werner, D. and Hohencr, P. (2003). Small-volume release of gasoline in the vadose zone: impact the additives MTBE and ethanol on groundwater quality. *Environ Sci. Technol.*, 37(10): 2127-2133.
- Day, B.; Van Heckenken, A.; Min, E. and Velsor, L. (2004). Role for cystic fibrosis transmembrane conductance regulator protein in a glutathione response to bronchopulmonary pseudomonas infection. *Infect. Immun.*, 72(4): 2045-2051.
- Ezzat, A. R.; Rouina, I. and Fares, N. H. (2001). Effect of chronic exposure to two types of gasoline vapours on the free amino acids profile and structure of cerebral cortex in the albino rats. *J. Egypt Ger. Soc. Zool.*, 34(A): 255-276.
- Fares, N. H.; Rouina, I. and Ezzat, A. R. (2001). Behavioral and structural studies on the effect of chronic exposure to gasoline vapours in the albino rat brain. *J. Egypt Ger. Soc. Zool.*, 34(C): 34 1-366.
- Griffith, O. W. and Meister, A. (1979). Glutathione interogen translocation turnover and metabolism. *Proc. Nail. Acad. Sic.*, 76: 5606-5616.
- Habig, W. M.; Pabst, M. J. and Jakoby, W. B. (1974). Glutathione-S-transferase: The first step in mercapturic acid formation. *J. Biol. Chem.*, 249:7130-7139.
- Hammerschmidt, S.; Buchler, N. and Han, W. (2002). Tissue lipid peroxidation and reduced glutathione depletion in hypochlorite-induced lung injury. *Chest*, 121(2): 573-581.
- Hartley, W. R.; England, A. and Jr, J. (1992). Health risk assessment of the migration of unleaded gasoline a model for petroleum products. England, A. J.; Eckenfelder, W. W. eds., 253: 65-72. Quoted from Rouina, I. A.

- (2000). Ph.D. Thesis, Department of Zoology, Faculty of Science, Aim Shams University, Egypt.
- Henderson, A. P.; Barnes, M. L.; Bleasdale, C.; Cameron, R.; Clegg, W.; Heath, S. L.; Lindstrom, A.; Rappaport, S.; Waidyanatha, S.; Watson, W. and Golding, B.; (2005). Reactions of benzene oxide with thiols including Glutathione. *Chem. Res. Toxicol.*, 21(2):265-270.
- Housset, B. (1994). Free radicals and respiratory pathology. *CR Seances Soc. Biol. Fit.*, 188(4): 321-333.
- Jaeschke, H. (1992). Enhanced sinusoidal glutathione efflux during endotoxin induced oxidant stress in vivo. *Am. J. Physiol.*, 263: 60-68.
- Jiang, Q.; Gu, Z.; Zhang, G. And Jiang, G. (2000). Glutamate-induced neuronal apoptotic-like cell death. *Brain Res.*, 887(2); 285-292.
- Kato, A.; Nagi, A. and Kagawa, J. (2000). Morphological changes in rat lung after long-term exposure to diesel emission. *Inhalation Toxicol.*, 12: 469-490.
- Kelly, F. J. (1999). Glutathione: In defence of lung. *Food Chem. Toxicol.*, 37:963-966.
- Kinnula, V. L. and Crapo, J. D. (2003). Superoxide dismutase in the lung and human lung diseases. *Am. J. Respir. Crit. Care. Med.*, 167(12): 1600-1619.
- Lall, S. B.; Das, N.; Das, B. P. and Gulahi, K. (1998). Biochemical and histopathological changes in respiratory system in rat following exposure to diesel exhaust. *Indian J. Exp. Biol.*, 36 (1): 55-59.
- Liu, J.; Hara, K.; Kashimura, S.; Kashiwagi, M.; Hamauaka, T.; Miyoshi, A. and Kageura, M. (2000). Head-space solid-phase microextra action and gas chromatographic-mass spectrometric screening for volatile hydrocarbons in blood. *J. Chromatgr. Biomed. Sci. Appl.*, 748(2): 401-406.
- McConnell, E. E. (1993). Environmental Health Criteria 150. BENZENE. World Health Organization. Geneva. pp: 107-109.
- Murray, R. K.; Gvanner, D. K.; Mayes, P. A. and Rodwell, V. W. (1988). HAPER'S BIOCHEMISTRY. 21th edition. Appleton & Lang. Norwalk.
- Nihlen, A.; Lof, A. and Johoson, G. (1998). Experimental exposure to methyl tertiary-butyl ether. I. Toxicol. Kinetics in humans. *Toxicol. Appl. Pharmacol.*, 148 (2): 274-280.
- Nishikawa, M.; Nobumasa, K.; Ito, T.; Kudi, M.; Kaneeko, T.; Suzuki, M.; Udaka, N.; Ikeda, H. and Okubo, T. (1999). Superoxide mediates cigarette smoke induced infiltration of neutrophils into airways through nuclear factors activation and IL-8 mRNA expression in guinea pigs in vivo. *Am. J. Respir. Cell. Mol. Biol.*, 20: 189-198.
- Pagalla, D. E. and Valentine, W. N. (1967). Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70:158.
- Pagano, A. and Barazzone, A. (2003). Alveolar cell death in hyperoxia-induced lung injury. *Ann. N. Y. Acad. Sci.*, 10: 405-416.
- Plopper, C. G.; Van Winkle, L. S.; Fanucchi, V. M.; Malburg, S. R.; Nishio, S. J.; Chang, A. and Buckpitt, A. R. (2001). Early Events in naphthalene induced acute Clara cell toxicity. *Am. J. Res. Cell. Mol. Biol.*, 24(3): 272-281.
- Pranjic, N.; Mujagic, H. and Pavlovic, S. (2003). Inhalation of gasoline and damage to health in workers at gas stations. *Med. Arch.*, 57(1): 17-20.
- Prins, G. K. and Loose, J. A. (1969). GLUTATHIONE. Chapter 4 BiochemiCal methods in red blood cell genetics. Academic Press. N.Y.D. London. pp: 126-129.
- Rahman, I.; Abidi, P.; Afaq, F.; Schiffmann, D.; Mossman, B.T.; Kamp, D. W. and Athar, M. (1999): Glutathione redox system in oxidative lung injury. *Crit. Rev. Toxicol.*, 29(6): 543-568.
- Rahman, I. and MacNee, W. (1999). Lung glutathione and oxidative stress implications in cigarette smoke-induced airway disease. *Am. J. Physiol.*, 277: L1067-L1088.
- Rouina, I.; Ezzat, A. R. and Fares, N. H. (2002). Effect of acute exposure to gasoline vapours on the free amino acids profile and of the structure of the cerebral cortex in albino rats. *J. Egypt Ger. Soc. Zool.*, 38(c): 11-31.
- Saville, B. (1958). A scheme for the colorimetric determination of microgram amount of thiols. *Analyst.*, 83:670. Quoted from Hegazy, H. G. (2000). Ph. D. Thesis, Department of Zoology, Faculty of Science, Aim Shams University, Egypt.
- Scala, R. (1988). Motor gasoline toxicity. *Fundam. Appl. Toxicol.*, 10: 553-562.
- Shinazaki, S.; Kobayashi, T.; Kato, K. and Sekiguchi, M. (1992). Pulmonary hemodynamic and lung function during chronic paraquat poisoning in sheep. *Am. Rev. Resp. Dis.*, 146:775-780.
- Sprong, R. C.; Winkelhuzen, J. A.; Aavsmann, J.M.; Van Oirschot, F. L. and Van Den. B. T. (1998). low dose acetylcysteine protects rats against endotoxin mediated oxidative stress, but high dose increases mortality. *Am. J. Respir. Crit. Care. Med.*, 157: 1283- 1293.
- Stroev, E. A. and Makarova, V. G. (1988). Study in peroxidation of biological membrane lipids. Chapter 15, In: Laboratory Manual in

- Biochemistry. English transition. pp:251-255.
- Tallarida, R. J. and Murray, R.B. (1987). "Manual of pharmacologic calculations with computer programs" 2nd ed. (c) 1981, 1987. By *Springer-Verlage*, New York, Inc.
- Tardif, R.; Liu, L. and Raizenne, M. (2004). Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. *Inhal. Toxicol.*, 16(4): 203-207.
- Tatrai, E.; Kovacikova, Z., Hudak, A.; Adomis, Z. and Ungvary, G. (2001b). Comparative in vitro toxicity of cadmium and lead on redox cycling in type II pneumocyte. *J. Appl. Toxicol.*, 21(6): 479-483.
- Travis, C.C.; Jeffery, L. Q. and Arms. A. D. (1990). Pharmacokinetics of benzene. *Toxico. Appl. Pharmacol.*, 102: 400-420.
- Venable, J. H. and Coggeshal, R. (1965). A simplified lead citrate stain for use in electron microscopy. *J. Cell. Biol.*, 25: 407-408.
- Vyskocil, A.; Tusl, M. and Obsal, J. (1988). A sub-chronic inhalation study with unleaded Petrol in rats. *J. Appl. Toxicol.*, 8: 239-242.
- Williams, P. R.; Cushing, C. A.; Sheehan, P. J. (2003). Data available for evaluating the risks and benefits of MTBE and ethanol as alternative fuel oxygenates. *Risk. Ana.*, 23(5): 1085-1115.
- Yuan, D.; Zhou, W. and Ye, S. (2000). Effect of leaded and unleaded gasoline on the mutagenicity of vehicle exhaust particulate matter. *J. Environ. Pathol. Toxicol. Oncol.*, 19(1-2): 41-48.
- Yunbo, L.; Periannan, K.; Jay, Z. and Michael, A. (1996). Role of Cu/Zn-superoxide dismutase in xenobiotic activation: II Biological effects resulting from the Cu-Zn superoxide dismutase-accelerated oxidation of benzene metabolite1, 4, hydroquinone. *Mol. Pharmacol.*, 49 (3): 412-421.
- Zanetti, G. (1979). Rabbit liver glutathione reductase: Purification and properties. *Arch. Biochem. Biophys.*, 198: 241.
- (3) High magnification of a part of the degenerated lining epithelium of the bronchiole of LG1 rat showing shrunk Clara cells with pyknotic (pn), karyorrhexic (Kx) and karyolytic (kn) nuclei. Macrophages (ma), amorphous materials (am), and red blood cells (RBCs) are seen in the lumen. Bar=13.3µm.
- (4) A terminal bronchiole of LG2 rat showing local destruction of the basal bronchiolar lamina (BL) and abnormal outgrowth of the inner bronchiolar wall into the bronchiolar lumen (arrow). Bar=34µm.
- (5) An alveolar septa of LG1 showing degenerated type II Pneumocytes with pyknotic (pn) nuclei and no secretory granules. Thickening of the air-blood barrier (arrows) is also seen. Bar= 13.3µm.
- (6) Alveolar region of LG2 rat showing a large multicellular area (arrow) with a central highly congested blood vessel (BV) and ruptured interalveolar septa forming large alveolar sacs (Las). Bar=68µm.

Figure 7-13: Electron micrographs of rat lungs.

6.Explanation of Figures

Figures1-6: Photomicrographs of semithin sections of rat lungs stained with toluidine blue.

- (1) Control bronchioles showing low columnar ciliated cells (CC) and Clara cells (CL) and bundles of smooth muscles (mu). Bar =34µm
- (2) Control alveoli showing type I (PI) and type II (PII) Pneumocytes in the alveolar wall and a free macrophage (ma).En,endothelial cell. Bar=13µm.
- (7) Alveolar walls of control lung showing alveolar sacs (as) and intervening septal wall. The septal wall consists of type I pneumocytes (PI) and type II pneumocytes (PII). Intervening the septal walls are several blood capillaries occupied by RBCs and lined by thin endothelial cells (En). Bar=2µm
- (8) Type II Pneumocytes of control lungs showing heterochromatic nucleus (N) with prominent nucleolus (nu), mitochondria (MI), several lamellar bodies (Lb) with lamellar pattern and microvilli. Bar=1µm.
- (9) The long term exposed groups (LG1) showing two alveolar spaces(as). The left alveolar space contains escape red blood cells (RBCs), active alveolar macrophage (ma) and platelets (PLt). The thin arrows indicate the vesicles and belb formation of the epithelial layer. Small lymphocytes (L) occupy the majority of blood capillaries. Proliferated cells (Pt) are occasionally located in this region. Necrotic type II pneumocyte (PII), a thickened air- blood barrier (thick arrow), and large masses of collagen fibers (CF) and elastic fibers (Ef) are seen. Bar=2µm.
- (10) Degenerated type II pneumocytes of LG1 rat with highly indented nucleus (N), many cytoplasmic vacuoles (V), fragmented endoplasmic reticulum (thick arrows) and scarce microvilli (thin arrow). Bar=1µm.
- (11) High power of part of type II pneumocyte of LG1 showing mitochondria with destructed cristae (thin arrow) and deformed inner membrane (small arrow), fragmented rough

- endoplasmic reticulum (RER), hypertrophied Golgi complex (Gc), and several free polyribosomes (thick arrow). Bar=0.5 μ m.
- (12) An alveolar region of LG2 showing dilated blood capillaries (Cap) with aggregated red blood cells (RBCs). Neutrophils (Nu) appear adhering to shrunk endothelial cells (En). Blood platelets (PLt) and necrotic type II pneumocyte (PII) are also seen. Bar=2 μ m.
- (13) High magnification of part of an alveolar septum of LG2 rat showing neutrophil (Nu) with electron dense granules (thin arrows), a fibroblast (F), proliferated cells (Pt) with indented euchromatic nucleus and masses of collagen fibers (CF). Bar=1 μ m.



