

Antioxidants in Shoe-makers Exposed to Organic Solvents

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Abstract: Workers in the shoe manufactures are routinely exposed to mixtures of organic solvents. The aim of the present study was evaluation of the hazardous effects of occupational exposure to organic solvents in shoe-makers on their liver enzymes and the hepatic tumor marker (AFP), with assessment of the role of oxidative stress (MDA). Additionally, the present work aimed to evaluate the protective roles of the antioxidant enzymes (SOD and GR) and some trace elements (Se, Zn and Cu). The present study included 47 shoe-makers exposed to organic solvents for more than 5 years, and 40 control workers, never occupationally exposed to organic solvents. The liver enzymes (ALT, AST, and GGT), tumor marker (AFP), antioxidants (SOD and GR), oxidative stress marker (MDA) and some trace elements (Se, Zn and Cu) levels were estimated. Statistical analysis of the results revealed that ALT, AST, GGT, AFP and MDA in the workers were significantly higher compared to their controls, while the SOD, GR and Se of the workers were significantly lower compared to their controls. Even the antioxidants levels were significantly decreased and the oxidative stress was significantly increased in the shoe-makers compared to their controls, there were no significant relationships between the levels of antioxidants and the trace elements on one hand and the levels of liver enzymes and AFP on the other hand in the shoe-makers, except in the Se. Se was significantly negative correlated with the liver enzymes (ALT and GGT) in the shoe-makers. On conclusion: occupational exposure to organic solvents in shoe-makers found to have hazardous effects on the liver enzymes, AFP, and oxidative stress, but the protective roles of antioxidants and trace elements (except Se) was not been proved.

Key words: Shoe-makers, organic solvents, liver enzymes, AFP, SOD, GR, MDA, Se, Zn and Cu

INTRODUCTION

Liver injury has long been associated with occupational exposure to a wide variety of chemicals. Its susceptibility to chemical injury is a result of its unique position within the circulatory system, and also because it is the primary organ for the bio-transformation of chemicals within the body^[1], as liver is the main organ involved in the metabolism of toxins and medicinal agents. Such metabolism is always associated with the disturbance of hepatocyte biochemistry and generation of reactive oxygen species (ROS)^[2]. The concentrations of ROS have to be controlled by several defense mechanisms, which involve also a number of antioxidant and detoxifying enzymes. Their induction reflects a specific response to pollutants^[3]. A balance between free radical reactions

and antioxidant activities is very important for normal liver functioning. This balance is altered in pathological processes^[4]. Lots of liver damages ranging from subclinical hepatitis to necroinflammatory hepatitis, cirrhosis, and carcinoma have been proved to associate with the redox imbalance and oxidative stress^[5]. Some trace elements; such as zinc, was proved to have hepato-protective properties^[6,7]. Additionally, Gurusamy^[8] found that development of hepatocellular carcinoma (HCC) has been linked to the levels of some trace elements.

The most common chemicals known to cause liver injury are the organic solvents. Organic solvents utilized in various industrial processes may be associated with hepatotoxicity^[9]. There is some evidence that organic solvents may express their toxicity by the way of ROS that was found to induce

cell damage^[10]. Moreover, higher incidence of cancer is suspected in subjects exposed to organic solvents characterized by reactive metabolic intermediates, which are assumed to induce oxidative damage on liver, kidney and haematopoietic system^[11].

Employees in the shoe manufacturers are routinely exposed to a mixture of organic solvents used in cleaning and as diluents in glues, primers, and degreasers^[12]. Exposure to organic solvents in shoe factories has been reported to increase the risks for acute and chronic health problems in shoe-makers^[13]. In shoes industry, the risk of hepatotoxicity due to exposure to organic solvents has already been suggested^[14]. Although, the role of organic solvent exposures in HCC is limited, prolonged exposure to organic solvents such as toluene and xylene may represent a risk factor for liver cancer^[15].

The aim of the present study was evaluation of the hazardous effects of occupational exposure to organic solvents in shoe-makers on their liver enzymes and the hepatic tumor marker (AFP), with assessment of the role of oxidative stress (MDA). Additionally, the present work aimed to evaluate the protective roles of the antioxidant enzymes (SOD and GR) and some trace elements (Se, Zn and Cu).

MATERIALS AND METHODS

The present study included 47 shoe-makers occupationally exposed to organic solvents for more than 5 years (workers group), and 40 workers never occupationally exposed to organic solvents (control group). The shoe-makers were selected from five small workshops. All the included subjects in the two groups were from urban area. After verbal consent, detailed personal, occupational and medical questionnaire was completed with the workers through personal interview. Subjects with history of viral hepatitis, bilharziasis, blood transfusion, or hospitalization for surgery were excluded from the study.

Samples Collection and Assay: Random blood samples were collected from all cases by sterile disposable syringes. Part of samples was left to clot and centrifuged, the separated serum was used for estimation of biochemical parameters and the other part was collected in clean tubes with EDTA as anticoagulant substance for estimation of GR and SOD.

Serum aminotransferases (ALT and AST) were estimated by the method of Provisional Recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes^[16]. Serum Gamma glutamyl transferase (GGT) was

measured using the method of Szasz^[17]. Enzymatic immunoassay for the determination of Alpha fetoprotein (AFP) in human serum was estimated according to Chiergatti *et al*^[18].

Determination of blood superoxide dismutase (SOD) activity was based on the method developed by McCord and Fridovich^[19], blood Glutathione reductase (GR) activity was determined according to oldberg and Spooner^[20]. Serum Malondialdehyde (MDA) was determined according to the method of Satoh^[21].

Serum trace elements, selenium (Se), zinc (Zn) and copper (Cu) were determined according to AOAC^[22], using Atomic Absorption Spectrophotometer (SOLAAR-UNICAM 989).

Statistical Analysis: The collected data and the laboratory results were computerized. Statistical analysis was done through SPSS version 14.0. The quantitative results were expressed as means \pm standard deviation (SD), and qualitative results as number (No.) and percent (%). Independent t-test, Pearson's Chi-square (χ^2), Likelihood Ratio, and Pearson's correlation coefficient were used in the analysis of the results. P-value \leq 0.05 was considered significant.

RESULTS AND DISCUSSION

Results: In table (1), the liver enzymes (ALT, AST, and GGT), and the tumor marker (APF) of the shoe-makers were significantly higher compared to their controls.

Figures 1 to 3 illustrate the percentages of cases with abnormal elevated liver enzymes in the examined two groups. The percentages of shoe-makers with abnormal elevated liver enzymes (ALT, AST, and GGT respectively) were significantly higher compared to their controls.

Table (2) shows that the antioxidants (SOD and GR), and the Se level of the shoe-makers were significantly lower compared to their controls, and the oxidative stress marker (MDA) was significantly higher in the shoe-makers than in the control group. While, there were no significant differences between the two examined groups according to the levels of Zn, Cu and Cu/Zn ratio.

Table (3) shows that there were no significant relationships of the duration of exposure to organic solvents in shoe-makers (after controlled for age and smoking index) with the levels of their liver functions (ALT, AST, and GGT), the tumor marker (AFP), the antioxidant GR and Cu/Zn ratio. While, there were inverse correlations of the duration of exposure with

Table 1: Comparisons of the liver enzymes and AFP between the two examined groups

	Controls (40)		Shoe-makers (47)		Independent t-test	
	Mean	SD	Mean	SD	t-test	P-value
ALT (U/L)	3.8	0.22	10.7	0.86	8.037	P< 0.0001
AST (U/L)	5.7	0.35	16.0	0.84	11.301	P< 0.0001
GGT (U/L)	5.7	0.58	19.3	3.81	3.37	P= 0.001
AFP (ng/ml)	4.9	0.54	14.2	2.59	3.735	P= 0.001

Table 2: Comparisons of the levels of antioxidants, oxidative stress, and trace elements between the examined two groups

	Controls (40)		Shoe-makers (47)		Independent t-test	
	Mean	SD	Mean	SD	t-test	P-value
Blood SOD (µgU/gHb)	1949.4	59.45	1046.3	192.35	24.841	P< 0.0001
Blood GR (Iu/ g Hb)	3.3	0.15	2.9	0.35	5.960	P< 0.0001
Serum MDA (nmol/ml)	1.5	0.19	2.7	0.66	9.552	P< 0.0001
Serum Se (ppm)	63.0	23.35	32.7	19.78	3.203	P< 0.005
Serum Cu (ppm)	285.2	60.57	319.0	48.55	1.447	NS
Serum Zn (ppm)	132.9	34.81	121.6	84.48	0.295	NS
Cu/Zn ratio	2.21	0.18	4.82	0.89	0.956	NS

Table 3: The relationships of the duration of exposure to organic solvents in the shoe-makers with their liver enzymes, AFP, antioxidants, oxidative stress and trace elements after controlled for age and smoking index

Controlled for Age & SI	Duration of exposure	
	r=	P-value
ALT	0.1	NS
AST	0.2	NS
GGT	0.2	NS
AFP	0.3	NS
SOD	-0.4	P= 0.05
GR	0.1	NS
MDA	0.4	P= 0.05
Se	-0.5	P< 0.05
Cu	0.5	P< 0.05
Zn	-0.4	P< 0.05
Cu/Zn ratio	0.3	NS

the levels of SOD, Se, and Zn, and positive correlations with MDA, and the Cu levels.

Table (4) shows that the levels of SOD, GR and MDA had no significant correlation with the liver enzymes, as well as with AFP in the shoe-makers. According to trace elements, there were significant inverse correlations of the Se levels with the levels of

ALT and GGT, but not with the levels of AST or AFP. While, the other trace elements had no significant relationships with the liver enzymes, as well as with AFP.

Table (5) shows that in the shoe-makers, there were no significant relationships between the trace elements (Se, Cu, Zn and Cu/Zn ratio) and antioxidants

Table 4: The relationships of the liver enzymes and AFP in the shoe-makers, with their levels of antioxidants, oxidative stress and trace elements

	ALT		AST		GGT		AFP	
	r=	P-value	r=	P-value	r=	P-value	r=	P-value
SOD	-0.2	NS	-0.2	NS	-0.2	NS	0.2	NS
GR	-0.1	NS	-0.1	NS	0.1	NS	-0.3	NS
MDA	0.3	NS	0.2	NS	0.3	NS	0.3	NS
Se	-0.3	P< 0.05	0.1	NS	-0.3	P= 0.05	0.3	NS
Cu	0.1	NS	0.1	NS	-0.3	NS	0.2	NS
Zn	-0.1	NS	-0.1	NS	-0.03	NS	-0.01	NS
Cu/Zn ratio	0.1	NS	0.1	NS	-0.1	NS	0.03	NS

Table 5: The relationships of the trace elements in the shoe-makers, with the levels of antioxidant and oxidative stress markers

	Se		Cu		Zn		Cu/Zn	
	r=	P-value	r=	P-value	r=	P-value	r=	P-value
SOD	0.03	NS	0.1	NS	0.2	NS	-0.02	NS
GR	0.1	NS	-0.1	NS	0.2	NS	-0.01	NS
MDA	-0.2	NS	0.02	NS	-0.4	P = 0.05	0.2	NS

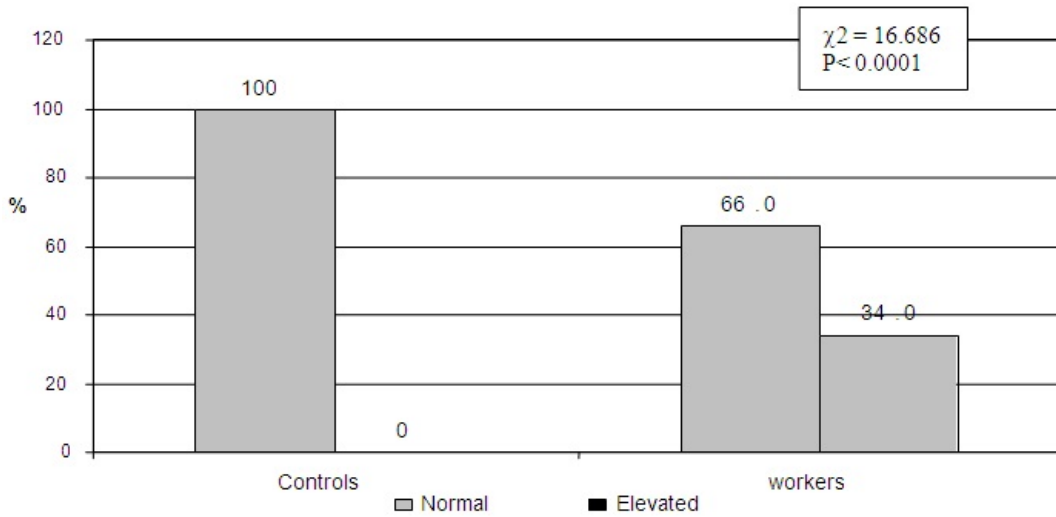


Fig. 1: Percentage of subjects with elevated ALT in the examined two groups

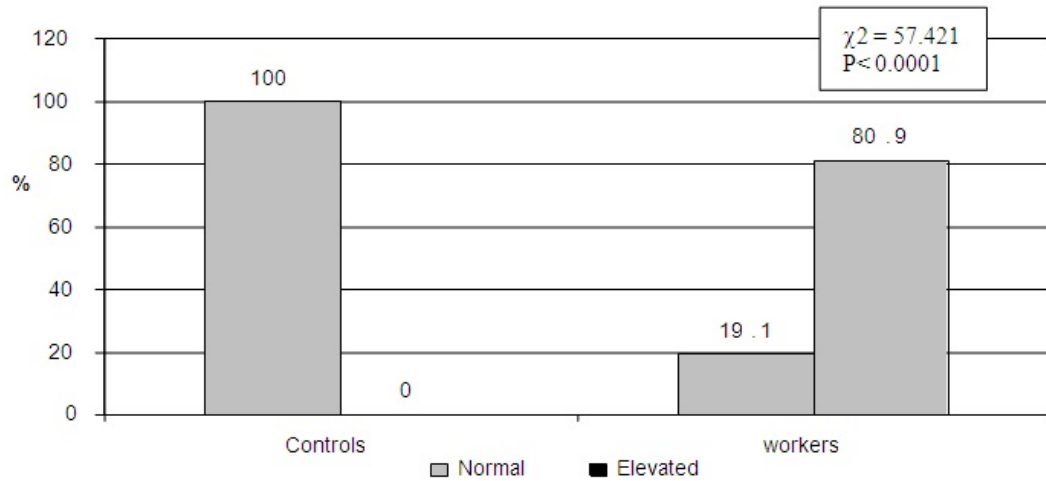


Fig. 2: Percentage of subjects with elevated AST in the examined two groups

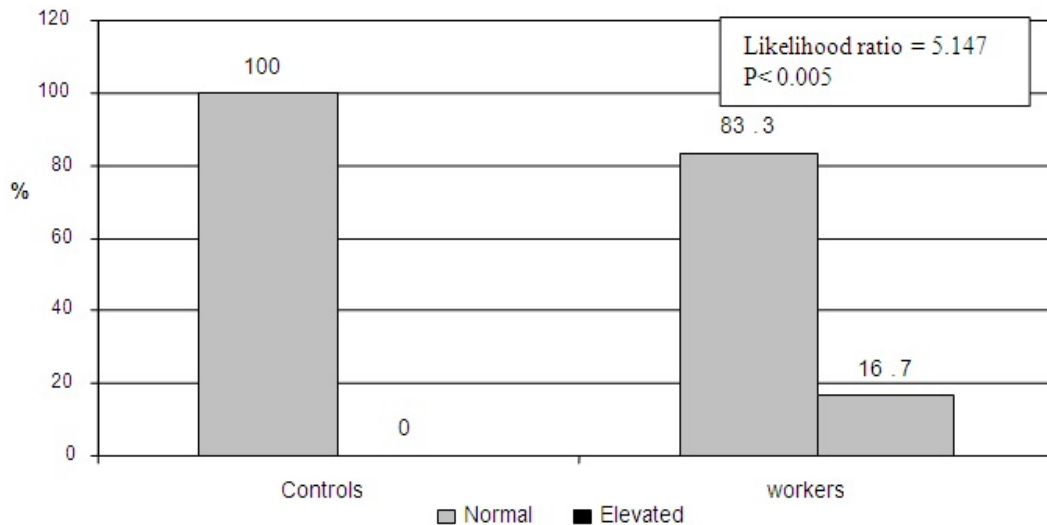


Fig. 3: Percentage of subjects with elevated GGT in the examined two groups

(SOD and GR), as well as oxidative stress marker (MDA), except between Zn and MDA. There was inverse correlation between Zn and MDA.

Discussion: Organic solvents is still an important occupational health problem due to its widespread usage, even in developed countries where there are strict rules and regulations about workplaces and working conditions^[23]. There are certain operations where organic solvents exposure is significant and represents a health hazard to the workers in shoe manufactures. These operations are mainly gluing, waxing, and polishing stations^[24,25].

Although the link between toxic hepatitis and exposure to organic solvents is relatively well documented, there is no specific laboratory or histological diagnostic findings for chemical-induced hepatitis^[26]. In general for detection of liver cellular injury, occupational medical surveillance programs have relied on measurement of the conventional liver enzymes. In screening for possible effects of hepatotoxicants, it is important to select the liver enzymes tests with the best combination of specificity and sensitivity. The aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are the most commonly measured enzymes that detect hepatocellular injury due to the toxicant's effect on all or part of the hepatocyte, including the cell membrane^[27]. For routine screening of chemicals that are known or suspected to cause hepatocellular injury, the ALT is considered to be the aminotransferase most specific for the liver, while, routine screening with "profiles" which also include GGT, bilirubin, LDH and protein determinations, provide limited additional information^[28]. Abnormal ALT can sound as an alarm

to alert medical staff to the possibility of some kinds of liver problem, which require further medical workup^[29]. Nasterlack *et al.*^[30] found that γ -glutamyl transpeptidase (GGT) in addition to ALT and AST are used as sensitive biomarkers for possible hepatocellular damage due to exposure to organic solvents.

In the current study, the liver enzymes (ALT, AST and GGT) of the shoe-makers were significantly elevated compared to their controls, and the percentages of the shoe-makers with abnormal elevated liver enzymes were significantly higher than in the control group. The liver enzymes of all the control subjects were within the normal levels.

Agreement with the present results, several studies proved that liver enzymes were significantly elevated in workers exposed to organic solvents compared to controls. The levels of ALT and AST enzymatic activities in the workers in shoe manufactures were found to be higher than that control group^[31]. Also, Tomei *et al.*^[14] showed similar results in shoe repairers who work in supermarkets, and use the glues which contain mixtures of organic solvents, the workers had higher levels of ALT and AST compared to their controls. Additionally, Ann *et al.*^[32] found that cumulative exposure to a mixture of organic solvents might cause a significant variability in the abnormality of liver enzymes (AST, ALT, and GGT) in male shipyard workers. In car painters exposed to organic solvents, statistical analysis also revealed a significant increase in AST level in the exposed workers compared with their controls^[33].

Contradictory, several studies have shown that, exposure to a mixture of organic solvents does not affect the levels of the liver enzymes, such as ALT, AST, and GGT^[34,36]. The controversial data existing in

relation to hepatotoxicity of organic solvents might be explained as a consequence of the different exposures or it may well be that the tests used for evaluating liver function might not be sensitive enough to detect any mild changes at an early stage^[37].

A number of blue-collar jobs were found to be significantly associated with primary liver cancer, including shoe fabrication^[38,39]. AFP is a biomarker often used for detection of carcinoma cells in the peripheral blood of the patients with HCC^[40]. In the current study, the concentration of AFP was significantly higher in the shoe-makers than in their controls. However, in HCC cases 84.3% of patients had increased level of AFP ranging from 32 ng/ml to more than 400 ng/ml^[41], the present study revealed that the mean value of AFP in the workers was with mean 14.2 ng/ml. Thus, we could not exclude increase the percent of liver injury in the shoe-makers compared to their controls, but we could not suspect that there was any case of HCC among the examined workers. Further follow-up every three months was recommended, especially for the suspicion workers.

Moreover, Schiodt *et al.*^[42] mentioned that serum concentrations of AFP were found to be elevated during liver injury, and have been suggested to be of prognostic importance in acute liver failure. So, the significant elevation in the levels of AFP in the shoe-makers in the current study could denote the magnitude of liver injury in the workers, and that monitoring of AFP might be used as screening marker for prediction of acute liver failure in workers exposed to organic solvents.

Unexpected, the present study revealed that there were no significant relationships between the duration of exposure to organic solvents in the shoe workers and the levels of liver enzymes (ALT, AST,GGT) and the levels of AFP. Contrary to the present results, Kaukiainen *et al.*^[43] found that AST and ALT correlated positively with cumulative exposure to the organic solvents for more than 5 years. But in agreement with the present study, Uuskulainen and his colleagues^[44] found the same results in shoe-repairers, they attributed the absence of these relations to the discontinuity of the exposure, as shoes repair work is not a repeated process, and thus the exposures may vary from day to day, depending on the work tasks, amounts of work, and chemicals used. This could be the same explanation in the present study, as although, the shoe-workers in the present study were exposed to organic solvents for more than 5 years, they were selected from small workshops, and their exposures vary from day to day according to the requests.

Typically, reactive oxygen species (ROS) are formed in cells through the reduction of oxygen by biological reducing agents, with the catalytic assistance

of electron transfer enzymes and redox active chemical species such as redox active organic chemicals and metals^[45]. ROS are key participants in the damage caused by toxic gases, such as organic solvent (e.g. toluene, xylene, ethanol, methanol and formaldehyde)^[46,47,48], and benzene which can be enzymatically bioactivated to reactive intermediates that can lead to increased formation of ROS^[49]. In the area of endogenous antioxidant defenses, excess amount of ROS leads to the depletion of the protective antioxidants, superoxide dismutase (SOD) and glutathione (GSH)^[50].

Superoxide dismutase (SOD) is an enzyme extensively used as a biochemical indicator of pathological states associated with oxidative stress^[51]. It is the only enzymes dismounting superoxide radicals. There are numerous mechanisms by which cells defend themselves against oxidants^[52]. While, glutathione reductase (GR) is the key enzyme of glutathione metabolism and is widespread in all tissues and blood cells. This enzyme catalyses reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH^[53,54].

In the current work, the antioxidants (SOD and GR) in the shoe-makers were significantly decreased compared to their controls, and this decrease increases significantly with the increase in the duration of exposure, as there were inverse correlations of the levels of SOD with the duration of exposure. Compatible to the present result, serum SOD activities were significantly lower in house painters exposed to organic solvents^[55]. Kumar and Singh^[56] mention that benzene, toluene and xylene are the chief industrial organic solvents and manifest their toxicity through elevation in lipid peroxidation and reduction in the antioxidant levels. Also, there was direct correlation of exposure to benzene, as an organic solvent, and the decrease in the levels of SOD in the human^[57]. These findings suggest that the decrease in SOD might be due to the major damage on the human body as a result of exposure to organic solvents, such as benzene, and that detection of the low levels of SOD might provide an earlier sign for the benzene damage.

Contrary to the current results, Wu *et al.*^[58] found that there was an increase in the activities of SOD in the serum of workers exposed to organic solvent, such as benzene, with the highest values recorded in workers with benzene poisoning. In addition, there was an increase in the SOD levels of people working with paint thinner compared with controls^[59]. However, Georgieva *et al.*^[60] found that workers exposed to a mixture of hydrocarbons, the SOD activities showed no significant changes.

In particular, more and more data are supporting the hypothesis that oxidative stress would be

determinant for the toxicity exerted by many xenobiotics, which create an imbalance of the homeostasis between oxidant molecules and antioxidant defense of the organism^[11,61]. Hepatotoxicity by oxidative stress may be achieved through a direct attack of ROS on essential biomolecules with loss of their biological functions and cell viability^[62,64]. ROS play a crucial role in the induction and progression of liver disease including acute hepatitis and HCC independent of etiology^[4,65,66]. The leading mechanism of free radical toxicity is the peroxidation of membrane phospholipids^[67,69]. Where free radical can damage cellular macromolecules, and therefore, may participate in hepatocellular injury^[70]. Malondialdehyde (MDA) is one of the end-products of the peroxidation of membrane lipids caused by ROS formation, especially by the superoxide ion. It is currently considered to be a basic marker of oxidative stress^[71].

In the current study, MDA was significantly elevated in the shoe-makers compared to their controls. This elevation in the MDA levels increases significantly with the increase in the duration of exposure, as there was a positive correlations of MDA with the duration of exposure. The marked rise in MDA concentration observed here indicates uncurved cellular damage induced by ROS induction. Several studies showed increase the levels of MDA in response to organic solvent^[46,47,51,72]. Similarly, Karagöçler *et al.*^[54] reported that MDA concentration, a measure of lipid peroxidation, was found to be significantly elevated in house painters occupationally exposed to organic solvents compared to controls. Agreement with the present results, in workers exposed to benzene serum MDA was increased^[56]. Halifeoglu *et al.*^[58] also found that inhalation of organic solvent may increase the lipid peroxidation. Additionally, workers exposed to a mixture of hydrocarbons had significantly higher MDA levels, and when these workers were given tablets containing vitamins, microelements, and bioflavonoids, in appropriate doses, the results after 40 days of supplementation demonstrated that the number of subjects with high values of MDA decreased significantly^[59].

Rahelić *et al.*^[73] suggested that correction of trace elements have a beneficial effect on the progression of liver cirrhosis. Shaban^[74], suggested that benzene, as an organic solvent, induce hepatic injury that was related to the formation of free radicals. She also proved that dietary supplement with Zn and Se in experimental animals prevented this toxicity. Se and Zn are essential trace elements involved in cellular protection against the deleterious effects of ROS^[75].

In the present study, Se was found to be significantly decreases in the shoe-makers compared to their controls. This decrease in the levels of Se increases significantly with the increase in the duration

of exposure, as there was an inverse correlations of the Se levels with the duration of exposure. Agreement with these results, it was found that chronic inhalation of volatile organic solvents can decrease the levels of Se, and thus, may lead to a decrease in the activity of antioxidant enzyme systems in adolescent abusers^[76]. Contradictory, Georgieva *et al.*^[60] found that Se were not significantly changes in workers exposed to a mixture of hydrocarbons.

In the current study, the levels of Cu and Zn, as well as the Cu/Zn ratio in the shoe-workers were not significantly different compared to their controls. But, there was an inverse correlations of the Zn levels with the duration of exposure, and a positive correlation of Cu levels with the duration of exposure. These results coincide with those obtained by Andrzejak *et al.*^[77], they found that in footwear factory workers exposed to toluene, benzene and trace amounts of chromium the levels of Cu and Zn in serum were within the normal values. Also compatible with the present results, El-Gazzar and his colleagues^[78] found that there were no significant variations detected in the levels of Cu and Zn between the workers exposed to benzene and toluene and the control subjects. Contrary to the current results, Dündaröz *et al.*^[76] found that chronic inhalation of volatile organic solvents can decrease the plasma levels of Zn.

The current study also found that the levels of GGT and ALT were significant inverse correlation with Se levels. While, there were no significant relationships between the levels of liver enzymes and the levels of Cu and Zn, as well as Cu/Zn ratio. Similarly, Navarro-Alarcón *et al.*^[79] proved that, in individuals with liver diseases (cirrhosis and hepatitis) GGT levels were significantly inverse correlated with serum Se levels, indicating that when the intensity of the hepatic injury increases (enhancement in serum GGT levels) concomitantly the serum Se levels decrease significantly. While, contrary to the present results Hyun *et al.*^[80] found that ALT and AST were significantly associated with serum levels of Cu and Zn, and the Cu/Zn ratio. Also, Pramoolsinsap *et al.*^[81] detected a significant correlation between serum Cu levels and GGT.

Moreover, the present results revealed that the Zn was found to be significantly inversely correlated with the MDA, as a marker of oxidative stress. Several studies proved that the same results^[82,83], they found a statistically significant negative correlation between Zn levels and the concentrations of MDA.

Thus on conclusion, exposure to organic solvents in the shoe-makers increases the risk of hepatotoxicity. Correction of antioxidants and trace elements concentrations may be recommended but under medical supervision. Periodic medical care was also recommended.

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