Protective Effect of Vitamin C on Some Biological Parameters of Blood and Liver Upon Mercury Exposure

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ABSTRACT

Mercury is considered one of the pollution problems as a toxic metal. The present study was carried out to investigate the protective effect of L- ascorbic acid (vitamin C) against chronic mercury exposure in white New Zealand male rabbits. The efficacy of vitamin C against induced mercury toxicity was evaluated by estimating some biochemical parameters in both blood plasma and liver tissues. Such parameters are generally used to evaluate the individual health status. Statistical analysis has been carried out using the SAS program.

The results indicated that inducing both low and high doses of mercury caused significant increases in blood glucose, total lipids, triglycerides, LDL and total cholesterol, whereas these treatments caused a significant (P<0.05) decrease in the HDL only after the long term exposure.

Values of AST , ALT ,and acid phosphatase (ACP) in plasma and liver were significantly elevated after the short as well as the long term of treatment, while significant decreases were observed in the alkaline phosphatase (ALP) and acetyl choline esterase (AChE) activity due to the long exposure to mercury. The results of oxidative stress markers (TBARS and GSH) showed significant hazardous effect of the mercury exposure.

Oral treatment with vitamin C decreased all the hazardous health effects caused by inducing mercuric acetate. Accordingly, addition of ascorbic acid as an antioxidant is recommended to be included in the human diets for its efficacy role in preventing the body from the mercury toxicity.

Key words: Mercury toxicity, vitamin C, biochemical parameters, blood plasma,liver enzymes.

INTRODUCTION

Heavy metals pollution is one of the worldwide health problems. Mercury is the most dangerous trace element, and chronic mercury exposure can cause adverse health effects. Mercury is used in different clinical and scientific purposes, therefore it is found in the air, soil, water and foods especially fish and shell fish. It is considered as one of the toxic substances on earth and it has the ability to accumulate in the food chain (Hounkaptin *et al.*, 2012, Engelberth *et al.,* 2013, Mok *et al.,* 2014).

Mercury is found in three forms, organic, inorganic, and metallic. All of these forms target the human body organs (Oliveira *et al.* 2012), and because it has the ability to soluble in fats, it targets the fatty tissues and organs such as brain and liver causing adverse effects (Diez *et al.,* 2009, Carman *et al.,* 2013).

The toxic effects of mercury on human and animal health have been reported extensively. It may exert its effect by helping producing free radicals and inhibiting the antioxidant enzymes, which have high efficiency against metal poison (Sharma *et al.,* 2005, Karapehlivana *et al.,* 2014).

It was found that treatment of rabbits and fish by mercury caused significant increases in the concentration of ALT and AST, and a significant decrease in the activity of ALP. These results indicated damage occurred in the liver and bone tissues (Ahmed, 1993, Abdel-Tawab *et al*., 2004, Sharma *et al.*, 2005, Wadaan, 2009, Patnaik *et al.*, 2010).

Vitamin C is an essential nutrient, and it has antioxidants property. Therefore it can be speculated that it could have a protective role in mercury toxicity through scavenging free radicals. Also, it has an important role in a great number of bio-

chemical processes such as synthesis of collagen, assists in metabolizing iron and helps in protecting vitamins A, E and unsaturated fatty acids, as well as glutathione (GSH).

Therefore the present study was undertaken to investigate the adverse health effects on some serum biochemical parameters caused by chronic exposure to mercury and the protective role of vitamin C against the mercury toxicity in white male rabbits.

MATERIALS AND METHODS

Materials

Thirty New Zealand white male rabbits (2-2.5 months old), weighing (2-2.5 Kg) were used. The animals were obtained from the laboratory of rabbit physiology research, Faculty of Agriculture, Alexandria University. All animals were kept 2 weeks without experimental treatment for acclimatization, and handled in accordance to the guide of experimental animals protection. The basal diet was purchased from the Global-IBEX-Limited-Company, Giza, Egypt. It consisted of 28% alfalfa meal, 44% corn and soybean meal, 23% wheat bran, 3% soybean oil, 0.5% mineral and vitamin mixture, and 0.4% sodium chloride. The approximate chemical composition of the basal diet was 12% crude fiber, 18% protein and 3% fat, and it provided about 2600 Kcal/ Kg. The doses of mercury acetate were given orally using a syringe 3 times a week.

Methods

Rabbits were randomly divided into 5 groups. One group (group 1) was kept as the control fed a basal diet. Group 2 and 3 were treated by a low dose of mercuric acetate (50 ppm/kg body weight) (Ahmed 1993), either without or with given vitamin C (8 mg / kg body weight) (Moumen *et al*. 2011), respectively. The last two groups (4 and 5) were treated by a high dose of mercury acetate (500 ppm/kg body weight) without injection (group 4) and with injection by vitamin C (group 5).

Three of the animals in each group were sacrificed after 4 weeks to study the effect of a short term exposure to mercury, while the other three animals were sacrificed at the end of the experiment (15 weeks) to investigate the effect of a long term exposure to mercury.

Blood samples were received in dry test tubes and centrifuged at 860 xg for 20 minutes. The obtained plasma was kept at -80 C for analysis. Plasma samples were analyzed for glucose, total lipids, cholesterol and triglycerides (TG) according to the methods of Zollner & Kirsch, (1962), Trinder (1969), Bucolo & David (1973) and Fossati & Prencipe (1982), respectively. High-density lipoprotein-cholesterol (HDL-c) was determined according to the methods of Grove, (1979), and Burstein *et al.* (1980). Low-density lipoprotein-cholesterol (LDL-c) was determined by calculation (cholesterol-(TG/5+HDL). All previous tested parameters were determined using commercial kits from Bio-systems S.A. (Spain), Diamond (Germany) and Randox (United Kingdom).

The method of Reitman& Frankel (1957) was used to assay the activities of ALT and AST, whereas the ALP and ACP activities were determined according to the methods of Kind & King, (1954), and Belfield & Goldberg, (1971), respectively.

Plasma thiobarbituric acid-reactive substances (TBARS) and reduced glutathione (GSH) were used to predict the level of oxidative stress (Jollow *et al*., 1974).

The data were statistically analyzed according to Steel & Torrie (1981). Statistical significance of the difference in values of control and treated animals was calculated by F test with 1% and 5% significance level. Then, the data were statistically tested by using Duncan's Multiple Range Test (SAS, 2004).

RESULTS AND DISCUSSION

Plasma Glucose

The data in Table (1) illustrate that exposure of the animals to mercury at both at low or high dose for short term insignificantly elevated the glucose level compared to the control group. Also, the long term exposure to low and high doses significantly ($P < 0.05$) elevated the glucose level by 12.9 % and 20.1%, respectively. Minoia *et al.* (2009) stated that insulin is the most hormone affected by mercury exposure through its ability to reduce hormone-receptor binding.

Presence of vitamin C reduced the glucose level to near that of the control group. Vitamin C could be able to antagonize the long term toxic effect of mercury upon the function of pancreas. Chen *et al.*,

Table 1: Effect of mercuric acetate alone and with vitamin C on plasma glucose (mg/100ml)

Group treatment	After 4 weeks	After 15 weeks
	Mean ± SD	Mean ± SD
(1) Control	104.54 ± 0.81[a]	107.57 ± 3.52 [b]
(2) Hg low dose	113.94 ± 6.46 [a]	111.73± 7.47 (12.9%)[a]
(3) Hg low dose + Vit. C	103.94 ± 1.21 [a]	105.89 ± 7.99 [b]
(4) Hg high dose	114.48 ± 2.35 [a]	129.17± 3.04 (20.1%)[a]
(5) Hg High dose + Vit. C	104.97± 6.84 [a]	101.24± 1.89 [b]

Data sharing a subscript letter in a column are not significantly different (P< 0.05)

(2010) and Chang *et al.,* (2011) mentioned that vitamin C, as an antioxidant, has the ability to inhibit the release of the corticosterone hormone which prevent the gluconeogenesis from non-carbohydrate resources. Also, vitamin C can bind mercury and prevent it from damaging tissues, and increase the anti-oxidant capacity of the cells and therefore helps in elimination of mercury from the body.

Blood Total Lipids

Exposure of the rabbits to mercury at low dose for short period of time had no significant effect on the blood total lipids, while high dose caused significant (P<0.05) elevation (14.6%) in the total lipids as compared to the control group (Table 2). Long term exposure significantly elevated the plasma total lipids by 12.9% and 30.3% due to low and high dose exposure, respectively. Bapu *et al.* (2003) mentioned that mercury is one of the agents which disturb cell lipid membranes, leading to elevation in the blood lipids.

The data in (Table 2) also, show that the levels of total lipids in the mercury-ascorbic acid groups decreased to be near that of the control group. It seems that ascorbic acid has a protective effect and has the ability to keep the total lipids to normal levels.

Blood Lipid Profile

The results of the blood lipid profile are presented in Table (3-A, 3-B, 3-C, 3-D). The triglyceride levels have been elevated in groups treated either by the low or the high dose for short as well as for long periods compared to the control group (Table 3-A). The percentage of increase was 41.6% after the short time of exposure. Giving the animals vitamin C decreased significantly these levels. Ismail & Ismail (2014*)* did not find any change in the serum triglycerides of rats treated for 5 weeks either in the presence of mercury alone or combined with ascorbic acid.

Total cholesterol (Table 3-B) and plasma LDL (Table 3-D) were significantly increased only after the long time of treatment with mercuric acetate, while the HDL (Table 3-C) decreased significantly (P<0.05) after the short as well as the long period of treatment.

The total cholesterol and plasma LDL levels have been returned to the levels of the control group when ascorbic acid was given. Also, the HDL values were significantly decreased in all of the animal groups as compared to the control, but the percentages of decrease were less in the groups treated with vitamin C (Table 3-D).

Table 2: Effect of mercuric acetate alone and with vitamin C on plasma total lipids (mg/100 ml)

Group treatment	After 4 weeks	After 15 weeks
	Mean ± SD	Mean ± SD
(1) Control	405.66 ± 6.55[b]	455.45 ± 55.51 [c]
(2) Hg low dose	438.68 ± 29.09[b]	514.15 ± 4.80 (12.9%) [b]
(3) Hg low dose + Vit. C	423.48 ± 12.91[b]	497.90 ± 23.08 [c]
(4) Hg high dose	464.88 ± 9.14 (14.6%)[a]	595.91±22.91 (30.3%) [a]
(5) Hg High dose + Vit. C	417.72 ± 23.62[b]	453.35 ± 27.69 [c]

Data sharing a subscript letter in a column are not significantly different (P<0.05)

Table 3-A: Effect of mercuric acetate alone and with vitamin C on plasma triglycerides (mg/100 ml)

Group treatment	After 4 weeks	After 15 weeks
	Mean ± SD	Mean ± SD
(1) Control	119.59 ± 1.70 [b]	112.52 ± 0.36 [b]
(2) Hg low dose	169.39 ± 3.19 (41.6%) [a]	125.03 ± 6.25 (11.1%) [a]
(3) Hg low dose + Vit. C	98.64 ± 1.21 [c]	106.40 ± 4.74 [b]
(4) Hg high dose	130.89 ± 1.51 [b]	128.57 ± 2.49 (14.3%) [a]
(5) Hg High dose + Vit. C	106.94 ± 4.71 [b]	110.20 ± 5.95 [b]

Data sharing a subscript letter in a column are not significantly different ($P< 0.05$)

Table 3-B: Effect of mercuric acetate alone and with vitamin C on plasma total cholesterol (mg/100 ml)

Group treatment	After 4 weeks	After 15 weeks
	Mean ± SD	Mean ± SD
(1) Control	119.50 ± 3.28 [b]	105.28 ± 2.61 [b]
(2) Hg low dose	129.94 ± 4.27 [b]	123.77 ± 5.56 [a] (17.6%)
(3) Hg low dose + Vit. C	122.89 ± 1.69 [b]	111.44 ± 4.42 [b]
(4) Hg high dose	135.09 ± 12.75 [a] (13.1%)	124.65 ± 3.19 [a] (18.4%)
(5) Hg High dose + Vit. C	112.20 ± 2.33 [b]	104.15 ± 4.90 [b]

Data sharing the same subscript letter in a column are not significantly different ($P<0.05$)

It is known that high levels of blood cholesterol and LDL are risk factors for cardiovascular disease, while HDL is involved in reverse cholesterol transport, which reduces tissues cholesterol levels and may provide a protective effects (Gross, 2008). Vitamin C has the ability to convert cholesterol to bile acids and therefore prevent the heart and blood vessels from damage (Reilly *et al.,* 2010*,* Barregrad*,* 2012 Hong *et al.,* 2013 , Engelking, 2015).

These results indicate that mercury has adverse effects on the blood lipid constituents, and on the other hand vitamin C has the ability to reduce these harmful effects and assists in retuning some biological processes to the normal and healthy situation.

Plasma and Liver Enzymes

Treatment either with low or high doses of mercuric acetate significantly ($P<0.05$) increased plasma and liver ALT and AST activities compared to the control groups at the end of the short as well as the long exposure (Table 4-A) and (Table 4-B), respectively. These increases were caused due to liver and other organs damage. Graz (2007*)* stated that ALT is more specific indicator of liver inflammation, while AST may be elevate in diseases affecting other organs, such as acute pancreatitis, acute hemolytical anemia, acute renal disease. On the other hand, these treatments did not cause any

Table (3-C): Effect of mercuric acetate alone and with vitamin C on plasma LDL (mg/100 ml)

Group treatment	After 4 weeks	After 15 weeks
	Mean ± SD	Mean ± SD
(1) Control	43.47 ± 4.58[b]	38.54 ± 4.49[b]
(2) Hg low dose	56.00 ± 4.27 [b]	64.20 ± 2.37 (64.1%)[a]
(3) Hg low dose + Vit. C	60.14 ± 1.81 [a]	47.62 ± 2.83[b]
(4) Hg high dose	69.49 ± 13.14 (48.3%)[a]	64.14 ± 3.63 (64.1%)[a]
(5) Hg High dose + Vit. C	50.17± 1.80 [ab]	44.84 ± 5.15[b]

Data sharing a subscript letter in a column are not significantly different (P<0.05)

Table (3-D): Effect of mercuric acetate alone and with vitamin C on plasma HDL (mg/100 ml)

Group treatment	After 4 weeks	After 15 weeks
	Mean ± SD	Mean ± SD
(1) Control	52.11 ± 1.77 [a]	44.24 ±1.86 [a]
(2) Hg low dose	39.86 ± 5.80 (-23.5%)[b]	34.57 ± 0.60 (- 21.9%)[b]
(3) Hg low dose + Vit. C	43.03± 0.84 (-17.4%)[b]	37.55 ± 1.21(- 15.1%) [b]
(4) Hg high dose	39.42 ± 0.27 (-24.4%)[b]	34.80 ± 0.22 (- 21.3%)[b]
(5) Hg High dose + Vit. C	40.64± 2.29 (-22.0)[b]	37.27 ± 1.23 (-15.8%)[b]

Data sharing a subscript letter in a column are not significantly different (P<0.05).

Table 4-A: Effect of mercuric acetate alone and with vitamin C on ALT

Group treatment	After 4 weeks	After 15 weeks
Plasma	Mean ± SD (IU/ ml)	Mean ± SD (IU/ ml)
(1) Control	23.13 ± 3.06 [c]	23.47 ± 0.53 [c]
(2) Hg low dose	27.71 ± 0.92 (19.8%) [b]	27.58 ± 1.20 (17.5%) [b]
(3) Hg low dose + Vit. C	25.93 ± 1.70 [bc]	23.56 ± 0.96 [c]
(4) Hg high dose	32.16 ± 0.11 (39.4%) [a]	33.97 ± 0.41 (44.7%) [a]
(5) Hg High dose + Vit. C	23.37 ± 0.38 [c]	25.62 ± 0.62 [bc]
Liver	Mean ± SD (IU/ g)	Mean ± SD (IU/ g)
(1) Control	107.53± 2.94 [b]	95.16 ± 2.12 [c]
(2) Hg low dose	108.04± 4.83 [b]	112.58 ± 3.04 (18.3%) [a]
(3) Hg low dose + Vit. C	106.44 ± 1.98 [b]	98.28 ± 1.32[c]
(4) Hg high dose	118.98 ± 1.94 (10.6%) [a]	119.06 ± 7.90 (25.1%) [a]
(5) Hg High dose + Vit. C	111.49 ± 4.49 [b]	104.59 ± 0.80 (9.9%) [b]

Data sharing a subscript letter in a column are not significantly different (P< 0.05).
ALT: Alanine Aminotransferase

significant changes in the activity of the plasma alkaline phosphatase (ALP) (Table 4-C). These results are in agreement with those of Bapu *et al.* (2003) and Ismail & Ismail (2014). Considering the level of alkaline phosphatase (ALP) activity in the liver tissue, the data in Table (4-C) show a significant (P<0.05) decrease in groups treated by low and high doses of mercury acetate for both short and long term of exposure.

Since liver is a principle target organ for mercury intoxication, therefore, mercury may cause liver cell lyses, which is probably responsible for the increase in ALT and AST activities and decrease in ALP concentration. Karapehlivan *et al.* (2014) observed severe degeneration in liver in animals injected with mercury.

The present results clearly indicate that increase in plasma ALT and AST and decrease in liver ALP can be used as potential enzyme biomarkers for mercury induced hepatotoxicosis which ultimately effects the general health by altering the functional and structural integrity of liver (Wadaan, 2009).

Moreover, in the case of Hg-ascorbic acid groups, the ALT and AST have not been varied significantly (P<0.05) compared to the control groups. These results are in agreement with other studies (Abdel-Tawab *et al.*, 2004, Wadaan 2009, Patnaik *et al.*, 2010, Al-Attar 2011, Kaoud *et al.*, 2012).

The results in (Table 4-D) illustrate that the high dose of mercury significantly (P<0.05) increased the activity of the acid phosphatase (ACP) in plasma after the short as well as the long period of exposure by (30.7%) and (104.8%), respectively. While the effect of the low dose treatment was only significant (P<0.05) after the long term treatment (61.9%). The elevation of acid phosphatase in the blood may be a sign of metastatic cancer. On the other

Table 4-B: Effect of mercuric acetate alone and with vitamin C on AST (IU/1 ml)

Group treatment	After 4 weeks	After 15 weeks
Plasma	**Mean ± SD (IU/ ml)**	**Mean ± SD (IU/ ml)**
(1) Control	26.27 ± 1.11 [b]	24.36 ± 1.36
(2) Hg low dose	31.20 ± 2.79 (18.8%) [a]	32.24 ± 0.44 (32.3%)
(3) Hg low dose + Vit. C	26.11 ± 1.52 [b]	26.62 ± 0.31
(4) Hg high dose	32.49 ± 0.28 (23.7%) [a]	37.08 ± 0.26 (52.2%)
(5) Hg High dose + Vit. C	27.52 ± 0.50 [b]	29.65 ± 0.85 (21.75)
Liver	**Mean ± SD (IU/ g)**	**Mean ± SD (IU/ g)**
(1) Control	97.58±2.31[b]	92.18± 1.21[d]
(2) Hg low dose	116.83 ± 2.25 (19.7%) [a]	100.11 ± 0.29 (6.8%)[c]
(3) Hg low dose + Vit. C	107.34 ± 2.27 (10.0%) [a]	95.09 ± 2.75 [d]
(4) Hg high dose	120.06 ± 1.45 (23.0%) [a]	114.35 ± 2.28 (24.1%) [a]
(5) Hg High dose + Vit. C	113.26 ± 4.09 (16.1%) [a]	104.22 ± 1.40 (13.1%)[b]

Data sharing a subscript letter in a column are not significantly different ($P< 0.05$).
AST: Aspartate Amino Transferase.

Table 4-C: Effect of mercuric acetate alone and with vitamin C on ALP (IU/1 ml)

Group treatment	After 4 weeks	After 15 weeks
Plasma	**Mean ± SD (IU/ ml)**	**Mean ± SD (IU/ ml)**
(1) Control	57.21 ± 2.99 [a]	44.93 ± 3.35 [a]
(2) Hg low dose	56.27 ± 5.21 [a]	42.98 ± 2.34 [a]
(3) Hg low dose + Vit. C	57.86 ± 0.11 [a]	46.52 ± 2.78 [a]
(4) Hg high dose	54.52 ± 0.73 [a]	39.68 ± 3.59 [a]
(5) Hg High dose + Vit. C	57.78 ± 0.34 [a]	44.55 ± 0.85 [a]
Liver	**Mean ± SD (IU/ g)**	**Mean ± SD (IU/ g)**
(1) Control	180.97 ± 25.62 [a]	166.62 ± 30.20 [a]
(2) Hg low dose	129.40 ± 20.65 (-28.5%) [b]	128.71 ± 14.63 (-22.8%)[c]
(3) Hg low dose + Vit. C	168.40 ± 24.90 [a]	138.45 ± 9.31(-16.9%) [b]
(4) Hg high dose	117.10 ± 13.04 (-35.3%) [b]	124.12 ± 10.74 (-25.5%) [c]
(5) Hg High dose + Vit. C	173.94 ± 22.60 [a]	140.53 ± 10.32 (-15.7%) [b]

Data sharing a subscript letter in a column are not significantly different ($P<0.05$).
ALP: Alkaline Phosphatase.

hand, no significant differences were observed in the concentration of the acid phosphatase in the liver tissues.

The data in Table (4-E) show that the two levels of chronic treatment significantly ($P< 0.05$) inhibited the activity of the plasma acetyl choline esterase (AChE) by (17.1%) and (17.5 %) (low dose) after 4 weeks and 15 weeks, respectively, while the percentage of inactivation were (32.4%) and (36.0%) when the animals were given the high dose after the two periods of treatment, respectively. The (AChE) inactivation leads to acetyl choline accumulation and disrupted neurotransmission (Colovic *et al.,* 2013). Although the ascorbic acid intake reduced the harmful effect of mercury, the differences remained significant compared to the control groups.

The mercury encourage the formation of free radicals in the body which deteriorate different enzymatic processes as well as mitochondrial function and therefore causes a decrease in the AChE activity (Joshi *et al.,* 2012).

Table 4-D: Effect of mercuric acetate alone and with vitamin C on ACP (IU/1 ml)

Group treatment	After 4 weeks	After 15 weeks
Plasma	**Mean ± SD (IU/ ml)**	**Mean ± SD (IU/ ml)**
(1) Control	18.89 ± 0.33 [b]	14.45 ± 0.66 [c]
(2) Hg low dose	17.78 ± 2.34 [b]	23.39± 2.84 (61.9%) [b]
(3) Hg low dose + Vit. C	17.31 ± 2.17 [b]	19.58 ± 1.39 [c]
(4) Hg high dose	24.69 ± 0.30 (30.7%)[a]	29.60 ± 3.36 (104.8%) [a]
(5) Hg High dose + Vit. C	18.60 ± 1.14 [b]	18.36 ± 0.44 [c]
Liver	Mean ± SD (IU/ g)	Mean ± SD (IU/ g)
(1) Control	20.68 ±1.48 [a]	14.49 ± 3.72 [a]
(2) Hg low dose	18.13 ± 1.31 [a]	11.93 ± 0.12 [a]
(3) Hg low dose + Vit. C	19.59 ± 2.83 [a]	12.42 ± 1.30 [a]
(4) Hg high dose	16.12 ± 2.44 [a]	11.87 ± 0.25 [a]
(5) Hg High dose + Vit. C	20.51 ± 5.12 [a]	14.89 ± 2.38 [a]

Data sharing a subscript letter in a column are not significantly different (P<0.05).
ACP: Acid Phosphatase.

Table (4-E): Effect of mercuric acetate alone and with vitamin C on AChE (IU/1 ml)

Group treatment	After 4 weeks	After 15 weeks
Plasma	**Mean ± SD (IU/ ml)**	**Mean ± SD (IU/ ml)**
(1) Control	2.99 ± 0.07 [a]	2.97 ± 0.09 [a]
(2) Hg low dose	2.48 ± 0.12 (-17.1%)[c]	2.45 ± 0.11 [b]
(3) Hg low dose + Vit. C	2.70 ± 0.10 (- 9.7%) [b]	2.65 ± 0.08 [b]
(4) Hg high dose	2.02 ± 0.08 (-32.4%) [d]	1.90 ± 0.11 [c]
(5) Hg High dose + Vit. C	2.41 ± 0.04 (-19.4%)[c]	2.38 ± 0.08 [b]

Data sharing a subscript letter in a column are not significantly different (P<0.05).
AChE: Acetyl Choline Esterase.

Oxidative Stress

Thiobarbituric acid reactive substance (TBARS) are produced by lipid peroxidation and considered as indicators of oxidative stress. The results in Table (5-A) show significant increases in the (TBARS) in plasma and liver of groups treated by mercuric acetate at the end of the short and long periods of exposure. Mercury toxicity is known to affect the redox status of the victims' tissues through increased production of free radicals leading to oxidative stress (Ercal *et al.,* 2001). This causes disturbances in the functions of many body organs.

The results in Table (5-B) illustrate that the reduced glutathione (GSH) in plasma significantly decreased by (15.0% and 21.3%) in animals treated for 4 weeks by low and high doses, respectively, while these decreases were (13.2% and 22.4%) after 15 weeks. The depletion of GSH from the liver tissue was higher compared to plasma. These depletions ranged between (14.5% - 35.4%) in liver compared to (11.1% - 28.1%) in plasma. Mercury compounds cause oxidative damage in liver cells, which has been characterized by depletion of reduced GSH, increased mitochondrial hydrogen peroxide production, and lipid peroxidation (Lund *et al*., 1993).

Khan *et al* (2012) explained that the decrease in GSH may be due to the formation of oxidized glutathione (GSSG) or mercuric glutathione complex.

CONCLUSION

Ascorbic acid showed a protective property against exposure to mercury. Therefore vitamin C as an effective antioxidant compound, it must be included the human daily diet in amounts not less than 90 mg for adults.

Table 5-A: Effect of mercuric acetate alone and with vitamin C on (TBARS)

Group treatment	After 4 weeks	After 15 weeks
Plasma	**Mean ± SD (nmol/ ml)**	**Mean ± SD (nmol/ ml))**
(1) Control	0.32 ± 0.01 [c]	0.36 ± 0.01[b]
(2) Hg low dose	0.36 ± 0.00 (12.5%) [b]	0.42 ± 0.01 (16.7%)[a]
(3) Hg low dose + Vit. C	0.32 ± 0.01 [c]	0.40 ± 0.00 (11.1%)[a]
(4) Hg high dose	0.41 ± 0.01 (28.1%) [a]	0.45 ± 0.01 (25.0%) [a]
(5) Hg High dose + Vit. C	0.33 ± 0.01[c]	0.37 ± 0.01 [b]
Liver	Mean ± SD (nomol / g)	Mean ± SD (nomol / g)
(1) Control	25.60 ± 1.44 [c]	29.64 ± 2.76 [c]
(2) Hg low dose	30.83± 0.41 (20.4%) [b]	35.79 ± 4.78 (20.7%) [b]
(3) Hg low dose + Vit. C	30.37 ± 0.81 (18.6%) [b]	33.93 ± 1.38 (14.5%) [b]
(4) Hg high dose	34.66 ± 2.00 (35.4%) [a]	38.82 ± 3.30 (31.0%) [a]
(5) Hg High dose + Vit. C	30.21 ± 0.22 (18.0%)[b]	30.30 ± 3.96 [c]

Data sharing a subscript letter in a column are not significantly different (P<0.05).
TBARS: Thiobarbituric Acid Reactive Substance.

Table 5-B: Effect of mercuric acetate alone and with vitamin C on (GSH)

Group treatment	After 4 weeks	After 15 weeks
Plasma	**Mean ± SD (mcmol / mg protein)**	**Mean ± SD (mcmol / mg protein)**
(1) Control	0.80 ± 0.02[a]	0.76 ± 0.01[a]
(2) Hg low dose	0.68 ± 0.01 (- 15.0 %)[b]	0.66 ± 0.03 (-13.2%)[b]
(3) Hg low dose + Vit. C	0.79 ± 0.02 a	0.75 ± 0.01[a]
(4) Hg high dose	0.63 ± 0.01 (- 21.3%)[b]	0.59 ± 0.01 (- 22.4%)[b]
(5) Hg High dose + Vit. C	0.73 ± 0.01 a	0.73 ± 0.01a
Liver	**Mean ± SD (mcmol / mg protein)**	**Mean ± SD (mcmol / mg protein)**
(1) Control	11.20 ± 0.18[a]	12.13 ± 0.05[a]
(2) Hg low dose	8.38 ± 0.15 (-25.18%)[b]	8.05 ± 0.32 (-33.6%)[b]
(3) Hg low dose + Vit. C	10.77 ± 0.24a	10.11 ± 0.31a
(4) Hg high dose	6.54 ± 0.11(- 41.6%)[c]	7.10 ± 0.05 (- 41.5%)[b]
(5) Hg High dose + Vit. C	9.33 ± 0.2 2 (-16.7%)[b]	9.90 ± 0.91[a]

Data sharing a subscript letter in a column are not significantly different (*P*> 0.05).
GSH: Reduced Glutathione.

REFERENCES

Abdel-Tawwab, M., Shalaby, A., Ahmad, M.H. & Khattab, Y.A. **2004**. Effect of supplementary dietary L-ascorbic acid (Vitamin C) on mercury detoxification, physiological aspects and growth performance of Nile Tilapia (*Oreochromis niloticus L.*) Egyptian Journal of Aquatic Biology and Fisheries, **4**: 159 -171.

Ahmed, M.Z.D. **1993**. Effect of Mercury Intoxication on Some Physiological Parameters Male Rabbits. M.Sc. Thesis. Faculty of Agriculture, Alexandria University.

Al-Attar, A.M. **2011**. Vitamin E attenuates liver injury induced by exposure to lead, mercury, cadmium and copper in albino mice. Saudi Journal of Biological Sciences, **18**: 395–401.

Bapu, L.C., Prakah, P.P. & Nivsarkar, M. **2003**. Organelle specific enzyme markers as indicators of methylmercury neurotoxicity and antidotal efficacy in mice. BioMetals, **16**: 279-284.

Barregarad, L. **2012**. Cardiovascular disease and diabetes–effects of methyl mercury, cadmium, and lead. Toxicology Letters, **211**: 4–23.

Belfield, A. & Goldberg, D.M. **1971**. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme, **12**: 561-573.

Bucolo, G.& David H. **1973**.Quantitative determination of serum triglycerides by use of enzymes. Clinical Chemistry, **19**: 476-482.

Burstein, M., Scholnick, H.R. & Morfin, R. **1980**. Rapid method for isolation lipoproteins from human serum by precipitation with polyanions. Journal of Clinical and Laboratory Investigation, **40**: 583-95.

Carman, K.B., Tutkin, E., Yilmaz, H., Dilber, C., ,Dalkiran, T., Cakiv, B., Arslantas, D. & Cesareti, Aykanat S.A. **2013**. Acute mercury poisoning among children in two Provinces of Turky. European Journal of Pediatrics, **173**: 821-827.

Chang, J.W, Chen, H.L, Sua, H.J, Liao, P.J, Guo, H. R. & Lee, C.C. **2011**. Simultaneous exposure of non-diabetics to high levels of dioxins and mercury increases their risk of insulin resistance. Journal of Hazardous Materials, **185**: 749–755.

Chen, Y.W., Huang, C.F., Yang, C.Y., Yen, C.C., Tsai, K.S. & Liu, S.H. **2010**. Inorganic mercury causes pancreatic β-cell death via the oxidative stress-induced apoptotic and necrotic pathways. Toxicology and Applied Pharmacology, **243**: 323–331.

Colovic, M.B., Krestic, D.Z., Lazarevic-Pasti, T.E., Bondzic, A.M. & Vasic, V.M. **2013**. Acetylcholinesterase inhibitors: Pharmacology and Toxicology Current Neuropharmacology, **1**: 315-335.

Dıez, S, Delgado, S., Aguilera, I., Astray, J., Beatriz, P., Torrent, M., Sunyer, J. & Bayona, J.M. **2009**. Prenatal and Early Childhood Exposure to Mercury and Methylmercury in Spain, a High-Fish-Consumer Country. Archives of Environmental Contamination and Toxicology, **56**: 615–622.

Engelberth,H, Teisl M, Frohmberg E, Butts K, Bell K, Sith, A. **2013**. Can fish consumption advisories do better? Providing benefit and risk information to increase knowledge. Environmental Research, **126**, 232–239.

Engelking, L.R. **2015**. LDL Receptors and HDL. Textbook of Veterinary Physiological Chemistry, 421-426.

Ercal, N., Gurer-Orhan, H., Aykin-Burns, N. **2001**. Toxic metals and oxidative stress. Part 1: mechanisms involved in metal-induced oxidative damage. Current Topics in Medical Chemistry, **1**: 529-539.

Fossati, P., Prencipe, L. **1982**. Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, **28**: 2077-2080.

Graz, D.C. **2007**. The role of existing and novel cardiac biomarkers for cardio-protection. Current Opinion in investigational Drugs, **8**: 711-717.

Gross, M.D. **2008**. Lipids,Oxidation, and Cardiovascular Disease. Atherosclerosis and Oxidant Stress. Book Chapter p.79-95. Ed Springer US. 599 Maarouf Tounes, Cherif Abdelnour and Nadjet Houaine.

Grove, T.H. **1979**. The effect of reagent pH on determination of high density lipoprotein cholesterol by precipitation with sodium phosphate –magnesium. Clinical Chemistry, **25**: 560-564.

Hong, A.D., Chob, S.H., Parkb, S.J., Kimb, S.Y. & Park, S.B. **2013**. Hair mercury level in smokers and its influence on blood pressure and lipid metabolism. Environmental toxicology and pharmacology, **36**: 103–107.

Hounkpatin, A.S.Y., Johnson, R.C., Guédénon, P., Domingo, E., Alimba, C.G., Boko, M. & Edorh, P.A. **2012**. Protective Effects of Vitamin C on Haematological Parameters in Intoxicated Wistar Rats with Cadmium, Mercury and Combined Cadmium and Mercury. International Research Journal of Biological Sciences, **1**: 76-81.

Ismail, S.M. & Ismail, H.A. **2014**. Protective effect of L-ascorbic acid (vitamin C) on mercury detoxication and physiological aspects of Albino rats. Research and Review Journal of Zoological Sciences.International Journal of Medical and Health Sciences Research, Conscientia Beam, **1**:126-132.

Jollow, D.J., Mitchell, J.R., Zampaglione, N. & Gillete, J.R, **1974**. Bromobenzene- induced liver necrosis, protective role of Glutathione and evidence for 3, 4- bromobenzene oxide

in the hepatotoxic metabolite. Pharmacology, **11**:151-169.

Joshi, D., Mittala, D.K., Shuklaa, S. & Srivastavb, A.K. **2012**. Therapeutic potential of N-acetyl cysteine with antioxidants (Zn and Se) supplementation against dimethyl-mercury toxicity in male albino rats. Experimental and Toxicological Pathology , **64**:103– 108.

Kaoud, H.A., Khaled, M.A., Rezk, M.A. & Khalf, M.A. **2012**. Bioremediation the toxic effect of mercury on liver histopathology, some hematological parameters and enzymatic activity in Nile tilapia, *Oreochromis niloticus*. Researcher. **4**: 60-70.

Karapehlivan, M., Ogun, M., Kaya, I., Ozen, H., Deveci, H.A. & Karaman, M. **2014**. Protective effect of omega-3 fatty acid against mercury chloride intoxication in mice. Journal of Trace Elements in Medicine and Biology, **28**: 94– 99.

Khan, H., Farid, M. & Umer, S. **2012**. Role of Glutathione in protection against mercury induced poisoning. Pakistan Journal of Pharmaceutical Sciences, **25**:395-400.

Kind, P.R.N. & King, E.J. **1954**. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-anti-purine. Journal of Clinical Pathology, **7**: 322-326.

Lund, B.O., Miller, D.M. & Woods, J.S. **1993**. Studies on Hg (II)-induced H_2O_2 formation and oxidative stress *in vivo* and *in vitro* in rat kidney mitochondria. Biochemical Pharmacology, **45:**2017–202.

Minoia, C., Ronchi, A., Pigatto, P. & Guzzi, G. **2009**. Effect of mercury on endocrine system. Critical Reviews in Toxicology, **39**: 538.

Mok, W.J., Hatanaka, C.Y., Seoka, D.T., Itoh, A.Y., Tsukamasa, A.M. & Ando, M. **2014**. Effects of additional cysteine in fish diet on mercury concentration. Food Chemistry, **147**: 340–345.

Moumen, Y., Abdennour, C. & Loudjani, F. **2011**. Influence of palm date and vitamin C supplementation on testicular functions of domestic rabbit *Oryctolagus Cuniculus* under mercury exposure. Annals of Biological Research**, 2**: 359-367.

Oliveira, S.C., Oliveira, V.A., Ineu, R.P., Silva, L.M. & Ereira, M.E. **2012**. Biochemical parameters of pregnant rats and their offspring exposed to different doses of inorganic mercury in drinking water. Food and Chemical Toxicology, **50**: 2382–2387.

Patnaik. B.B., Roy, A., Agarwal, S. & Bhattacharya, S. **2010**. Induction of oxidative stress by non-lethal dose of mercury in rat liver: Possible relationships between apoptosis and necrosis. Journal of Environmental Biology, **6**:413 – 416.

Reilly, S.B., Kathleen, M., McCarty, S.D., Steckling, N. & Lettmeier, B. **2010**. Mercury Exposure and Children's Health. Curr Probl Pediatr Adolesc Health Care, **40**:186 - 215.

Reitman, S. & Frankel, S. **1957**. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, **26**: 56-63.

Steel. R.G.D. & Torrie J.H. **1981**. Principle and Procedures of Statistics. A Biochemical Approaches, 2ed Ed, McGraw-Hill Book Company, NY, USA, pp.281-300.

SAS Institute Inc. **2004**. User's Guide., NC. SAS/ ETS 9.1.

Sharma, M.K., Patni, R., Kumara, M. & Kumara, A. **2005**. Modification of mercury-induced biochemical alterations in blood of Swiss albino mice by *Spirulina fusiformis*. Environmental Toxicology and Pharmacology, **20**: 289–296.

Trinder, P. **1969**. Determination of glucose in blood using glucose oxidase with an alterative oxygen acceptor. Annals of Clinical Biochemistry, **6**: 24-27.

Wadaan, M.A/M. **2009**. Effect of mercury exposure on blood chemistry and liver histopathology of male rats. Journal of Pharmacology and Toxicology, **1**: 1 – 6.

Zollner, N. & Kirsch, K. **1962**. Über die quantitative Bestimmung von Lipoiden (Mikromethod) mittels der vielen naturlichen Lipoiden (allen bekannten Plasmalipoiden) gemeisamen Sulphophospho-vanillin Reaction. Z. ges. exp. Med, **135**: 545-561.(in German).

التأثير الوقائي لفيتامين C علي بعض المعايير الحيوية في الدم والكبد عند التعرض للزئبق

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تهدف الدراسة إلي تحديد التأثير الوقائي لفيتامين C علي بعض المعايير الحيوية في كل من الدم و الكبد عند تعرض ذكور الأرانب النيوزيلاندية للزئبق. تم تقدير فاعلية فيتامين C ضد تسمم الزئبق من خلال تقدير جلوكوز و دهون الدم و بعض إنزيمات الدم و الكبد، والتي تعتبر مؤشرا للحالة الصحية. عولجت البيانات احصائياً باستخدام برنامج ASA.

أظهرت النتائج حدوث ارتفاع معنوي في كل من جلوكوز الدم، و الدهون الكلية، والجلسريدات الثلاثية، LDL، والكولستيرول الكلي، عند تعرض الحيوانات للزئبق سواء لفترة قصيرة أو طويلة، بينما حدث انخفاض معنوي في HDL فقط بعد التعرض لفترة طويلة. ارتفعت معنويا مستويات كل من AST وALT وإنزيم ACP في كل من البلازما و أنسجة الكبد عند معاملة الحيوانات بالزئبق سواء لفترة قصيرة أو طويلة، بينما حدث انخفاض معنوي في نشاط إنزيمي ALP و Ach E. كما أظهرت النتائج علامات الإجهاد التأكسدي (TBARS و الجلوتاثيون) تأثيرات معنوية خطيرة بسبب التعرض للزئبق. أدت المعاملة الفمية بفيتامين C إلي خفض جميع التأثيرات الصحية الخطيرة الحادثة بسبب المعاملة بخلات الزئبق. وعلي ذلك، فإنه يوصي باضافة حمض الأسكوربيك كمضاد للأكسدة لوجبات الإنسان لتأثيره في وقاية الجسم من تسمم الزئبق.