EVALUATION OF DMPS AND DMSA EFFICACY IN THE TREATMENT OF LEAD TOXICITY

MANAL M. SAYED[*], D.A. SALEM[**], A.A. SHARKAWY[**] and O.A. SHEHATA[*]

[*]Animal Health Research Institute, Assiut Lab.
[**]Dept of Forensic Med. and Toxicology, Faculty of Veterinary Medicine, Assuit Univ.

ABSTRACT

Received at: 1/3/2012

Accepted: 31/3/2012

The current study was designed to evaluate the efficacy of both DMPS (2-3-Dimercapto-1-Propane Sulfonic acid) and DMSA (Meso-2,3-Dimercapto Succinic acid) in treatment of long-term lead toxicity through assessment of some biochemical indices and levels of lead in liver, kidney and brain of exposed rats. One hundred twenty male albino rats were divided into four equal groups. The first group (I) was used as control, the 2nd, 3rd and 4th (II, III, IV group respectiveIy) were exposed to 100 ppm lead acetate in drinking water for 12 weeks. Groups III and IV were treated with DMSA and DMPS respectively after 2, 4, 6, 8, 10, 12 weeks 24 hours prior to sampling. The investigated parameters were ALT, AST, γ–GT, LPO, NO and SOD. Lead levels in liver, kidney and brain were estimated. The results revealed that, both DMPS and DMSA induced many positive effects on lead exposed rats. These effects include, decrease the levels of LPO and NO especially in the first six weeks of the experiment, increase the level of SOD enzyme in both serum and brain tissues at the last six weeks of the experiment, decrease the lead levels in the tissues of the kidney and liver in group III allover the experimental period, while in group IV the decrease was only significant at the first weeks, and significant decrease in the brain lead levels in group III at the last six weeks of the experiment. These results indicated that Both DMSA and DMPS improve the biochemical, free radicals and antioxidant parameters in their groups in comparison with Lead treated group. DMSA have a better effect in reducing lead levels in liver and kidney during the whole period of the experiment and reduced lead level in brain tissue at the last sex weeks of the experiment.

Key words: *Lead, DMPS, DMSA, Lipid peroxidase, Antioxidants.*

تقييم كفاءة الدمسا والدمبس فى علاج التسمم بالرصاص

منال محمد سيد ، ضيفى احمد سالم ، احمد عبد الباقى شرقاوى ، عمر عادل شحاته

صممت هذه الدراسة لتقييم الدمسا (DMSA) والدمبس (DMPS) فى علاج التسمم طويل المدى بالرصاص. تم فى هذه الدراسة استخدام ١٢٠ من الجرذان البيضاء قسمت إلى أربع مجموعات متساويه. استخدمت المجموعة الأولى (I) كضابط للتجربة والثانية والثالثة والرابعة (II,III,IV) تعرضت لخلات الرصاص فى مياه الشرب لمدة ١٢ أسبوع. وتم علاج المجموعتين (III, IV) بمركبات الدمسا والدمبس على التوالى بعد ٢ ، ٤ ، ٦ ، ٨ ، ١٠ ، ١٢ أسبوعا وتم أخذ

العينات للفحص بعد ٢٤ ساعة من العلاج. تم فى هذه الدراسة قياس الخمائر الكبدية (AST, ALT, Y-GT) وبعض الشقائق الحرة (LPO, No) ومضادات الأكسدة (SOD) فى السيرم وأنسجة المخ وكذلك قياس مستويات الرصاص فى الكبد ، الكلى والمخ وأظهرت النتائج مايلى: قدرة المركبين على تقليل تركيز LPO, NO فى المجموعتين (III, IV) عنها فى المجموعة المتعرضة للرصاص خاصة فى الأسابيع الستة الأولى من التجربة. زيادة تركيز SOD فى المجموعة III,IV عنها فى المجموعات الأخرى. انخفاض مستوى الرصاص فى كل من أنسجة الكلى والكبد فى المجموعة III خلال الفترة الكاملة للتجربة بينما كان الانخفاض فى المجموعة IV خلال الأسابيع الأولى فقط. انخفض مستوى الرصاص فى أنسجة المخ خلال الأسابيع الستة الأخيرة وذلك فى المجموعة IV فقط.

INTRODUCTION

The main threats to human health from heavy metals are associated with exposure to cadmium and lead. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries (Senze *et al.,* 2009). Poisoning by toxic chemicals can cause serious stock losses. Historically, lead and arsenic have been the most common causes of inorganic chemical poisoning in farm animals (Byrne, 2007). Lead intoxication cause damage to membrane-associated enzymes such as sodium-potassium pumps result in red blood cell fragility and renal tubular injury. Lead may also alter zinc-dependent enzyme processes and interfere with GABA production or activity in the CNS (Haschek and Rousseaux, 2002). It damages the small blood vessels, causing bleeding, and deprives the nerves, the brain and other organs from oxygen. Lead severely damages the kidney and liver tissue. (Siddiqui and Gayatri, 2008).

Tatjana *et al.* (2003) and Suradkar *et al.* (2009) demonstrated that increased Aspartate aminotransferase (AST and Alanine aminotransferase (ALT) which might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by lead acetate. Increase in Gamma-glutamyl Tansferase is an indication of hepatotoxicity and oxidative damage in the hepatocytes. Sivaprasad *et al.* (2003) reported that rats exposed to lead in drinking water for 5 weeks showed lowered activities of catalase, superoxide dimutase, glutathione perioxidase and reduced glutathione. Abdel Aal and Hussein (2008) recorded that there was a significant increase in the lipid peroxidation and significant decrease in the serum total antioxidant level in lead treated animals.

Chelation is a chemical process in which specific chemical antidote reacts with metal protein complex, combined with the metal and leaves the protein free. Most of chelators contain two thiol groups; therefore, they attract metal to combine with them. The combined metal chalet's forms stablele complex and mostly excreted through urine (Osweiler, 1996).

The study aims to evaluate the efficacy of DMPS (2-3-Dimercapto-1-Propane Sulfonic acid) and DMSA (Meso-2,3-Dimercapto Succinic acid) as a chelating agents in case of long-term exposure to lead.

MATERIALS and METHODS

a) Chemicals:

- DMPS (2,3-Dimercapto-1-propanesulfonic acid sodium salt), monohydrate , Purity: 95% and DMSA (meso-2,3-Dimercapto succinic acid) of 98% purity were obtained from Sigma Chemical Co., Germany

- Lead acetate was obtained from B.D.H laboratories chemicals division Poole, England.

b) Animals:-

One hundred and twenty male albino rats weighting 100-150 g of 10-12 weeks old were obtained from the Laboratory Animal House, Faculty of Medicine, Assuit University. Rats were housed in plastic cages, five rats each and acclimatized to laboratory condition two weeks before the experiment and fed commercial pellet rat feed. Food and water were available add libitum, suitable temperature and lighting cycle of 12 hours (light/dark) were also in consideration.

c) Experimental Design:-

The obtained rats were randomly divided into 4 groups (30 each), I, II, III and IV. The last 3 groups (II, III and IV) were exposed to lead acetate in drinking water at a concentration of 100 ppm for the whole period of the experiment (12 weeks). The lead acetate concentration was used according to Sharkawy and Abd-el ghaffar (1999).

- Group I: was used as negative control.

- Group II: Animals were used as positive control.

- Group III: were exposed to DMSA in a dose of (135 mg/kg body weight orally by intubation) 24 hours prior to sampling

- Group IV: Animals were exposed to DMPS in a dose of (200mumol/kg body weight IP) 24 hours prior to sampling (Twarog and Cherian, 1984).

d) Sampling:-

Five rats were taken randomly from each group at 2, 4, 6, 8, 10 and 12 weeks post-lead exposure.

Blood samples were collected from ocular vein of these rats as follows:

Whole blood samples in vacutainer tubes without anticoagulant to obtain serum for estimation of biochemical parameters (AST, ALT, γ–GT, lipid peroxides, superoxide dismutase and nitric oxide.) Liver, kidney and brain samples were collected from each rat for tissue lead level estimation. The brain samples were also used for evaluation of free radicals as (lipid peroxide, superoxide dismutase and nitric oxide).

e) Biochemical analysis

-Serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were estimated as described by Henery (1960), using commercial kits supplied by (Diamond Diagnostics. Hannover, Germany).

-Gamma - GT (γ-GT) concentration was measured spectrophotometrically according to the method of Tietz, (1994) by using commercial kit obtained from (Chema Diagnostica. Italy).

-Lipid peroxidation determination (LPO) as Thiobarbituric acid reactive Substances (TBARS) were determined according to the method of Ohkawa *et al.* (1979).

-Nitric oxide (NO) was measured as nitrite concentration colorimetrically using the method of Ding *et al.* (1988).

-Superoxide dismutase activity (SOD): activity in serum and tissue cytosols was determined according to the method of Misra and Fridovich (1972).

f) Estimation of tissues lead level:

- Preparation of the samples:

Liver, kidney and brain samples were digested according to the method of Yeager *et al.* (1971).

Measurement of lead:

Lead level was estimated using Graphite atomic absorption spectrometer ZEEnit 700 P analytikjena (Germany) at the Central Lab in the Faculty of Veterinary Medicine-Assiut University was used (Szkoda and Zmudzki (2005).

3. Statistical analysis:

Statistical analysis of data was conducted using SAS statistical package (1990).

RESULTS

Enzymes activities:

AST was decreased in serum at the 12^{th} week in group II and significant increase at the 2^{nd} week in groups III and IV in relation to the group II (Table 1). The same result was obtained in serum ALT as significant decrease in group II at 10^{th} and 12^{th} weeks and significant increase in groups III and IV at the 2^{nd} and 4^{th} weeks in relation to the group II (Table 2). The gamma-GT values showed no significant change in group II in comparison with the group I. On the other hand a significant decrease in group III and IV was recorded at the $2^{nd,}$ 4^{th}, and 6^{th} weeks in comparison with both groups I and II (Table 3).

Free radicals and Antioxidants:-

LPO in brain homogenate (Table 4) showed significant increase during the whole period of the experiment in group II and at 2^{nd}, 4^{th}, 6^{th} and 8^{th} week in group III. In group IV showed significant decrease in comparison to group II

and III all over the experiment period. The serum LPO showed significant increase in group II in comparison with group I during the whole period of the experiment. At 2nd, 4th and 6th weeks, groups III and IV showed significant increase than group I. and decreased in comparison with group II (Table 5).

Nitric oxide in brain cytosol showed a significant increase in group II in comparison with group I. Groups III and IV showed a significant decrease when compared to group II at the whole period of the experiment (Table 6). No significant change was recorded in the nitric oxide in serum except a significant increase in the group II at the last two periods (10th and 12th weeks) and a significant decrease in group III in comparison with group II (Table 7).

Superoxide dismutase (SOD) in brain cytosol revealed a significant increase in group III at 8th week in comparison with group II. Also significant increase in group IV was recorded in comparison to group III. (Table 8). Serum suproxide dismutase (SOD) showed no significant changes in all investigated groups (Table 9).

Lead concentration in tissues

Lead levels in liver showed significant increase in group II during the whole period of the experiment. Group III and IV showed significant decrease in liver lead levels in comparison with group II. At the 6th, 8th, 10th and 12th weeks, a significant increase in lead levels was recorded in group IV in comparison with group III (Table 10).

Lead levels in the kidney at the whole period of the experiment showed a significant increase in group II in comparison with group I. A significant decrease was recorded in both group III and IV in comparison with group II at the whole period of the experiment. The lead level was significantly decreased at the 2nd, 4th and 6th weeks and significantly increased at 8th, 10th and 12th weeks in group IV when compared with group III (Table 11).

The result of lead levels in the brain tissues revealed a significant increase in group II in comparison with group I at 6th, 8th, 10th and 12th weeks. A significant decrease was recorded at 8th, 10th and 12th weeks in group III in comparison with group II (Table 12).

Table 1: Efficacy of DMSA and DMPS on AST in serum (IU/l)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	24.79± 2.670	31.92± 1.549	30.91± 4.244	28.71± 3.263	28.35± 3.656	33.57± 2.775
Group II (lead)	29.04± 2.679	29.25± 3.124	32.58± 1.612	28.24± 5.431	24.67± 4.002	19.74± 4.222
Group III (lead+DMSA)	38.71± 0.445*a	30.21± 3.040a	28.92± 5.324	29.10± 1.604	33.93± 0.586	31.26± 1.591a
Group IV (lead+DMPS)	37.23± 1.014*a	29.29± 2.666	23.13± 3.157	23.63± 0.697	25.17± 3.242	26.91± 3.586

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 2: Efficacy of DMSA and DMPS on ALT in serum (IU/l)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	36.80± 3.918	30.06± 3.226	30.72± 1.916	32.90± 3.246	29.47± 1.891	34.62± 0.937
Group II (lead)	26.09± 3.523	22.99± 3.967	29.10± 2.306	26.24± 0.733	23.62± 1.104*	21.36± 1.797*
Group III (lead+DMSA)	32.59± 1.332*a	29.01± 3.115a	30.26± 2.198	31.19± 4.388	27.36± 2.295	29.94± 3.184a
Group IV (lead+DMPS)	30.72± 2.331*a	29.98± 2.595a	26.52± 3.154	26.78± 0.365	25.89± 1.583	30.72± 1.580a

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 3: Efficacy of DMSA and DMPS on gama-GT in serum (IU/l)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.837± 0.061	0.851± 0.131	0.873± 0.043	0.788± 0.096	0.647± 0.116	0.827± 0.140
Group II (lead)	1.263± 0.281*	0.840± 0.158	0.977± 0.130	0.763± 0.035	0.877± 0.162	0.867± 0.034
Group III (lead+DMSA)	0.610± 0.082*a	0.527± 0.073*a	0.617± 0.063*a	0.567± 0.096	0.710± 0.015	0.760± 0.012
Group IV (lead+DMPS)	0.557± 0.024*a	0.580± 0.074*a	0.463± 0.034*a	0.460± 0.021*a	0.873± 0.061	0.853± 0.018

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 4: Efficacy of DMSA and DMPS on Lipid peroxide in brain homogenate (nmol/mg protein)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.357± 0.253	0.193± 0.067	0.207± 0.081	0.387± 0.255	0.210± 0.107	0.303± 0.080
Group II (lead)	2.167± 0.233*	2.557± 0.202*	2.200± 0.252*	2.100± 0.503*	2.300± 0.100*	2.100± 0.346*
Group III (lead+DMSA)	1.933± 0.441*a	2.233± 0.788*a	1.467± 0.233*a	1.133± 0.481*a	0.367± 0.067a	0.163± 0.015a
Group IV (lead+DMPS)	0.127± 0.087ab	0.043± 0.012ab	0.197± 0.054ab	0.090± 0.026ab	0.123± 0.038a	0.027± 0.003a

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 5: Efficacy of DMSA and DMPS on Lipid peroxide in serum (nmol/mg protein)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I control)	0.060± 0.025	0.077± 0.030	0.037± 0.026	0.080± 0.006	0.073± 0.009	0.070± 0.010
Group II (lead)	0.693± 0.202*	0.433± 0.176*	0.733± 0.273*	0.633± 0.067*	0.620± 0.099*	0.733± 0.088*
Group III (lead+DMSA)	0.107± 0.047*a	0.177± 0.113*a	0.270± 0.217*a	0.100± 0.021a	0.094± 0.055a	0.067± 0.012a
Group IV (lead+DMPS)	0.227± 0.037*a	0.210± 0.049*a	0.250± 0.076*a	0.050± 0.010a	0.103± 0.038a	0.067± 0.015a

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 6: Efficacy of DMSA and DMPS on Nitric oxide in brain cytosol (nmol/mg protein)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	7.27± 0.384	6.87± 1.844	6.33± 1.155	6.09± 1.158	6.83± 1.369	6.47± 0.974
Group II (lead)	21.50± 6.574*	22.15± 0.953*	22.55± 1.703*	19.33± 4.116*	20.94± 2.541*	15.23± 1.157*
Group III (lead+DMSA)	7.07± 0.558a	8.23± 0.736a	7.23± 1.212a	7.17± 0.267a	7.60± 0.961a	6.17± 0.606a
Group IV (lead+DMPS)	7.30± 1.201a	8.63± 1.068	8.17± 0.505a	6.80± 1.079a	6.70± 1.185a	12.43± 2.226*b

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 7: Efficacy of DMSA and DMPS on Nitric oxide in serum (nmol/mg protein)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	12.36± 2.486	12.57± 2.090	12.80± 1.698	13.93± 2.434	14.63± 0.775	13.52± 2.438
Group II (lead)	14.40± 1.457	17.34± 1.354	17.10± 0.666	16.84± 2.299	19.23± 0.751*	22.77± 2.797*
Group III (lead+DMSA)	9.67± 1.592	14.07± 1.233	14.67± 1.304	15.67± 0.590	15.00± 0.493a	14.00± 1.550a
Group IV (lead+DMPS)	12.67± 1.272	13.70± 1.429	12.73± 3.242	15.60± 2.982	18.07± 0.353*b	18.93± 0.884

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 8: Efficacy of DMSA and DMPS on SOD in brain cytosol (nmol/mg protein)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.74± 0.019	0.81± 0.061	0.76± 0.064	0.79± 0.054	0.86± 0.088	0.86± 0.062
Group II (lead)	0.97± 0.050	0.91± 0.019	0.84± 0.030	0.73± 0.021	0.75± 0.026	0.77± 0.007
Group III (lead+DMSA)	0.71± 0.133	0.64± 0.074a	0.60± 0.036*a	1.11± 0.090*a	0.85± 0.076	0.90± 0.047
Group IV (lead+DMPS)	1.00± 0.144	0.98± 0.112b	0.97± 0.054*b	0.99± 0.067a	0.96± 0.062	1.06± 0.098a

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 9: Efficacy of DMSA and DMPS on SOD in serum (nmol/mg protein)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.27± 0.009	0.33± 0.025	0.26± 0.035	0.26± 0.029	0.26± 0.047	0.24± 0.056
Group II (lead)	0.27± 0.003	0.22± 0.023	0.20± 0.006	0.16± 0.012	0.18± 0.007	0.16± 0.013
Group III (lead+DMSA)	0.27± 0.024	0.24± 0.044	0.21± 0.021	0.21± 0.020	0.25± 0.034	0.23± 0.015
Group IV (lead+DMPS)	0.23± 0.028	0.27± 0.049	0.19± 0.015	0.20± 0.031	0.19± 0.009	0.18± 0.019

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 10: Efficacy of DMSA and DMPS on liver Lead concentration (ppm)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.587± 0.113	0.660± 0.093	0.643± 0.052	0.550± 0.130	0.560± 0.075	0.627± 0.141
Group II (lead)	1.283± 0.258*	2.000± 0.153*	2.133± 0.285*	2.497± 0.234*	2.717± 0.052*	2.383± 0.188*
Group III (lead+DMSA)	0.753± 0.075a	0.807± 0.038a	0.557± 0.276a	0.833± 0.318a	1.373± 0.127*a	1.293± 0.294a
Group IV (lead+DMPS)	0.710± 0.052a	0.773± 0.033a	2.100± 0.231*b	1.933± 0.318*b	1.967± 0.291*ab	2.067± 0.186*b

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 11: Efficacy of DMSA and DMPS on kidney Lead concentration (ppm)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.660± 0.127	0.667± 0.022	0.720± 0.085	0.780± 0.017	0.700± 0.104	0.783± 0.028
Group II (lead)	7.400± 0.586*	6.673± 1.231*	7.147± 0.472*	6.480± 1.175*	6.467± 1.146*	7.167± 0.745*
Group III (lead+DMSA)	3.867± 0.120*a	3.800± 0.379a	3.500± 0.208*a	2.027± 0.130a	2.267± 0.318a	2.357± 0.222a
Group IV (lead+DMPS)	2.353± 0.247*ab	2.800± 0.635ab	2.547± 0.296*ab	4.467± 0.145*ab	5.200± 0.208*b	5.933± 1.004*b

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

Table 12: Efficacy of DMSA and DMPS on brain Lead concentration (ppm)

Exposed groups	Post-exposure time (weeks)					
	2	4	6	8	10	12
Group I (control)	0.833± 0.044	0.883± 0.017	0.830± 0.082	0.890± 0.031	0.827± 0.079	0.783± 0.088
Group II (lead)	1.140± 0.092	1.397± 0.255	1.860± 0.567*	2.417± 0.263*	2.393± 0.298*	2.403± 0.223*
Group III (lead+DMSA)	1.133± 0.145	1.000± 0.153	1.027± 0.156	1.123± 0.153a	1.127± 0.073a	1.437± 0.148*a
Group IV (lead+DMPS)	1.113± 0.286	0.920± 0.214	1.367± 0.145	1.543± 0.255*	1.883± 0.507*	1.233± 0.088*

* Significant at $p \leq 0.05$ to 0.01 in comparison with the control group
a indicates significant difference between II and both III and IV
b indicates significant difference between III and IV

DISCUSSION

The use of large number of chelating agents in treatment of lead toxicity initiated us to evaluate DMSA and DMPS. The present study was designed to evaluate the two chelators through exposure of male albino rats for 12 weeks to lead acetate in 3 groups. One kept as control, the 2nd was treated with DMSA and the 3rd treated with DMPS 24 hours prior to sampling. Changes of many parameters as enzymes activities, free radicals, antioxidant, lead levels were evaluated.

AST activity was decreased at the 12th week in group II although it was significantly increased at the 2nd week in both group III and IV in relation to the group I. The same behaviour was noticed in ALT activity. The gamma-GT values showed no significant change in group II in comparison with the control group. On the other hand, significant decrease in group III and IV was recorded at the 2nd, 4th, and 6th weeks in comparison with both group I and II. Suradkar *et al.* (2009) reported that increased AST and ALT might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by lead acetate. These findings are in accordance with Shalan *et al.* (2005). Increase in Gamma-GT is an indication of hepatotoxicity and oxidative damage in the hepatocytes (Tatjana *et al.,* 2003). Abdel Aal and Hussein (2008) who found in the treatment with DMSA or ALA, they improved the increased hepatic enzyme levels and this improvement was highly significant when ALA and DMSA were given

in combination. This coincide with Shalan *et al.* (2005) who mentioned that administration of lead acetate in diet for 6 weeks resulted in elevations of serum GPT, GOT, and ALP, as recorded in this study in the 2nd week in groups D and P.

LPO level in brain homogenate showed significant increase during the whole period of the experiment in group II and from the 2nd to the 8th week in group III. Group IV showed significant decrease in LPO level in comparison to group II and III all over the experiment period. The serum LPO showed the same picture as brain homogenate. At 2nd, 4th and 6th weeks group III and IV showed significant increase in serum LPO than group I and at the same time the measured values were lower than that group II. In male calves exposed to lead acetate Patra and Swarup (2000) reported that lipid peroxide level was recorded to be significantly higher by day 21 and 28 of exposure. Nitric oxide in brain cytosol showed significant increase in group II in comparison to group I. Both group III and IV showed a significant decrease when compared to group II at the whole period of the experiment. No significant change recorded in the level of nitric oxide in serum except a significant increase in the group II at the last two periods (10th and 12th weeks). This was in agreement with Heydari *et al.* (2006) who mentioned that lead administration in rats for 4, 8, and 12 weeks resulted in decrease serum NO level which may attributed to either decreased its production or enhanced its inactivation by free radical formation. Vaziri *et al.* (1997) reported that glutathione depletion and lead exposure have been independently shown to depress nitric oxide in animal models. Heydari *et al.* (2006) investigated that short-term (4 and 8 weeks) and subchronic (12 weeks) effects of lead treatment on responsiveness of vascular adrenergic system and level of nitric oxide metableolites, that is, total nitrates and nitrites (NOx). They found marked elevation of blood pressure accompanied by significant reduction in serum NOx levels.

SOD in brain cytosol revealed a significant increase in group III at 8th week in comparison with II group. Also significant increase in group IV was recorded in comparison to group III. Serum SOD showed no significant changes in all investigated groups. One of the most effective intracellular enzymatic antioxidants is SOD. It catalyzes the dismutation of $O^{2\bullet-}$ to O^2 and to the less-reactive species H_2O_2. While this enzyme was isolated as early as 1939, it was only in 1969 that McCord and Fridovich proved the antioxidant activity of SOD (McCord and Fridovich, 1969).

Lead level in liver tissues showed significant increase in group II during the whole period of the experiment. Group III and IV showed significant decrease in lead levels in comparison with group II. At the 6th, 8th, 10th and 12th weeks a significant increase in lead levels was recorded in group IV in comparison with group III. Lead levels in the kidneys at the whole period of the experiment showed significant increase in group II in comparison with group I. A significant decrease was recorded in both group III and IV in comparison with group II. The lead level was significantly decreased at the 2nd, 4th and 6th weeks and significantly increased at 8th, 10th and 12th weeks in group IV when compared with III group. Brain tissues revealed a significant increase of lead level in group II in comparison with group I at 6th to the end of experiment. A significant decrease was recorded at the last three periods in group III in comparison with group II. These results are in agreement with Okonishnikova *et al.* (1976) who reported that DMSA given s/c to rabbits previously challenged with Pb acetate not only increased the excretion of circulating Pb but also removed Pb from tissue and bone, also the disturbances in porphyrin metableolism usually seen with Pb intoxication were prevented**.** Friedheim *et al.* (1978) also stated that, when DMSA given orally reduced the Pb concentration of the blood from 97 to 43 µg/dl.

Also our results agree with results of (Xu Y *et al.,* 2011) who stated that DMSA effectively decreased the Pb levels of the femur, brain, kidney, liver, and blood, greatly enhanced urination, and increased the Pb levels of both urine and feces, while causing no redistributions of Pb to the other organs,

especially to the brain. With respect to lowering the bone and brain Pb, This benefit was attributed to its high transmembrane ability. The absence of apparent clinical signs on exposed rats in all groups (II, III and IV) is in agreement with Kirk (1986), who stated that chronic lead poisoning in dogs and cats is sometimes overlooked, because the signs can be insidious in onset and subtle in nature mimicking a variety of other elments.

In conclusion Both DMSA and DMPS improve the biochemical, free radicals and antioxidant parameters in their groups in comparison with Lead group. DMSA have a bitter effect in reducing lead levels in liver and kidney during the whole period of the experiment and reduced lead level in brain tissue at the last sex weeks of the experiment.

REFERENCES

Abdel Aal, K.M. and Hussien, A.M.R. (2008): Therapeutic efficacy of alpha lipoic acid in combination with succimer against lead-induced oxidative stress, hepatotoxicity and nephrotoxicity in rats. Ass. Univ. Bull. Environ. Res., Vol. 11, No. 2: 87-98.

Byrne, D. (2007): Lead poisoning in livestock. www.dpi.nsw.gov.au.

Ding, A.H.; Nathan, C.F. and Stuchr, D.J. (1988): Release of reactive nitrogen intermediate and reactive oxygen intermediate from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. J. Immunol., 141: 2407-2412.

Friedheim, E.; Graziano, J.H.; Popovac, D.; Dragovic, D. and Kaul, B. (1978): Treatment of lead poisoning by 2,3-dimercaptosuccinic acid. Lancet, 2: 1234-36.

Haschek, W.M. and Rousseaux, C.G. (2002): Handbook of toxicologic pathology, ed. 2, San Diego, Academic Press.

Henery, J. (1960): Interpretation of Clinical Laboratory tests. Am. J. Clin. Path. 34-38.

Heydari, A.; Norouzzadeh, A.; Khoshbateen, A.; Asgari, A.; Ghasemi, A.; Najafi, S. and Badalzadeh, R. (2006): Effects of short term and subchronic lead poisoning on nitric oxide metableolites and vascular responsiveness in rat. Toxicol. Letters, 166 (1): 88-94.

Kirk, R.W. (1986): Current veterinary therapy IX, Philadelphia, WB Saunders.

McCord, J.M. and Fridovich, I. (1969): Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein), J. Biol. Chem., 244: 60409–60455.

Misra, H.P. and Fridovich, I. (1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissue by thiobarbaturic acid reaction. Anal. Biochem., 95: 351-358.

Okonishnikova, I.E.; Rozenberg, E.E. and Rezina, I.A. (1976): The therapeuticprophylactic effect of succimer in experimental subacute lead acetate poisoning. Gig. Tr. Prof. Zabol., 8: 24-28.

Osweiler, G.D. (1996): Toxicology, The National Veterinary Medical series for independent study. Williams and Wilkins. USA.

Patra, R.C. and Swarup, D. (2000): Effect of lead on erythrocytic antioxidant defence, lipid peroxide level and thiol groups in calves http://www.sciencedirect.com

Senze, M.; Monika, K. and Przemysaw, P. (2009): Metals in chosen aquatic plants in a lowland dam reservoir. J. Elementol., 4(1): 147-156.

Shalan, M.G.; Mostafa, M.S.; Hassouna, M.M.; El-Nabi, E.E. and El- Refaie (2005): Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicol., 206(1): 1-15.

Sharkawy, A.A. and Abdel-Ghaffar, S.Kh. (1999): influence of DMSA and EDTA on lead mobilization and redistribution in albino rats. Assiut Vet. Med. J. Vol. 40 No. 80: 242-259

Siddiqui, M.F. and Gayatri, R.R. (2008): Lead an emerging threat to livestock. Veterinary world, Vol. 1(7): 213-216.

Sivaprasad, T.R.; Nagaraj, M. and Varalakshmi, P. (2003): Combined efficacies of lipoic acid in combination with 2,3-dimercaptosuccinic acid on leadinduced erythrocyte membrane peroxidation and antioxidant status in rats. Hum. Exp. Toxicol., 4: 183-192

Suradkar, S.G.; Ghodasara, D.J.; Vihol, P.; Patel, J.; Jaiswal, V. and Prajapati, K.S. (2009): Haemato-Biochemical alterations induced by lead acetate toxicity in wistar rats. Veterinary world, Vol. 2(11): 429-431.

Szkoda, J. and Żmudzki, J. (2005): Feasibility of microwave digestion for determination of trace elements in biological material by atomic absorption spectroscopy methods. Bromat. Chem. Toksykol., 28: 369-375.

Tatjana, J.; Gordana, K.; Dusica, P. and Ivana, S. (2003): Effects of captopril on membrane associated enzymes in lead induced hepatotoxicity in rats. Acta. Fac. Med. Naiss., 20(3): 183-188.

Tietz, N.W. (1994): Textbook of Clinical Chemistry. 2 nd ed. W. B. Sounder Co.; Philadelphia, P. 851.

Twarog, T. and Cherian, M.G. (1984): Chelation of lead by dimercaptopropane sulfonate and a possible diagnosis. Toxicol. Appl. Pharmacol., 72: 550-556.

Vaziri, N.D.; Ding, Y.,Ni.Z. and Gonick, H.C. (1997): Altered nitric oxide metableolism and increased oxygen free radical activity in lead-induced hypertension: effect of lazaroid therapy. Kidney Int., 52: 1042-1046.

Xu, Y.; Wang, Y.; Wang, L.; Zhao, M.; Zhang, X.; Hu, X.; Hou, B.; Peng, L.; Zheng, M.; Wu, J.; Peng, S. (2011): Lead detoxification activities of a class of novel DMSA--amino acid conjugates. Chem. Res. Toxicol. Jun 20, 24(6): 979-84.

Yeager, D.W.; Cholak, J. and Hendersen, E.W. (1971): Determination of lead in biological and related material by atomic absorption spectrophotometery. Environ. Sci. Technol., 5: 1020-1022.