

PROTECTIVE EFFECT OF L-ASCORBIC ACID (VITAMIN C) ON MERCURY DETOXICATION AND PHYSIOLOGICAL ASPECTS OF ALBINO RATS

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ABSTRACT

The present study was carried out to investigate the protective effect of L-ascorbic acid (A.A.), against mercury toxicity in albino rats. Twenty four males were divided into three groups. The control was fed a basic diet, whereas the other two groups were treated either by Hg alone (1g HgCl₂/Kg food) or Hg-ascorbic acid (1g HgCl₂/Kg food + 5% ascorbic acid), respectively, for 35 consecutive days. The following serum parameters were estimated; alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), urea, creatinine, uric acid, triglycerides, total cholesterol, HDL-cholesterol and LDL cholesterol. The present data obtained found that a significant decrease in serum ALP activity in mercury treated group, accompanied by a significant increase in serum ASAT. Furthermore, serum urea concentration was significantly higher in the mercury group compared to the control. However, the concentration of urea and the activity of ALP and ASAT of the Hg+ascorbic acid group were not statistically different from the control. Moreover, no significant variations were recorded concerning ASAT, creatinine and uric acid. The lipid profile; triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were not significantly varied between the three groups, despite the observed elevated concentration of triglycerides in Hg+ascorbic acid group. In conclusion, ascorbic acid may partially help in the protection against mercury intoxication and it could also be considered a safe nutritional source.

Keywords: Hg, Lipid profile, creatinine, triglycerides, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol, Ascorbic acid, Serum biochemical parameters.

Contribution/ Originality

This study is one of very few studies which have investigated protective effect of l-ascorbic acid mercury detoxication and physiological aspects and The paper's primary contribution is finding that ascorbic acid may partially help in the protection against mercury intoxication and it could also be considered a safe nutritional source.

1. INTRODUCTION

Food contamination by toxic trace elements is nowadays an evident health problem worldwide for general population [1]. Mercury is the most dangerous trace element. Acute or

chronic mercury exposure can cause adverse effects during any period of known safe level of exposure. Ideally, neither children nor adults should have any mercury in their bodies because it provides no physiological benefit. Prenatal and postnatal mercury exposures occur frequently in many different ways [2], due to its physico-chemical properties, it is found in the air, water, soil and food, polluting, however, the different ecosystems. Mercury is found to alter the physiological and the biochemical functions of living organisms [3], and cause a wide range of clinical symptoms in occupationally exposed workers [4]. The toxic effects of Hg on human and animal health have been reported extensively [5]. When binding to cell components, mercury provokes the oxidative stress, leading to the formation of a number of toxic substances [6]. Moreira, et al. [7] support the concept of Hg-induced cardiovascular toxicity. Thus, lipids are amongst the target molecules to be oxidized by mercury [8, 9]. Consequently, mercury can alter membrane lipid structure and functions [10] and even inhibits lipid synthesis in the nervous system [11].

Ascorbic acid (vitamin C) is an essential nutrient in feeds, and is an indispensable nutrient required to maintain the physiological processes of different animals [12]. Small amount of this vitamin is sufficient to prevent and cure scurvy; however, larger amount may be essential to maintain good health during environmental adversities, situation of physiological stress and conditions of infectious and parasitic diseases [13, 14]. Ascorbic acid (vitamin C) is essential for producing collagen and bone minerals, assists in metabolizing iron and helps in activation of vitamin D. It also assists in reducing the harmful effects of hormones produced by the adrenal gland during prolonged periods of stress [15]. Also, it has an important role in a great number of biochemical processes such as synthesis of collagen which is an intercellular protein and principal constituent of skin, scales, mucosa, cartilaginous tissues, bones and conjunctive tissue formation, which involves all the organs of the body [13]. Agrawal, et al. [16].

The objective of the present work is therefore, to evaluate the beneficial roles of ascorbic acid on serum biochemical parameters in mercury contaminated diet of albino rats. Such parameters are generally representing the health status of the individuals.

2. MATERIALS AND METHODS

Twenty four male albino rats weighing 100-150g were divided into 3 groups. Animals were put in the animal house under standard conditions of temperature, light and humidity and food was given ad libitum. The control was fed a basic diet, while the other two groups were treated either by Hg (1g HgCl₂/Kg food) or Hg-ascorbic acid (1g HgCl₂/Kg food + 50g/Kg food of ascorbic acid). After five weeks continuous treatments, animals were decapitated and blood was received in dry test tubes, and then centrifuged at 5000 rpm/15 minutes. The automate apparatus METROLAB 2300 (Random Access Clinical Analyzer) was used to measure serum alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), urea, creatinine, uric acid, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol.

Statistical analysis has been carried out by student *t*-test to compare between paired groups, whereas the one way analysis of variance (ANOVA) was used to compare between groups. Results are expressed as mean \pm SD and the statistical test was considered significant at $p < 0.05$ level.

3. RESULTS

Results of the serum enzymes ALP, ASAT, ALAT are in Table (1) and Fig(1), urea, creatinine and uric acid presented in Table (2) and Fig(2) representing liver and kidney functions the results showed that the activity of ALP was significantly reduced in rats exposed to mercury alone compared to the control. Contrary, the serum ASAT activity was increased significantly in the mercury treated group compared to the control. Accordingly, serum urea concentration was also significantly increased in mercury group. When comparing between the three groups, ANOVA has revealed a significant variations in ALP, ASAT and urea. However, the ASAT, creatinine and uric acid were not significantly changed in all cases. Meanwhile, in the Hg-ascorbic acid group, the ALP, ALAT, ASAT, urea, creatinine and uric acid have not been varied significantly compared to the other two groups.

Table-1. Comparison of ALP, ASAT and ALAT activities (mean±SD) in the serum of rats in the control (G1), in Hg (G2) and in Hg+ascorbic acid (G3) after 5 consecutive weeks.

	Group1	Group 2	Group 3
ALP (IU/L)	63.4 ± 4.6 (a)	40.2 ± 3.8	52.2 ± 4.2 (d)
ASAT (IU/L)	24.1 ± 2.8 (a)	37.8 ± 3.4 (c)	32.8 ± 2.4 (d)
ALAT (IU/L)	31.5 ± 3.4	28.8 ± 1.6	29.2 ± 2.2

Letters differ significantly at $p < 0.05$. a: G1 vs G2 c: G2 vs G3; d: G1 vs G2 vs G3

Table-2. Comparison of urea, creatinine and uric acid concentrations (X±SD) in the serum of male rats in the control (G1), in Hg (G2) and in Hg+ascorbic acid (G3) after 5 consecutive weeks.

	Group1	Group 2	Group 3
Urea (g/L)	0.25 ± 0.06 (a)	0.38 ± 0.038	0.34 ± 0.04 (d)
Creatinine (mg/L)	9.4 ± 1.8	10.2 ± 2.4	10.3 ± 2.6
Uric acid (mg/L)	11.1 ± 2.4	11.3 ± 2.1	10.8 ± 1.6

Letters differ significantly at $p < 0.05$. a: G1 vs G2; d: G1 vs G2 vs G3

Table-3. Comparison of triglycerides, total cholesterol, HDL- and LDL- Cholesterol concentrations (X±SD) in the serum of male rats in the control (G1), in Hg (G2) and in Hg+ascorbic acid (G3) after 5 consecutive weeks.

	Group1	Group 2	Group 3
Triglycerides (g/L)	1.14 ± 0.086	1.21 ± 0.082	1.32 ± 0.048
Total cholesterol (g/L)	0.54 ± 0.08	0.51 ± 0.034	0.56 ± 0.042
HDL-cholesterol (g/L)	0.22 ± 0.034	0.19 ± 0.046	0.21 ± 0.022
LDL-cholesterol (g/L)	0.12 ± 0.008	0.14 ± 0.004	0.12 ± 0.006

The results of the lipid profile; triglycerides, total cholesterol, HDL-cholesterol and LDL cholesterol are presented in Table (3) and Fig (3). The most noticeable result was the triglyceride concentrations, which have been increased in the Hg-ascorbic acid group, but it was not statistically significant compared either to the control, or to the mercury group.

4. DISCUSSION

Mercury contamination generates a prooxidative environment, leading to oxidative damage, which in turn could disturb some cell functions. However, in the mercury exposed male rats, the activity of alkaline phosphates was decreased, whereas that of ASAT was increased, with no change in ALAT level.

In this case, mercury may affect intestinal functions by inhibiting the enzyme activity during absorption processes; especially serum alkaline phosphates is mainly originated from intestines, liver and bones. Accordingly, [Bapu, et al. \[17\]](#) have observed a decrease in the alkaline phosphates in brain and spinal cord of mice exposed to organic mercury. On the other hand, the liver cell lyses, is probably responsible on the observed increase in ASAT activity, since liver is among the principal target organ for mercury intoxication. Mercury is one of the agents which disturb cell lipid membranes [\[18\]](#), leading to the release of hydrolases [\[17\]](#). Also mercury impairment in intracellular calcium which lead to oxidative stress and as important events in mercury induced neurotoxicity [\[19\]](#). [Karapehliyan, et al. \[20\]](#) were noticed severe degeneration in liver and kidney in animals injected with mercury. It seems that Ascorbic acid has a bouncing effect on alkaline phosphates activity. Accordingly, ascorbic acid showed marked antihepatotoxic activity against carbon tetrachloride intoxicated rats by returning both serum ASAT and ALAT to normal levels [\[21\]](#). A contradictory result was reported by [Endo, et al. \[22\]](#), where the activity of serum ALAT of rat was increased, accompanied with no change in both ASAT and alkaline phosphates in mercury contaminated whale red meat [\[5\]](#).

In this study, the exposure to mercury has increased serum urea concentration after 5 weeks, confirming, however, the presence of renal functional disturbances, as a result of mercury accumulation, particularly mercury can cause anuria [\[23\]](#).

It appears that the period of exposure to mercury could affect kidney functions differently. Thus after a short period of seven days, serum urea was reduced in rat given mercury contaminated whale red meat [\[22\]](#), while it increased in mice received a single dose of mercury during five consecutive days [\[24\]](#). Urea augmentation in this study could also come from protein catabolism acceleration because of oxidative stress provoked by mercury. Accordingly, This antioxidant activity of ascorbic acid (vitamin C) makes it a hunter of free radicals, thus preventing the autointoxication of immunological cells such as macrophages which are the first processors of the information about the alien bodies and maximizing the defense of animals [\[25\]](#).

Beyond phagocytosis mechanism, ascorbic acid (vitamin C) is involved in leukocyte migration and retarded hypersensitivity. It also participates in the mutagenic proliferation of lymphocytes, in the increase of the level of serum complements and in the production of interferon; therefore it is considered as anti-infection vitamin [\[26\]](#). On the other hand, ascorbic acid (vitamin C) has been shown to enhance also the urinary elimination of metal to reduce hepatic and renal burden of metal [\[27, 28\]](#). Serum triglycerides in the present study have not been changed significantly either in the presence of mercury alone, or combined with of ascorbic acid. It is known that triglycerides are essential source of energy for cellular functions. Importantly, total cholesterol and LDL-cholesterol levels have not increased after ascorbic acid supplementation this agree with

[7] Y7Y3 . To know that blood cholesterol and LDL levels are well-established risk factors for cardiovascular disease, while HDL is involved in reverse cholesterol transport, which reduces tissue cholesterol levels and may provide a protective effect [29].

Accordingly, ascorbic acid was found to have antihyperlipidemic properties [30, 31]. The brain is particularly sensitive to oxidative attack because of its high level of unsaturated lipids and high rate of oxidative metabolism [32], and mercury inhibits lipid synthesis in the nervous system [11], causing a reduction in tissue cholesterol levels [33].

In the same regard, other authors claimed the importance of vitamin C to aquatic organisms. Thus, [28] found that ascorbic acid has been shown to have protective and therapeutic effects against copper intoxication, however it reduced fish mortality and poisoning signs, lowered metal content of tissues and prevented the inhibition of GOT and LDH activities and also found that ascorbic acid enhanced transport of copper to kidney. Moreover, many investigators used ascorbic acid (vitamin C) for disease preventing. Huq, et al. [34] was also found toxic signs, body weight, hemato-biochemical and post mortem lesions were found to be slight (+) or mild (++) and/or improved in rest three groups of mice following treatment with vitamin E, vitamin C and combination of vitamin E and vitamin C. Eya [35] showed that a concentration of 46 mg ascorbic acid/kg of diet would prevent broken-skull disease in African catfish anomaly and allow optimum synthesis of vertebral collagen. Leonardo, et al. [36] studied the effect of different levels of dietary ascorbic acid (vitamin C) on the occurrence of ectoparasites of Nile tilapia larvae and found that the optimum level of ascorbic acid (1000 mg/kg diet) was efficient for reduction of ectoparasites occurrence.

Thus the present study could recommend that high levels of ascorbic acid (vitamin C) (>500 mg/kg diet) must be used to be efficient in toxicity reduction, preventing disease and enhancing animals tolerance to environmental stress.

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