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# Biochemical Study of the Effects of Some Heavy Metals on Oxidant / Antioxidant Status in Gasoline Station Workers /Basra-Iraq

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***Abstract-*** This work aims to study the effects of exposure to the vapors of motor gasoline (vehicles fuel), on the antioxidants, trace elements and oxidative stress (MDA), on workers of gasoline station. Results showed that increased duration of the work in the filling station lead to significantly increase in levels of Pb, Hg, Cd and MDA, decreased levels of Se, Cu, Zn, Mg, SOD, CAT, GPx, GRx, GST, ALAD, GSH and TAC. Furthermore, MDA showed positive correlation ($P < 0.001$) with Pb, Hg, Cd, negative correlation ($P < 0.001$) with SOD, ($P < 0.01$) with GRx and ALAD, ($p < 0.05$) with Se, Zn, Mg, GSH, CAT and GRx and non-significantly ($P > 0.05$) negative correlation with Cu and of GST. Level of Pb was negatively correlation with GRx, ALAD ($P < 0.01$), while ($p < 0.001$) with TAC, GSH, GST, GPx and SOD, and ($P < 0.05$) with CAT. Also, Hg gives negative correlation ($P < 0.001$) with TAC and SOD, ($P < 0.05$) with CAT, GPx, GRx, ALAD and ($P > 0.05$) with GSH and GST. Moreover, Cd showed negative correlation with levels of TAC and SOD ($p < 0.001$), ($p < 0.05$) with GSH and ALAD, CAT and GPx, and ($P > 0.05$) with GRx, GST in workers, compared to healthy control.

Keywords:Gasoline station workers, Heavy metals, Trace elements, Antioxidants, Oxidative stress.

## INTRODUCTION

Gasoline or petrol, is a complex manufactured mixture that does not exist naturally in the environment. It consists mostly of several hundred hydrocarbons obtained by the fractional distillation of petroleum that have boiling points from approximately 40°C to 180°C. The hydrocarbons present in the gasoline mixture include alkanes, or straight chain C5 to C12 compounds also known as paraffins, iso-paraffins, or branched-chain compounds of the same size; alkenes, or olefins, which are unsaturated linear and branched-chain hydrocarbons; and naphthenics, or saturated cyclic hydrocarbons. Also included in the gasoline is aromatic compounds (principally benzene, toluene, ethyl-benzene, and xylene) 1.

Exposure to gasoline may occur by:Breathing vapors at a service station when filling the car's fuel tank, Working at a service station, Using equipment that runs on gasoline, such as snow thrower, Drinking contaminated water and Being close to where gasoline has spilled or leaked into the soil. Certain workers have a greater risk of exposure to gasoline vapors. These include service station attendants, drivers of gasoline tank trucks, workers at bulk loading terminals and marine loading docks, workers who remove and service underground storage tanks and gasoline pipelines, workers who find and clean up gasoline spills and leaks, and refinery workers 2.

Several studies show that inhaling or swallowing large amounts of gasoline can cause death 3-4. Inhalation of ≥5,000 ppm gasoline vapor (20,000 ppm for 5 minutes) has been shown to be lethal. It has been postulated that the cause of death following inhalation or ingestion of high concentrations of gasoline is either central nervous system depression due to asphyxia leading to respiratory failure, or cardiac sensitization to circulating catechol amines leading to a fatal arrhythmia 4.

Damage to cells caused by free radicals, is believed to play a central role in the aging process and in disease progression5. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure 6.The human antioxidant defence system consists of both enzymatic and non-enzymatic systems.

There are several enzymtic systems that catalyze reactions to neutralize free radicals and reactive oxygen species. These enzymes include: Superoxide Dismutases (SOD), Catalases (CAT), Glutathione Peroxidases (GPx), GlutationeReductases (GRx) and Glutathione Transferases (GST). These form the body’s endogenous defence mechanisms to help protect against free radical-induced cell damage (11) .These enzymes also require co-factors such as selenium (Se), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) for optimum catalytic activity. SOD, CAT, Gpx, GRx, GST and some minerals like Se, Mn, Cu and Zn are known as the first line defence antioxidants.Whilenon-enzymatic defense systems are scavenger antioxidants GSH by binding ROS. Glutathione plays a key role in maintaining proper function and preventing oxidative stress in human cells.

The aim of the work is to obtain the effects of pollution (gasoline station workers) and the effect of pollution period on heavy metals (Pb, cd and Hg), essential trace elements (Se, Zn, Mg and Cu) and biochemical parameters (TAC, SOD, CAT, GRx, GPx, GST, GSH and ALAD). Also study correlation between (Pb, Cd, Hg and MDA) and the levels of essential trace element (Se, Zn, Mg and Cu) and some biochemical parameters (TAC, SOD, CAT, GRx, GPx, GST, GSH and ALAD).

## MATERIAL AND METHOD

This study was carried out on peoples who work in gasoline stations from different areas of Basra governorate (Andalus Station, Jubailah Station, Dinar Station and Shabib Station). The study was included 50 workers exposed to different gasoline derivatives for not less than 1 years with an age range between 18 to 56 years (Mean ± SD = 30.45 ± 9.31) years and controlled with 50 healthy individuals (non-workers) aged between 18 to 50 years (Mean ± SD = 30.27 ± 10.37) to assess the health of gasoline station workers.

About (10 ml) of the blood was drawn from a forearm vein of each fasting worker in gasoline station and control subjects. (5 ml) were added into EDTA containing polypropylene tubes and shacked gently to be used for measurement the concentration of Se, Pb, Hg, Cd and ALAD. The remaining (5 ml) of whole blood transferred to plain tube and allowed to clot on ice , and then centrifuged in (402 Xg) for (15 minutes). The obtained sera immediately use in digestion procedure for determining Mg, Zn, Cu and estimate other parameters included (SOD, CAT ,GST ,GPx , GRx , MDA and TAC), and others of sera were stored in deep freezing at (-20°C) until using. The concentrations of Se was determined using hydride generation method [7]. The concentrations of Mg, Zn and pb in standards and samples solutions were measured by flame atomic absorption spectrometry (AAS) (GBC 933 Plus) [8] while the concentrations of Cu, Cd were measured by flameless atomic absorption spectrometry (AA500-PG)[8-9]. Moreover Hg was measured by Atomic Absorption Spectrometry using cold vapor apparatus AAS (Shimadzu AA-630-12) [10]. MDA,TAC, SOD, CAT,GRx, GST,GPx, ALAD and GSH were measured according to the methods of (Burtis & Ashwood,1999)[11],(Koracevic et al., 2001)[12], (Winterboun et al., 1975)[13,] (Mueller et al., 1997)[14], (Delides et al., 1976)[15], (Habig et al.,1974)[16], (Rotruck et al., 1984) [17], (Katsumaro, 1974) [18] and (Burtis&Ashwood, 1999) [11] respectively

## STATISTICAL ANALYSIS

The data were statistically analyzed according to the t-Student's test using the variance analysisVersion 17, SPSS Inc, Chicago). The obtained results were expressed as (mean values and standard deviations)are presented in tables. The level of $p < 0.05$ was considered as statistically significant, and as a highlysignificant at $p < 0.01$.

## RESULTS AND DISCUSSION

Table I shows the comparison of various trace elements and heavy metals of blood in workers with healthy control group. A highly significant ($p<0.01$) decrease in Se, Zn, Cu and Mg with ($p < 0.05$) was recorded in gasoline station workers, whereas heavy metals (pb, Hg and Cd) were found to be elevated ($p< 0.001$).

The results of different biochemical parameters in gasoline station workers and control group were listed in table (2).The results showed that the level of MDA in serum was increased in workers compared to healthy controls ($p<0.001$). Also, the results found there is a significant decrease of biochemical parameters with the workers of gasoline station when compared with the control group in: TAC ($P< 0.05$),Cu/Zn-SOD ($P<0.001$), CAT ($P<0.001$), GRx($P<0.001$), GST ($P< 0.001$), GPx ($P< 0.001$), ALAD ($P< 0.01$) and GSH ($P<0.001$).

Trace elements play a vital role in human body to perform the functions properly. These elements should be present in the body in appropriate amounts and must be available for reacting with other elements to form critical molecules as well as to participate in various important chemical reactions [19]. Then, decreased levels of whole blood Se in gasoline station workers may be due to increased oxidative stress that requires increased utilization of Se as antioxidant by binding with vitamin E and as a cofactor of GPx to scavenge free radical, which acting to detoxify tissue peroxidation [20]. These observations are in concordance with the findings of other studies [21].

Also, The present results indicated that the lower levels of serum Zn in gasoline station workers may be due to reduced levels of antioxidant enzyme SOD. The antioxidant properties of zinc have been demonstrated in vitro zinc may exert its antioxidant effect by decreasing the susceptibility of essential -SH groups of proteins to oxidation and by competing with pro-oxidant metals such as iron and copper for biological binding sites [22]. The lower levels of Cu concentration in serum of gasoline station workers may be due to the interaction of hydroquinone, the one metabolites of benzene, with Cu and Zn components of SOD enzyme and release Cu of the enzyme. Then the reaction between the released Cu and $H_2O_2$ generates reactive oxygen species and initiate lipid oxidation chain reactions, So Cu deficiency leads to a two fold increase in the peroxide processes [23].

Decreased levels of Mg might be due to its high utilization rate, suggesting that this Mg may be used to protect against oxidative stress. It has been established that oxidative stress is induced by both the increases in free radicals and the disturbance of the free radical scavenging system in worker of a gasoline station. On the other hand, it is possible that reduced level of Mg reflects low intake, excessive sweating or due to interaction Mg with heavy metal [24] which resulted in decreasing of antioxidant defense system in gasoline station workers.

The air in the work environment usually contains a number of chemicals like heavy metals, which are inhaled and absorbed by the body, pose a potential risk to workers health. The high levels of Pb, Hg and Cd in workers possible that the results from the inhalation of air contaminated with these metals, eating foods contaminated as a result of some bad habits when workers such as not washing hands contaminated, or enters the body through the skin and not use the protective clothes [25]. Also, elevated level of Cd come from smoking during occupational exposure as well as in the case of iron, calcium and protein deficiency in the body will increase the absorption of Cd and thus increases the concentration of Cd in the body and cause lipid peroxidation by increasing free radicals ROS and cause many of diseases [26].

The decrease of antioxidant refers to its utilization which prevents the cellular damage from free radicals, therefore a decrease in TAC, SOD, CAT, GRx, GST, GPx and GSH was observed. The increases in MDA level in worker of gasoline station may explain that the gasoline undergoes hepatic metabolism, generating hydroquinone, phenol and other compounds with the ability of redox cycling, which might cause excess generation of ROS [27]. Therefore the lipid peroxidation increased, that means increased of MDA.

Moreover, it has been observed that decreased level of serum GSH in workers could represent an adaptive response to increased oxidative stress and free radicals generation that oxidized thiol group of GSH and decline reduced glutathione level. The inhibition of ALAD activity is one of the most sensitive diagnostic indicators of lead exposure [28]. In the present study, there was a significant decrease in the whole blood ALAD activity levels (18.164± 0.866 Vs. 24.90 ± 1.205μmol/L, $P < 0.01$) in workers. The whole blood ALAD is a enzyme contain thiol groups that is particularly susceptible for oxidation [29]. Therefore, decreasing in the activity of whole blood ALAD results from the oxidation of the -SH group of ALAD by ROS which is generated in gasoline station workers as a result of exposed workers to numerous heavy metals.

Depending on the period workers in gasoline station, the group was subdivided into two groups as listed in table 3. The results were indicated that Pb, Cd, Hg and MDA were increased significantly with increasing of the period of gasoline station workers, Increase the accumulation of these elements within the body and led increase of ROS and MDA. For other hand, the results indicated that trace element Se, Zn, Cu and Mg decreased significantly with the increase of the period of gasoline station workers. Low levels of Se, Zn, Cu and Mg in workers could represent an adaptive response to increased oxidative stress and free radical generation which results from exposure to gasoline and heavy metals.

Moreover, the results indicated that TAC was decreased significantly with increasing of the period of gasoline station workers. The increase level of heavy metals from pollution as the period of pollution increased might increase ROS and decrease TAC [30]. In contrast to our study[31]. Also, the results show that an increase of the period of pollution, cause excess generation of ROS, therefore the enzymatic and non-enzymatic antioxidants levels decreased to reduce the oxidative stress that might be produced from the ROS.

The correlation coefficient between determined parameters in workers of gasoline station were conducted and presented in Table 4 and we found a negative and significant correlation between serum MDA level and level of elements (Se, Zn and Mg) in gasoline station workers ($r = -0.320, -0.331, -0.287, r = -0.351, r = -0.310$ and $r = -0.033$ with $P < 0.05$) respectively. Moreover, serum MDA level showed a negative correlation, without significance with Cu level and GST($r = -0.146$ and $r = -0.121$ $P > 0.05$).

These results possible to explain the elements (Se, Zn, Cu and Mg) that possess antioxidant properties in addition to being an integral part of antioxidant enzymes such as GPx, SOD, so the increased generation of ROS in workers at filling stations lead to increased lipid peroxidation and MDA level, therefore decreased levels of these elements in the workers as a result of their use in protecting cells against the harmful excitement caused by ROS. Moreover, the present results revealed that the serum MDA was positively and highly significantly correlated with Pb, Hg and Cd in workers of gasoline station ( $r = 0.723$ with $P < 0.0001$ and $r = 0.455$, $r = 0.536$ with $P < 0.001$) respectively. This positive correlation could be explained through the ability of these element to generate ROS by depletion glutathione, and protein-bound SH groups, resulting in the production of reactive oxygen species. As aconsequence, increased lipid peroxidation (increased MDA)[32]. Moreover, the Cd can compete with metals in protein binding sites [33] leading to the release of $Fe^{+2}$ and $Cu^{+2}$ ions, causing increased production of reactive oxygen species and increased of MDA end products of lipid peroxidation process.

The results confirmed that Pb level was negatively and highly significantly correlated with Se, Zn and Cu ($r = -0.511$, $r = -0.616$ and $r = -0.476$, $P < 0.01$) respectively . Futhermore, a negative correlation with no significant, has been shown between pb and Mg level ($r = -0.245$, $P > 0.05$). These results may be due to that Pb can bind to Se and form highly bonded Se-Pb complexes, which have been proposed as a mechanism for Se protective effect in Pb toxicity [34]. These results were probably due to that Pb can interact with trace metals (especially Zn) at the stage of their intestinal absorption and distribution in tissues as well as with their biological functions. Also found increased Pb concentration causes, a decrease in Cu absorption from the gastrointestinal tract [35].

Also in this study it is observed that the correlation between the level of Pb and TAC, SOD were highly significant negative ($r = -0.39$, $r = -0.733$, $p < 0.001$) and significant with CAT ($r = -0.295$, $p < 0.05$). The reason is probably due to an increased level of Pb in the blood causes increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage, that mean decreased level of TAC this agree with similar results [36].

The decreased SOD activity in gasoline station workers is probably due to the interaction of pb with Cu molecule. As SOD is a Zn-Cu containing enzyme, hence pb exposure induced Cu deficiency resulted in decreased erythrocyte SOD activity. Zn, which serves as a cofactor for SOD enzymes, is also replaced by Pb, which is another factor behind the inactivation of SOD [37]. CAT contains heme as the prosthetic group, the biosynthesis of which is inhibited by Pb and resulted in decrease erythrocyte CAT activation [38]. Furthermore, Pb concentration showed a negative correlation, highly significance with GRx, ALAD, GST, GPx and GSH ($r = -0.392$, $r = -0.587$ with $P < 0.01$ and $r = -0.395$ , $r = -0.381$, $r = -0.455$ with $p < 0.001$) respectively. Depressed levels of GRx, GST and GPx were all found to correlate with depressed GSH levels in occupationally-exposed workers [39]. Another reason GRx has a disulfide bond in its active site, but Pb interferes with the disulfide bond and inhibits the enzyme. This inhibition prevents the reduction of GSSG, making cells more susceptible to oxidative damage [40]. ALAD is a thiol-containing enzyme. Pb binds to the -SH group of this enzyme, making it inactive. Also decreased activity of ALAD result from interaction Pb with enzyme functional groups (Zn). ALAD affinity for lead is 25 times that for zinc, lead and zinc appear to compete for a single metal-binding site [41].

From results of this study noticed that there was a negative and significant correlation between Hg level in blood and level of Se ($r = -0.170$, $P < 0.05$). Also, found Hg level was negatively and highly significantly correlated with another elements in serum Zn, Mg and Cu ($P < 0.001$, with $r = -0.579$, $r = -0.299$ and $r = -0.401$) respectively. Furthermore, Hg level showed a negative and high significant correlation with TAC and SOD ($r = -0.300$ and $r = -0.534$, $P < 0.001$) respectively. Also, a significantly negative correlation was observed between Hg level and activity of CAT, GRx, GPx, ALAD in workers of gasoline station ($r = -0.201$, $r = -0.213$, $r = -0.291$ and $r = -0.342$, $p< 0.05$). Furthermore, there was a negative correlation , but not significant ( $r = -0.147$ and $r = -0.188$, $P > 0.05$) between Hg level and levels of GST and GSH, Table 4. Se can achieve a protective effect ,either by directly binding to Hg or serving as a cofactor for GPx, and thereby facilitating its ROS scavenging activity [42].

Hg is known to markedly alter the metabolism and function of some essential trace elements, such as Se, Zn, Cu and Mg by competing for ligands in the biological system. In such cases, a deficiency of a certain essential element may result because of interference with its absorption [43].The scavenger role of antioxidant enzymes in removing toxic electrophiles helps the cell to maintain its internal environment to a limited extent at lower concentration of Hg. An increase in the oxidative stress may be due to a decrease in the antioxidant defenses or due to an increase in the processes that produce oxidants[44]. In this study, Hg initiates lipid peroxidation by generating free radicals and thereby interfering with the antioxidant system of the cell. This toxicity may be due to mercury-induced alterations in membrane integrity via the formation of ROS and the perturbation of antioxidant defense mechanisms. Hg also inhibits the activities of free radical quenching enzymes such as CAT, SOD and GPx [45].

GSH is an important intracellular antioxidant that spontaneously neutralizes several electrophiles and reactive oxygen species, whereas GSH/GSSG ratio maintains the redox status of the cell. In addition, GSH can also be a substrate for GPx, GRx and GST, and is involved in the enzymatic detoxification reactions for ROS. Most of these enzymes become inactive also due to direct binding of the metal to the enzymes' active sites, if the sites contain –SH groups [46]. The decline in levels of erythrocyte ALAD of gasoline station workers might be due to ability heavy metal mercury to oxidize -SH group in this enzyme or from competes Hg with Zn in ALAD, making ALAD inactive .

The results of this study show that there was a negative and highly significant correlation between level of Cd and levels of Se and Cu ($r = -0.368$ and $r = -0.441$, $P < 0.001$). Also found significant negative correlation between Cd and Zn in gasoline station workers ($r = -0.95$, $P< 0.05$). Moreover, there was a negative with no-significant correlation between Cd and Mg ($r = -0.207$, $P > 0.05$), Table 4.

Cd interacts with Se may be induced by heavy metals exhibiting a high affinity for Se. Heavy metal influence bioavailability, uptake, transport, and physiological activity of Se. Se used as an antioxidant to protect cell from ROS generated by Cd and because ability of Cd to interact with Se and caused reduce level of Se and inhibition activity of GPx that containing Se in the active site. The reduced level of Cu and Zn with increased level of Cd may result from Cd susceptibility to displace Cu and Zn [47], this leads to reduce concentration of these metals. And a lot of studies show the ability Cd to interaction with Zn, Cu, Mg, Se and other metals [48], thus reduce its concentration in the body. Furthermore, Cd showed a negative and highly significant correlation with TAC and SOD ($P < 0.001$ with $r = -0.55$ and $r = -0.553$). This result may explain that Cd caused oxidative stress by ROS generation from replacing Fe and Cu in numbers of cytoplasmic and membrane proteins, which in turn would release and increase the concentration of unbound Fe and Cu ions. These free ions caused oxidative stress via the Fenton reaction[49], thus reduce the enzymetic and no-enzymatic antioxidant to protect cell from oxidative stress and that leads to decreased total antioxidant capacity with increased Cd level in blood of workers. In this result, we have demonstrated that Cu, Zn-SOD activity is strongly inhibited by Cd. It has also been demonstrated that Cd can replace Zn to reduce SOD activity [50]. Cd is similar to another heavy metals like Pb and Hg, all have electron-sharing affinities that can result in formation of covalent attachments with thiol groups of proteins, when GSH is depleted by any metal, GSH synthesizing systems start making more GSH from cysteine via the γ- glutamyl cycle, however, if GSH depletion continues because of chronic metal exposure. Several enzymes in antioxidant defense systems may protect this imbalance.

## CONCLUSION

Conclusion was drawn from the study that, an increase exposure of petroleum pollutants and heavy metals Pb, Cd, Hg led to an increase in the oxidative stress MDA for station workers. This is due to decrease in the trace elements, enzymatic antioxidant and non - enzymatic levels and an increase in the oxidants especially in the last period of work and decrease levels of trace elements and TAC, SOD, CAT, GPx, GST, GRx, GSH, ALAD with inceased period of working. This behaviors might suggest that workers subject to petroleum pollutants must supply with antioxidants dose especially with increasing period of exposure.

## RECOMMENDATION

Further study may be taken other parameters such as red blood cell counts, hemoglobin, haematocrit mean corpuscular hemoglobin and platelet counts and also had the determination of kidney function tests (urea, creatinine and uric acid) and liver function enzyme activities (aspartate aminotransferase and alanine aminotransferase) in worker exposure to gasoline.

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**Table Title**

Table 1. Levels of Essential trace element and Heavy metal in serum and whole blood of gasoline station workers and healthy control.

Table 2. Levels of Enzymatic and Non–Enzymatic Antioxidants in serum and whole blood of gasoline station workers and healthy control.

Table 3. The biochemical parameters of gasoline station workers at different periods of working, and control .
Table 4. Coefficient of correlation (r) between determined parameters in workers of gasoline station (n = 50).

Table 1. Levels of

| | | | Se | Zn | Cu | Mg | pb | Hg | cd |
|---|---|---|---|---|---|---|---|---|---|
| | | | (ng/ ml) | **(µg/ml)** | **(µg/ml)** | **(µg/ml)** | **(µg/ml)** | **(ng/ ml)** | (µg/ml) |
| | Mean ± SD | | **49.129±2.011** | **0.637±0.0283** | **0.2471±0.038** | **13.96± 1.009** | **0.704 ± 0.017** | **29.635±4.28** | 0.0531 ± 0.0022 |
| | SE | | **0.2844** | **0.0040** | **0.0053** | **0.1427** | **0.0024** | **0.6053** | 0.00031 |
| | Range | | **66.20 – 30.50** | **1.19- 0.13** | **0.4 - 0.14** | **19.14- 8.59** | **1.04-0.35** | **37 -21** | 0.1 – 0.03 |
| | 95 % C.I | Lower | **43.5471** | **0.5584** | **0.1416** | **11.1590** | **0.6568** | **17.7547** | 0.0469 |
| | 95 % C.I | Upper | **54.7121** | **0.7155** | **0.3526** | **16.7609** | **0.7512** | **41.5173** | 0.0591 |
| C n= 50 | Mean ± SD | | **68.679±3.204** | **0.892±0.0412** | **0.6333 ±0.044** | **15.5±1.325** | **0.2674± 0.013** | **14.70±2.636** | 0.0177 ± 0.0011 |
| P–value | | | HS | HS | HS | S | HS | HS | HS |

elements in serum and whole blood of gasoline station workers and healthy control.

| | | | MDA | TAC | SOD | CAT | $GR_X$ | GST | $GP_X$ | protein | GSH | ALAD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (μmol/L) | **(mmol/L)** | **(μmol/L)** | **(K/ml)** | **(μmol/L)** | **(μmol/L)** | **(U/mg protein)** | **(mg/ml)** | **(μmol/L)** | (μmol ALA/L of erythrocyte) |
| Gasoline station workers n =50 | **Mean ± SD** | | **5.744 ±0.132** | **1.153 ± 0.035** | **1.1258±0.031** | **0.258±0.01** | **14.532± 1.324** | **3.616 ± 0.1315** | **4.747±0.181** | **0.521±0.050** | **1.054 ± 0.0416** | 18.164± 0.866 |
| | **SE** | | **0.0187** | **0.0050** | **0.0044** | **0.0014** | **0.1872** | **0.01859** | **0.0256** | **0.0071** | **0.0058** | 0.1224 |
| | **Range** | | **7.99 – 3.20** | **1.79 – 0.18** | **1.52 – 0.36** | **0.48- 0.20** | **39.90- 2.89** | **6.56 – 1.90** | **7.76 -2.04** | **2.08 -0.03** | **1.86 -0.37** | 16.2-12 |
| | **95 % C.I** | **Lower** | **5.3775** | **1.0540** | **1.0392** | **0.2297** | **10.8566** | **3.2509** | **4.2445** | **0.0379** | **0.9385** | 15.7610 |
| | | **Upper** | **6.1104** | **1.2515** | **1.2124** | **0.2863** | **18.2074** | **3.9810** | **5.2494** | **0.0662** | **1.1695** | 20.5671 |
| C n=50 | **Mean ± SD** | | **2.254 ± 0.568** | **2.543 ± 0.034** | **1.83±0.1997** | **0.7157±0.073** | **36.168±1.934** | **7.576 ± 0.6815** | **9.267±0.4056** | **0.831± 0.02** | **1.85± 0.145** | 24.9± 1.205 |
| P–value | | | HS | S | HS | HS | HS | HS | HS | HS | HS | HS |

C: Health Control, n: Number of subjects, Values represent means ± SD (Standard deviation), SE: Standard Errors, Range: is the difference between the highest and lowest values in the set, , C.L: Confidence limits (Lower and Upper ), P- value: N.S (P> 0.05), S (P< 0.05), HS (P< 0.01) indicate the level of significance in comparison with the corresponding control value.

Table 2. Levels of Enzymatic and Non –Enzymatic Antioxidants in serum and whole blood of gasoline station workers and healthy control.

C: Health Control, n: Number of subjects, Values represent means ± SD (Standard deviation), SE: Standard Errors, Range: is the difference between the highest and lowest values in the set, , C.L: Confidence limits (Lower and Upper ), P- value: N.S (P> 0.05), S (P< 0.05), HS (P< 0.01) indicate the level of significance in comparison with the corresponding control value.

Table 3. The biochemical parameters of gasoline station workers at different periods of working, and control

| **Parameter** | **Control group n=50** | | **(1-5 year) n=32** | | **(> 5 year) n=18** | |
|---|---|---|---|---|---|---|
| | **mean** | **SD** | **mean** | **SD** | **mean** | **SD** |
| **Se (ng/ml)** | 68.679 | 3.204 | 50.083 | 2.31 | 48.175 | 1.981 |
| **Zn (µg/ml)** | 0.892 | 0.0412 | 0.646 | 0.0301 | 0.628 | 0.0252 |
| **Cu (µg/ml)** | 0.6333 | 0.0443 | 0.2462 | 0.0214 | 0.2480 | 0.029 |
| **Mg (µg/ml)** | 15.5 | 1.325 | 14.504 | 0.7401 | 13.416 | 0.331 |
| **Pb (µg/ml)** | 0.2674 | 0.0132 | 0.699 | 0.0192 | 0.7092 | 0.01454 |
| **Hg (ng/ml)** | 14.70 | 2.636 | 26.68 | 3.822 | 32.59 | 3.311 |
| **Cd (µg/ml)** | 0.0177 | 0.0011 | 0.0356 | 0.00133 | 0.0698 | 0.0031 |
| **MDA (µmol/L)** | 2.254 | 0.568 | 5.46 | 0.185 | 6.028 | 0.1237 |
| **TAC (mmol/L)** | 2.543 | 0.0342 | 1.219 | 0.0448 | 1.087 | 0.0305 |
| **SOD (U/ml)** | 1.83 | 0.1997 | 1.1607 | 0.02525 | 1.091 | 0.0425 |
| **CAT (K/ml)** | 0.7157 | 0.0734 | 0.3172 | 0.0104 | 0.1982 | 0.016 |
| **GRx (U/L)** | 36.168 | 1.934 | 14.86 | 0.9942 | 14.205 | 1.8639 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **GST (U/L)** | 7.576 | 0.6815 | 4.1491 | 0.1398 | 3.084 | 0.0946 |
| **GPx (U/mg protein)** | 9.267 | 0.4056 | 6.0105 | 0.1708 | 4.485 | 0.1612 |
| **GSH (µmole/L)** | 1.85 | 0.1450 | 1.2907 | 0.0494 | 0.8173 | 0.0314 |
| **δ-ALAD (µmol ALA /min/ L of erythrocyte)** | 24.900 | 1.205 | 20.932 | 0.423 | 15.396 | 0.636 |

Table 4. Coefficient of correlation (r) and P-valuebetween determined parametersin workers of gasoline station (n = 50)

| **parameter** | **MDA** | | **Pb** | | **Hg** | | **Cd** | |
|---|---|---|---|---|---|---|---|---|
| | **r=** | **P-value** | **r=** | **P-value** | **r=** | **P-value** | **r=** | **P-value** |
| **Se** | -0.320 | S | -0.511 | HS | -0.170 | S | -0.368 | HS |
| **Zn** | -0.331 | S | -0.616 | HS | -0.579 | HS | -0.441 | HS |
| **Cu** | -0.146 | N.S | -0.476 | HS | -0.401 | HS | -0.441 | HS |
| **Mg** | -0.287 | S | -0.245 | N.S | -0.299 | HS | -0.207 | N.S |
| **Pb** | 0.723 | HS | --- | --- | --- | --- | --- | --- |
| **Cd** | 0.536 | HS | --- | --- | --- | --- | --- | --- |
| **Hg** | 0.455 | HS | --- | --- | --- | --- | --- | --- |
| **TAC** | -0.671 | HS | -0.39 | HS | -0.300 | HS | -0.55 | HS |
| **SOD** | -0.792 | HS | -0.733 | HS | -0.534 | HS | -0.553 | HS |
| **CAT** | -0.351 | S | -0.295 | S | -0.201 | S | -0.217 | S |
| **GRx** | -0.310 | S | -0.392 | HS | -0.213 | S | -0.224 | N.S |
| **GST** | -0.121 | N.S | -0.395 | HS | -0.147 | N.S | -0.251 | N.S |
| **GPx** | -0.684 | HS | -0.381 | HS | -0.291 | S | -0.330 | S |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **GSH** | -0.033 | S | -0.455 | HS | -0.188 | N.S | -0.200 | S |
| **ALAD** | -0.675 | HS | -0.587 | HS | -0.342 | S | -0. 365 | S |

P- value: N.S (P> 0.05), S (P< 0.05), HS (P< 0.

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