ROLE OF ASCORBIC ACID SUPPLEMENT IN REDUCING OXIDATIVE STRESS AND HEPATOTOXICITY IN LEAD INTOXICATION

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ABSTRACT

Objective: The present study was conducted to measure the oxidative stress and hepatotoxicity in lead intoxicated sprague dawley rats with and without supplementation of ascorbic acid.

Study Design: Randomized Control Trial.

Place of Study: Physiology Department, Army Medical College, Rawalpindi. (From Oct 2007 to Sep 2008)

Material and Methods: One hundred and five male rats (age, 90-120 days; weight 200 - 250 gm) were divided into three groups each having 35 rats. Rats of group 1 and group 2 were given weekly injections of sodium acetate (10 mg /kg body weight) and lead acetate (10 mg /kg body weight) respectively, whereas rats of group 3 were administered lead acetate(10 mg /kg body weight) through weekly injections and ascorbic acid in drinking water (500 mg/l). After 6 weeks, 4 ml of blood was drawn from each rat by cardiac puncture. The blood was allowed to clot and serum was separated for estimation of serum malondialdehyde (MDA) levels on spectrophotometer; and serum alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) levels on Merck Micorlab 200.

Results: Lead intoxication of rats revealed that serum MDA levels were raised to 7.8 ± 0.48 µmol/l (control, 3.2 ± 0.39µmol/l), ALT levels to 76.26 ± 5.88 IU/l (control, 44.1 ± 3.26) and AST levels to 258.06 ± 13.30 IU/l (control, 156.2 ± 4.97). Ascorbic acid supplementation significantly lowered serum MDA levels (3.8 ± 0.34 µmol/l), ALT levels (52.26 ± 4.57 IU/l) and AST levels (188.13 ± 12.91 IU/l).

Conclusion: Ascorbic acid supplementation ameliorates lead intoxication probably by reducing the oxidative stress, thus preventing the development of hepatotoxicity, but this amelioration is not equal to the control.

Keywords: Lead intoxication, ascorbic acid, malondialdehyde, alanine aminotransferase, aspartate aminotransferase.

INTRODUCTION

Lead is among the oldest known and most commonly used heavy metal. It has been established that lead accumulates in every tissue of the body and produces effects on almost all the body systems especially liver, RBCs, nervous system, gonads and kidneys.

The proposed mechanism of intoxication is the generation of reactive oxygen species (ROS)[1-2]. Excess ROS, generated in lead toxicity produce oxidative stress by disturbing the balance of prooxidants and antioxidants in favour of prooxidants. Lead also causes direct depletion of antioxidant reserve and decreasing body's ability to neutralize naturally produced free radicals in the body. The most important antioxidant system depleted by lead involves glutathione. In addition to acting as an important antioxidant for quenching free radicals, glutathione is a substrate responsible for the metabolism of specific drugs and toxins through glutathione conjugation in the liver. The sulfhydry[1] complex (- SH) of glutathione directly binds to the toxic metal that has high affinity for this groups. Mercury, arsenic and lead effectively inactivate the glutathione molecule thus making it unavailable as an antioxidant or as a substrate in liver[3]. In lead intoxication, both the excessive generation of ROS as well as the depletion of the antioxidant reserves, cause accumulation of ROS in hepatocytes. These ROS initiate lipid peroxidation in cell membranes, inactivate sulfhydryl antioxidants, damage nucleic acids and inhibit DNA repair in hepatocytes[4].

Many epidemiologic and clinical trials have indicated that dietary intake or

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supplementation with antioxidant vitamins, is associated with reduction in the incidence of morbidity and mortality of chronic diseases[5]. This is probably because of prevention of damage caused by ROS in these chronic diseases. Among these antioxidant supplements, ascorbic acid appears to be more effective in minimizing the toxicity of lead[6,7]. A lot of research is underway to evaluate the role of antioxidant vitamin supplementation in decreasing the toxicity of lead. The present study was designed to investigate the effect of ascorbic acid supplementation on the oxidative stress and hepatotoxicity in lead intoxicated rats.

MATERIAL AND METHODS

These randomized controlled trials were conducted in the Physiology Department, Army Medical College Rawalpindi from Oct 2007 to Sep 2008. Male Sprague Dawley healthy rats of 90-120 days age and 200 - 250 grams weight were purchased from National Institute of Health (NIH), Islamabad. The rats were shifted from the breeding area of NIH to the rat cages in a separate room. Rats were randomly divided into three groups with thirty five rats in each group. The three groups were handled as follows:

Control: Rats were fed normal standard rat diet without any supplementation and were given weekly intraperitoneal injections of sodium acetate 10 mg /kg body weight [8].

Lead intoxicated: Rats were given weekly intraperitoneal injection of lead acetate 10 mg /kg body weight for 6 weeks and were fed standard diet [8].

Lead intoxicated, with ascorbic acid supplementation: Rats were intoxicated by weekly intraperitoneal injection of lead acetate 10 mg/kg body weight for 6 weeks, and were given drinking water containing ascorbic acid 500 mg/l[8].

After 6 weeks of intraperitoneal injections and ascorbic acid supplementation to the respective groups, intracardiac blood samples of 35 rats were taken in one day. Four ml of blood was drawn with the help of 5 ml disposable syringe and transferred in a plain tube. Blood in the plain tubes was centrifuged for separation of serum in cold centrifuge machine (Model 5810R; Eppendorf, Germany). After cold centrifugation, serum was pippeted out[9]. Approximately 1.5 ml of serum was obtained from each blood sample. It was placed in serum tubes (Eppendorf, Germany) which were labeled with the group number and sample number. Serum was stored at – 80 °C in a deep freezer (Model DFU- 446 CE, Operon, Korea) till analysis. Serum was later used to determine MDA, ALT and AST levels by using commercial kits on spectrophotometer and Microlab 200.

Serum Malondialdehyde (MDA) levels were estimated by thiobarbituric acid reactive substances (TBARS) assay. MDA is dialdehyde of malonic acid which is a standard marker of oxidative stress. MDA present in the sample reacts with thiobarbituric acid (TBA) present in the kit under high temperature (90-100°C) and acidic conditions. Concentration of MDA-TBA Adduct formed was estimated by measuring the absorbance by using spectrophotometer, which depends on the concentration of MDA in the sample[10].

Estimation of serum alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) levels was done by using commercial kits on Merck Microlab 200. For estimation of ALT and AST levels these parameters were selected on the 'TEST SELECTION' display screen. Microlab 200 was then programmed for these two parameters. Wave length of 340 nm, temperature of 37 °C and aspirated volume of 100 micro litres were selected. The programming was saved and was used for determining the results of selected parameter. The serum was mixed with predetermined volume of the kit reagents and was aspirated by the sipper tube. The enzymes ALT and AST catalyzed the reaction of serum and the reagents and in this process NADH present in the kit reagents was used up. The rate of decrease in concentration of NADH was measured photometrically and was proportional to catalytic concentration of ALT or AST present in the sample, depending on the kit used.

Statistical analysis: Data were entered into SPSS 11. Mean and standard deviation of all the parameters were calculated. Significance of the differences between the three groups was determined by using One-Way Analysis of Variance (ANOVA) followed by Post-Hock (Tukey) test. P value < 0.05 was considered as significant.

RESULTS

Age and weight of rats of the three groups were recorded at the start of the project. Mean age of control group was 92 ± 3 days , lead group was 92 ± 5 days and ascorbic acid group was 91 ± 5 days. Mean weight of control group was 228 ± 5 grams, lead group was 227 ± 7 grams and ascorbic acid group was 226 ± 6 grams. There was no statistically significant difference in age ($p = 0.554$) and weight ($p = 0.388$) between the groups at the beginning. All rats remained alive and healthy throughout the study.

Comparison of serum MDA, ALT and AST levels between the groups is given in Table 1. Serum MDA, ALT and AST levels in the ascorbic acid group are significantly higher than control group but are significantly lower than lead group. Statistical differences of serum MDA, ALT and AST levels between the groups is given in Table 2.

DISCUSSION

Results of the study reflected that lead intoxication produced statistically significant changes in MDA, ALT and AST levels in Sprague Dawley rats. Ascorbic acid supplementation (in vivo) significantly influenced the outcome of lead intoxication on aforementioned parameters. The present study compared these parameters in healthy control rats, leads intoxicated rats and lead intoxicated rats taking ascorbic acid supplement and also

Table-1: Comparison of serum MDA, ALT and AST levels between different groups .

Variables	Control group (n =35)	Lead Treated group (n=35)	Lead with Ascorbic acid treated group (n=35)	*p*- Value*
Malondialdehyde (MDA) (µmol / l)	3.2 ± .39	7.8 ± .48	3.8 ± .34	<0.001
Alanine Aminotransferase (ALT) (IU / l)	44.1 ± 3.26	76.26 ± 5.88	52.26 ± 4.57	<0.001
Aspartate Aminotransferase (AST) (IU / l)	156.2 ± 4.97	258.06 ± 13.30	188.13 ± 12.91	<0.001

All values are presented as mean ± SD

* p value is determined by One Way ANOVA.

Table-2: Statistical differences of serum Malondialdehyde (MDA), Alanine Aminotransferase (ALT) and Aspartame Aminotransferase (AST) levels between different groups using Post-Hock (Tukey) test.

Comparison between	*p*- Value		
	MDA	ALT	AST
Control and Lead	<0.001	<0.001	<0.001
Control and ascorbic acid treated	<0.001	<0.001	<0.001
Lead and ascorbic acid treated	<0.001	<0.001	<0.001

with the studies already published by different authors.

Serum MDA levels in control group of Sprague Dawley rats was 3.2 ± 0.39 µmol / l which was consistent with the published data[11]. During health, the reactive oxygen species (ROS) are neutralized by the defense system of the cell which prevents abnormal lipid peroxidation in the body, hence MDA levels are found in the narrow range in healthy subjects.

Intraperitoneal injections of lead acetate in the Sprague Dawley rats led to increased serum MDA level (7.8 ± 0.48 µmol / l) which was consistent with the documented levels in lead intoxicated rats[12]. Ascorbic acid supplementation in the present study has resulted in statistically significant improvement in MDA levels when compared to the lead intoxicated group. These findings are similar to the study by Eun Young et al. in which they concluded that antioxidants supplementation ameliorated the oxidative stress and decreased the damage produced by it[13]. It is therefore believed that ascorbic acid slowed down the lipid peroxidation caused by oxidative stress in lead intoxication. Neutralization of reactive oxygen species (ROS) and restoration of antioxidant reserves has been documented to contribute in lowering the oxidative stress by ascorbic acid supplementation. MDA levels were not affected by ascorbic acid supplementation in the studies on healthy Sprague Dawley rats. Jacob et al had concluded that antioxidants supplementation did not affect the markers of oxidative damage in healthy subjects[14]. However, lowered MDA levels in the present study have revealed that ascorbic acid supplementation is effective to reduce the oxidative stress caused by lead. Therefore, it may be inferred that although ascorbic acid supplementation did not affect MDA levels in healthy subjects but it does prevent the development of oxidative stress in conditions when prooxidants are abnormally increased in conditions like lead intoxication.

The levels of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) recorded in the control group of this study (ALT, 44.1 ± 3.26 IU / l and

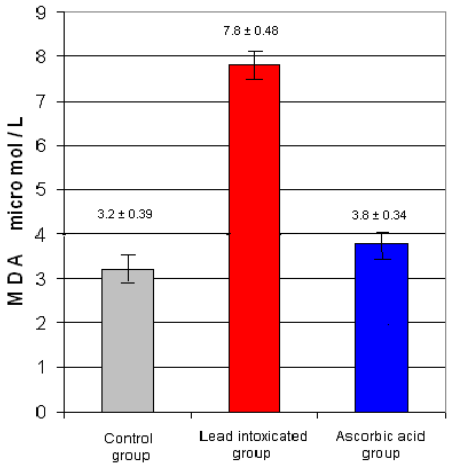

Fig1. Comparison of serum malondialdehyde (MDA) levels in ascorbic acid supplemented (500 mg/l in drinking water) and lead intoxicated (10 mg /kg body weight, intraperitoneal injection) groups with the control group.

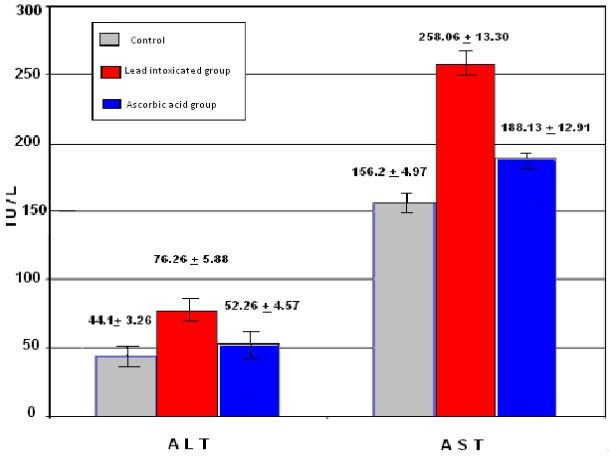

Fig.2. Comparison of serum alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) levels in ascorbic acid supplemented (500 mg/l in drinking water) and lead intoxicated (10 mg /kg body weight, intraperitoneal injection) groups with control group.

AST 156.2 ± 4.97 IU / l) are consistent with the levels in healthy Sprague Dawley rats as documented in other international studies[15,16]. Serum ALT and AST levels have been used by many researchers to assess the damage to hepatocytes, by various toxins and environmental pollutants. When hepatocytes are damaged, it causes release of these enzymes (ALT and AST) into the plasma which are used as markers of hepatotoxicy[17]. Hepatotoxicity of lead has been manifested by elevation of ALT and AST levels beyond the physiological range

in lead intoxicated group (ALT ; 76.26 ± 5.8 IU/l and AST; 258.06 ± 13.30 IU/l). Quantitative estimation of damage to the hepatocytes had been documented by Moussa et al in 2008 by measuring serum ALT and AST levels which were raised from 24.85 ± 0.60 IU / l to 32.19 ± 0.35 IU / l and from 59.71 ± 1.09 IU / l to 90.50 ± 3.0 IU / l respectively, after giving lead acetate[18]. Shalan et al, also used serum ALT and AST levels as markers of hepatotoxicity and studied the amelioration of lead toxicity of rat liver by ascorbic acid and silymarin supplements[17].

Hepato-protective effect of ascorbic acid supplementation during lead exposure has been reflected by statistically significant reduction in the serum ALT and AST levels in the ascorbic acid group as compared to the lead intoxicated group. Earlier Shalan et al. in 2005 worked to find out amelioration of lead toxicity on rat liver by ascorbic acid and silymarin supplements. Their work reflected that lead exposure raised the serum levels of ALT and AST and supplementation of diet with ascorbic acid and silymarin ameliorated these changes[17]. This work reflected that ascorbic acid if given with hepato-protective agents like silymarin, can result in significant amelioration of hepatotoxicity produced by lead. The results of our study have revealed that ascorbic acid alone can cause significant amelioration of damage to the hepatocytes inflicted by lead.

CONCLUSION

It is concluded from the data of present study that lead exposure leads to an increase in oxidative stress and hepatotoxicity, manifested by the rise in levels of serum MDA, serum ALT and serum AST whereas, ascorbic acid supplementation significantly ameliorates the oxidative stress and hepatotoxicity inflicted by lead intoxication. Hepatoprotective effect of ascorbic acid can be due to its ability to scavenge free radicals and neutralization of ROS, which reduces the lipid peroxidation of hepatocytic cell membranes.

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