PROTECTIVE EFFECT OF DL- METHIONINE IN LEAD TOXICITY OF MALE GUINEA PIGS

DildeepVaradan
Assistant Professor, Kerala Veterinary & Animal Sciences University
BabithaVazhoor
Assistant Professor, Kerala Veterinary & Animal Sciences University
Ram Sharan Dass
Principal Scientist, Indian Veterinary Research Institute, IVRI, Deemed University, India
Anilkumar Garg
Principal Scientist, Indian Veterinary Research Institute, IVRI, Deemed University, India

Abstract: Lead is a heavy metal and can cause accidental poisoning in domestic and wild animals, as well as humans.In recent years, the lead consumption in India was around 2.5 lakh tons, out of which about 75 percent is being used in lead-acid battery and inverters. The present study was conducted to assess the serum enzyme and thyroid hormone status consequent to dietary lead exposure in male guinea pigs and the ameliorative effect of dietary addition of DL-methionine against lead toxicity. Thirty two healthy male guinea pigs of about 40 days of age were divided into four groups of 8 animals each. Group G1 was fed on basal diet; G2- Basal diet + 20 ppm Pbas lead acetate; G3- Basal diet + 20 ppm Pb + 20 ppm DL-methionine; G4- Basal diet + 20 ppm Pb + 40 ppm DL-methionine. Experimental feeding with lead for 105 days resulted in significant alterations in the serum enzyme activities and thyroid hormone status. Statistically significant ($P<0.05$) higher activity of ALT and AST enzymes were obtained in lead supplemented G2 and G3 groups when compared to control group G1, indicating hepatotoxicity . However, in G4 were forty ppm DL-methionine was supplemented, the activities of enzymes ALT and AST were statistically comparable to G1. The activity of sorbitol dehydrogenase (SDH) enzyme (U/L) was significantly ($P<0.05$) higher in G2 and G3, indicative of liver damage. The enzyme activity was found to be lower in G3 and G4 when compared to G2 indicating the hepato protective effect of DL-methionine in the diet of guinea pigs. Results revealed a statistically ($P<0.05$) significant reduction in T_3 and T_4 values in group G2 and G3 when compared to group G1, indicating thyrotoxicity. The T_3 and T_4 values in G1 (control) and G4 (40 ppm DL-methionine supplemented group) were statistically ($P>0.05$) higher indicating the ameliorative effect of 40 ppm of DL-methionine supplementation against lead toxicity. The T_4:T_3 ratio was found to be enhanced in group G2 and G3 when compared to group G1 and G4. The study revealed a significant ameliorative effect of DL-methionine against lead toxicity at the hepatic and thyroid level.

Keywords: Lead, DL-Methionine, Hepatotoxicity, Thyrotoxicity.

Introduction: Lead (Pb) is one of the most ubiquitous heavy metals that have been detected virtually in almost all settings of environmental and biological systems. The quantity of lead used in the 20th century far exceeds the total Pb consumed in all previous eras (Nriagu, 1998). Anthropogenic activities such as mining, smelting and use of automobile fuels have substantially altered the natural ecological distribution of lead in the environment leading to globally elevated levels of lead in air, water and soil. The environmental health of developing countries like India and most of the under developed countries is in a state of disorder mostly due to industrial emission and automobile smoke in and around urban and sub-urban areas, risking the human and animal population to the potential exposure of environmental lead (WHO, 1989). In recent years, the lead consumption in Indiaamounts to2.5 lakh tons, out of which about 75 percent is being used by lead-acid battery sector. Almost all automobile batteries contain lead and it is also being used in underground cable sheath, alloys and pigments. Lead is being used in batteries of UPS and power inverters and its business is increasing day by day. The

atomic symbol for lead is Pb, which is derived from Latin word *plumbum*. Lead (atomic number, 82; atomic weight, 207.19, specific gravity, 11.34) is a bluish to silvery grey heavy metal, which is soft, pliable and has no characteristic taste or smell (NRC, 2005).

Comparatively safer agents such as vitamin C and E, amino acids and trace minerals are being proposed in modern therapeutic management of lead intoxication in human and veterinary medicine (Patra *et al.,* 2011). Lead is a sulfhydryl reactive metal, which disrupts the structure and function of numerous important proteins through direct binding to free sulfhydryl groups. Lead is having high affinity for glutathione (GSH) and lead-GSH complex, gets excreted through urine. This depletes the cells of their GSH and decreases the antioxidant capacity of the animal. Methionine/cysteine when supplemented in diet can provide the sulfhydryl groups for glutathione formation (Quig, 1998). Hence DL-methionine added in the diet can have significant ameliorative effect against low level dietary lead toxicity in animals. Hence the present study was conducted to study the ameliorative effect of DL- methionine against low level dietary lead in guinea pigs.

Materials and Methods: Thirty two healthy guinea pigs (*Caviaporcellus*) of about 40 days of age were procured from Laboratory Animal Resource (L.A.R) Section of I.V.R.I, Izatnagar. For the purpose of adaptation to the new environment, these animals were maintained for 7 days on a standard diet comprised of concentrate mixture and green fodder (berseem) before the start of the proper experiment. These animals were then divided into four groups of 8 animals in each group on the basis of their body weight (449.3 ± 13.0 g) following randomized block design (RBD).

All the experimental animals were housed in a well ventilated room. Strict management and hygienic practices were adopted throughout the experimental period. Animals of different groups were kept in 4 separate galvanized iron trays. Rice husk was used as the bedding material in the trays. The bedding material was changed daily. Clean drinking water was provided *ad libitum* twice a day at about 9:30 A.M. and 3:30 P.M. daily. Experimental feeding lasted for a period of 105 days.

Basal diet consisted of concentrate mixture (27% ground maize, 25% Bengal gram, 5% soybean meal, 10% fish meal, 30% wheat bran, 2% mineral mixture and 1% common salt and 0.02% vitamin C) and berseem fodder .All the animals were fed to meet their nutrient requirement as per NRC (1995). Lead was added in the diet as aqueous solution of lead acetate [Pb $(C_2H_3O_2)_2$. $3H_2O$] and aqueous solution of DL-methionine was added into the concentrate mixture daily.

Group	Treatment
G1(control)	Basal diet
G2	Basal diet + 20 ppm Pbas lead acetate
G3	Basal diet + 20 ppm Pb + 20 ppm DL-methionine
G4	Basal diet + 20 ppm Pb + 40 ppm DL-methionine

At the end of experimental feeding period, about 6ml of blood was collected from each animal through cardiac puncture in the morning after ether anesthesia (before watering and feeding). Out of it, 2ml blood was taken into clean and dry test tube and kept in slanting position for 45 minutes to separate out the serum. AST and ALT in blood serum was determined using diagnostic kit manufactured by Span Diagnostic Limited, Surat, India. Serum sorbitol dehydrogenase (SDH) activity was estimated by the method of Agarwal (1991). The enzyme activity was measured by following the rate of change of absorbance at 340 nm in a spectrophotometer at 30° C. The reaction mixture (1.5 ml) contained NADH (0.03mM) and fructose (200mM) in triethanolamine-HCL buffer (150 mM, pH 7.4). One unit of enzyme activity was defined as the amount of enzyme which produced 1 µmol NAD per minute.

$$\text{Specific activity} = \frac{(\Delta A x V)}{((ex\ d\ x\ \Delta t\ x\ v\ x\ C_{protein})}$$

Where,

Δ A- Absorbance

V-Assay volume
e – Extinction coefficient (6.22)
d – Light path (1 cm)
Δt - time
v – sample volume
$C_{protein}$ – Protein concentration (mg/ml)

Tri-iodothyronine (T_3) and Thyroxin (T_4) concentration in serum was estimated by RIA kits (Immunotech, Radiova Czech Republic). The radioimmunoassay of total T_3 and T_4 is a competitive immunoanalytical determination. Unknown serum samples and standards were incubated together with ^{125}I-triiodotyrosine or ^{125}I-thyroxine in monoclonal anti-T_3 antibody-coated tubes. After incubation the contents of the tubes were aspirated and the bound activity was measured in a gamma counter (Packard, USA). The concentration of T_3 and T_4 were reversely proportional to the radioactivity measured. The values of unknown samples were read after the calibration curve was developed.

Results: Statistically significant (P<0.05) higher activity of ALT and AST enzymes were obtained in lead supplemented G2 and G3 groups when compared to control group G1. However, in G4 were forty ppm methionine was supplemented, the activities of enzymes ALT and AST were statistically comparable to G1. The activity of sorbitol dehydrogenase (SDH) enzyme (U/L) was significantly (P<0.05) higher in G2 and G3, indicative of liver damage. The enzyme activity was found to be lower in G3 and G4 when compared to G2 indicating the hepato protective effect of 20 and 40 ppm methionine supplementation in the diet of guinea pigs.

Results revealed a statistically (P<0.05) significant reduction in T3 and T4 values in group G2 and G3 when compared to group G1, but the T3 and T4 values in G1 (control) and G4 (40 ppm methionine supplemented group) were statistically (P>0.05) higher indicating the ameliorative effect of 40 ppm of methionine supplementation against chronic lead toxicity. The T4:T3 ratio was found to be enhanced in group G2 and G3 when compared to group G1 and G4.

Discussion: Higher ALT activity is indicative of liver damage since ALT and aspartate amino transferases (AST) are liver specific enzymes used in testing liver function. Forty ppm DL-methionine supplemented group G4 showed serum ALT activity statistically comparable to group G1 indicating the ameliorative effect of 40 ppm DL-methionine on liver. It was also observed that supplementation of 20 ppm DL-methionine in the Pb exposed animals could not exert any effect in serum ALT activity in group G3 as it was similar to group G2.

AST enzyme activity was also observed to be more in group G2 and G3 as compared to group G1, and the enzyme activity was comparable between group G1 and G4. Forty ppm DL-methionine supplemented group G4 showed AST activity statistically comparable to group G1 indicating the ameliorative effect of 40 ppm DL-methionine in liver. It was also observed that supplementation of 20 ppm DL- methionine in the Pb exposed animals could not exert any effect on serum AST activity in group G3 as it was similar to group G2. Protective effect of methionine at the liver function levels were earlier reported by Flora *et al.* (1991).

Liver is the major source of sorbitol dehydrogenase and it has been used as a marker enzyme in clinical diagnosis of liver diseases and hepatotoxicity in animals (Agarwal, 1991). Activity of sorbitol dehydrogenase enzyme (SDH) (U/L) was significantly (P<0.05) higher in the serum of group G2 and G3, as compared to G1, indicative of liver damage. Serum enzyme activity was found to be lower in group G4 as compared to group G2 and G3 indicating the hepato protective effect of 40 ppm DL-methionine in the diet. It was also observed that supplementation of 20 ppm DL-methionine in the Pb exposed animals could not exert any effect on serum SDH activity in group G3 as it was similar to group G2.

There was statistically significant (P<0.05) reduction in serum tri-iodothyronine (T3) and thyroxin (T4) values in group G2 and G3 as compared to control group G1. But the T3 and T4 values in group G1

(control) and G4 (40 ppm DL-methionine supplemented group) were statistically higher indicating the ameliorative effect of 40 ppm DL-methionine against chronic lead toxicity. Supplementation of 20 ppm DL-methionine in the Pb exposed animals could not exert any effect on serum T3 and T4 levels in group G3 as it was similar to group G2. Reports regarding the protective effect of DL-methionine on the serum thyroid hormone levels are scanty.

Yoshizuka*et al*. (1991) explained that lead can inhibit 5′-D activity through binding to sulfhydryl group of this enzyme. Decreased concentration of serum T3 in lead treated rats might be due to decreased conversion of T4 to T3 due to inhibition of type-I iodothyronine 5′-monodeiodinase(5′-D), the enzyme responsible for the peripheral deiodination of T4 to T3, as suggested by Chaurasia*et al.* (1996). However, the findings of the present study are contradictory to the findings of Swarup*et al.* (2007), who reported a positive correlation of T3 and T4 levels with respect to blood lead levels. Reports regarding the ameliorative effect of DL-methionine on thyroid hormone levels in different species of animals are scanty in the literature. Hence it can be concluded that dietary addition of 40 ppm DL-methionine can significantly reduce the hepatotoxic and thyrotoxic effects of low level lead toxicity in guinea pigs.

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Table1: Serum Enzyme Profile of Guinea Pigs in Different Groups

Attributes	Groups					
	G1	G2	G3	G4	SEM	P value
ALT (U/ml)*	36.3^{b}	39.9^{a}	39.6^{a}	36.4^{b}	0.34	0.000
AST (U/ml)*	33.7^{b}	40.3^{a}	38.6^{a}	35.4^{b}	0.71	0.001
SDH (U/L)*	10.5^{c}	16.9^{a}	15.3^{b}	14.3^{b}	0.46	0.000

*Means bearing different superscripts in a row differ significantly (P< 0.05)

Table 2: Serum Thyroid Hormone Profile

Attributes	Groups					
	G1	**G2**	**G3**	**G4**	**SEM**	**P value**
T3 (nmol/L)*	0.41^{a}	0.30^{c}	0.30^{c}	0.35^{b}	0.01	0.001
T4 (nmol/L)*	46.84^{a}	43.36^{c}	43.29^{c}	45.76^{a}	0.56	0.049
T4:T3*	113.43^{c}	147.52^{a}	149.95^{a}	132.17^{b}	8.11	0.034

*Means bearing different superscripts in a row differ significantly (P< 0.05)

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