BIOCHEMICAL CHANGES ON BLOOD ENZYMATIC ANTIOXIDANTS AFTER CO-EXPOSURE TO LEAD AND CADMIUM

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ABSTRACT

The present study was carried out to evaluate the pathological changes induced by lead acetate and cadmium chloride toxicity in 50 male albino rats which were uniformly divided into five different groups. Group I received only drinking water as control while, in group II rats were given Lead acetate, in group III rats were given cadmium chloride. In group IV and V rats were given lead and cadmium salt with antioxidant thiocitic acid as a treatment. Animals were examined after five and ten weaks for biochemical changes. The blood of each animal was collected and biochemical assays were conducted. L-Malondialdhyde (LMDA), catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPx) and reduced Glutathione (GSH) was determined in serum. The obtained results revealed that, a significant increase in serum LMDA level in lead intoxicated rats. However, administration of thioctic acid (alpha-lipoic acid) in lead intoxicated rats exhibited a significant decreased in this parameter. On the other hand, a significant decreased in serum CAT, SOD and GPx activities, and GSH concentration were observed in lead intoxicated rats. While, administrations of thioctic acid in lead intoxicated rats resulted in significant increase in these parameters. In case of exposure to cadmium, the obtained results revealed that, a significant increase in serum L-MDA level, and SOD activity in cadmium intoxicated rats. However, administration of thioctic acid showed a significant decreased in level of both L-MDA and SOD parameters. On the other hand, a significant decreased in serum CAT, GPx activities, and GSH concentration in cadmium intoxicated rats. While, the treatment with thioctic acid resulted in significant increase in these parameters. In conclusion, this study highlights a real problem of public health and the use of alpha lipoic Acid (thioctic acid) as a treatment for these changes lead to significant improvement.

Keywords: Pathological effect, Lead acetate, Cadmium chloride, Albino rats, Thiocitic acid.

1. INTRODUCTION

Heavy metals are individual metals and metal compounds that can impact human health. Two common heavy metals are discussed in this study: lead (Pb) and cadmium (Cd). These are all naturally occurring substances which are often present in the environment at low levels. In larger amounts, they can be dangerous. Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Working in or living near an industrial site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of exposure and health impacts because of hunting and gathering activities **(Sabine and Wendy, 2009).**

Some heavy metals, e.g. lead, even at low levels are toxic for microorganisms. As a rule, heavy metal has a negative effect on the growth of water microorganisms as it can greatly depress their numbers. On one hand, the number of microorganisms depends on the total content and concentrations of particular forms of heavy metals. On the other hand, it is conditioned by several other factors, quantity and quality of organic matter, especially carbohydrate rich organic matter, pH, total exchange capacity, nutrient availability, moisture, temperature and oxygen availability. Heavy metals shift the structure of microbial populations, impoverish their diversity, and affect species composition, reproduction and activity of indigenous microorganisms **(Majid, 2010).**

Otherwise, cadmium is a very toxic and is an important environmental pollutant which is present in the soil, water, air, food and cigarette smoke. It is present as an industrial pollutant, a food contaminant and as one of the major constituents of cigarette smoke **(Godt *et al.*, 2006).**

One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. A growing amount of data provide evidence that metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules **(Leonard *et al.*, 2004).** One of the best evidence supporting this hypothesis is provided by the wide spectrum of nucleobase products typical for the oxygen attack on DNA in cultured cells and animals **(Chen *et al.*, 2001).**

Antioxidant molecules are thought to play a crucial role in counteracting free radical induced damage to macromolecules and has been found to heel the free radical mediated cell damage **(Flora *et al.*, 2008).**

Alpha-lipoic acid is naturally occurring compound that is synthesized by plants and animals, including humans **(Self et al., 2000)**.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Experimental animals:

Fifty healthy white male albino rats, 8-10 weeks old and weighting 140-180 gm were used in the experimental investigation of this study. Rats were obtained from the Egyptian company for production of vaccines, sera, and drugs (Vacsera), Helwan branch. Animals were housed at faculty of veterinary medicine, Mansoura university in separate metal cages, fresh and clean drinking water was supplied ad-libitum.

2.1.2. Experimental design

The rats were divided according to their weight into five equal groups after accommodation to the laboratory conditions one control and four experimental groups. These groups, each one consisting of ten animals, placed in individual cages and classified with their administrated dose as follows:

Group I: (control group):

Rats of this group received drinking water with no drugs, served as control for all experimental groups.

Group II: (lead only exposed group)

Rats of this group received lead acetate 1/20 of LD_{50} (25mg/Kg b.wt) orally once per day over a period of 10 weeks as applied by **(Debosree *et al.*, 2012).**

Group III: (Cadmium only exposed group):

Rats of this group received cadmium chloride 1/20 of LD_{50} (5.0 mg/kg. body weight) orally and once per day over a period of 10 weeks as recommended by **(Van *et al.*, 1981).**

Group IV: (lead acetate with thioctic acid "ALA" treated group)

Rats of this group received lead acetate orally and daily (25mg/Kg b.wt) and treated with Thioctic acid "Alpha-lipoic acid" at a dose of (54 mg/kg body weight orally /day) **(Gruzman *et al.*, 2004).**

Group V: (Cadmium Chloride with thioctic acid "ALA" treated group):

Rats of this group received cadmium chloride (5.0 mg/kg. body weight) and treated daily with Thioctic acid "Alpha-lipoic acid" at a dose level of (54 mg/kg body weight orally /day) **(Gruzman et al., 2004).**

2.1.4. Sampling for Blood:

Blood samples were collected in dry, clean test tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 10 minute. The serum was separated by automatic pippte and received in dry strile tubes, then used for operating and determination of the parameters related to serum biochemical antioxidants. Samples were collected from all animals groups, control and four experimental groups two times along the duration of experiment at five and ten weeks from the beginning of rats exposure to lead, cadmium and antioxidant administrated.

2.2. Methods and Instrumentation:

2.2.1- Serum Analyses for Biochemical Enzymatic Antioxidants:

1) Determination of Serum L-Malondialdhyde (L-MDA):

L-MDA concentration was determined according to the method adapted by Mesbah **(Mesbah *et al*., 2004)**.

2) Determination of Serum and superoxide dismutase (SOD) activity:

superoxide dismutase activity was determined using NADH oxidation method described by Paoletti **(Paoletti and Macali, 1990).**

3) Determination of Serum Reduced Glutathione (GSH):

Erythrocytes reduced glutathione concentration was determined according to the methods described by Beutler **(Beutler *et al.*, 1963).**

4) Determination of Serum Glutathione Peroxidase Activity (GPx):

Serum GPx activities were determined according to the method described by Paglia **(Paglia *et al.*, 1967)**

5) Determination of Serum Catalase Activity (CAT):

Serum catalase activity was determined according to the method described by Xu **(Xu *et al.*, 1997**).

2.2.2. Statistical analyses

All statistical analyses were done by statistical software package “SPSS 15.0 for windows, SPSS Inc. Chicago, Illinois” and the GraphPad Prism package; v.5.0 (GraphPad Software, San Diego, CA). Animal’s baseline characteristics were descriptively summarized and reported as mean ± standard error of mean (SEM). Student’s test was used to compare continuous variables. All tests were two-tailed. The result of the t-values was then checked on student's-t-table to find out the significance level (*P* value) as Pearson and Hartly reported **(Pearson and Hartley, 1951).** Values were considered statistically significant when $p < 0.05$.

3. RESULTS

The obtained data in table (1) revealed that, lead intoxicated rats after ten weeks, showed significant decrease in serum SOD, CAT, GSH and GPx accompanied with significant increase serum MDA when compared with normal control group. Treatment with alpha-lipoic acid to lead intoxicated rats caused significant decrease in serum MDA concentration when compared with lead intoxicated group as shown in table (2).

Table 1. Effect of lead toxicity on MDA and some antioxidants serum levels after 10 weeks of treatment.

Parameter [a]	After 10 weeks of treatment		*P* value
	Controls	Pb treated animals	
MDA (Mmol/L)	34.7±1.0	182.7±1.9	<0.0001
SOD (U/L)	35.8±1.0	14.8±1.0	<0.0001
GSH (ng/mL)	8.9±0.4	1.2±0.1	<0.0001
GPx (ng/mL)	38.7±1.0	14.8±1.0	<0.0001
CAT (Mmol/L)	67.2±1.7	22.1±1.4	<0.0001

Continuous variables were expressed as mean ± SEM. Pb= lead; MDA= malondialdehyde; SOD= superoxide dismutase; GSH= reduced glutathione; GPx= glutathione peroxidase; CAT= Catalase; *P*>0.05 is considered not significant, *P*<0.05 considered significant.

Table 2. Effect of thioctic acid on some antioxidants serum levels in lead intoxicated rats after 10 weeks of treatment

Parameter [a]	After 10 weeks of treatment		*P* value
	Pb treated animals	Pb+TA cotreated animals	
MDA (Mmol/L)	182.7±1.9	52.5±1.4	<0.0001
SOD (U/L)	14.8±1.0	36.7±1.4	<0.0001
GSH (ng/mL)	1.2±0.1	5.7±0.5	0.0002
GPx (ng/mL)	14.8±1.0	27.1±1.1	0.0001
CAT (Mmol/L)	22.1±1.4	55.1±1.7	<0.0001

In case of cadmium, the obtained data in table (3) revealed that, serum catalase (CAT), GSH and GPx were significantly decreased and serum LMDA concentration and superoxide dismutase (SOD) activity were significantly increased in cadmium intoxicated rats when compared with normal control group. Treatment with alpha-lipoic acid and to cadmium intoxicated rats caused a significant increased in serum CAT, GSH and GPx level with significant decrease in SOD activity and L-MDA concentration when compared with cadmium exposed non treated group as shown in Table (4).

Table 3. Effect of cadmium toxicity on MDA and some antioxidants serum levels after 10 weeks of treatment.

Parameter [a]	After 10 weeks of treatment		*P* value
	Controls	Cd treated animals	
MDA (Mmol/L)	34.7±1.0	78.9±1.6	<0.0001
SOD (U/L)	35.8±1.0	40.0±1.2	0.0200
GSH (ng/mL)	8.9±0.4	2.6±0.1	0.0042
GPx (ng/mL)	38.7±1.0	18.5±1.1	0.0692
CAT (Mmol/L)	67.2±1.7	34.5±1.7	0.0061

Table 4. Effect of thioctic acid on some antioxidants serum levels in cadmium intoxicated rats after 10 weeks of treatment.

Parameter [a]	After 10 weeks of treatment		*P* value
	Cd treated animals	Cd+TA cotreated animals	
MDA (Mmol/L)	78.9±1.6	54.4±1.0	<0.0001
SOD (U/L)	40.0±1.2	36.5±1.2	0.0100
GSH (ng/mL)	2.6±0.1	4.2±0.3	0.0042
GPx (ng/mL)	18.5±1.1	22.5±1.5	0.0692
CAT (Mmol/L)	34.5±1.7	48.6±1.9	0.0061

4. CONCLUSION

The obtained results revealed that, lead intoxicated rats showed significant increase in serum L-MDA concentration when compared with normal control group. **(Omobowale *et al.*, 2014)** observed that, Treatment with lead gave rise to significant increase in MDA and H_2O_2 generation both in the liver and the erythrocytes following exposure to lead. level of MDA was significantly increased with respect to the control. And this could be due to lead induced inhibition of radical scavenging enzymes like GST and SOD **(Haleagrahara *et al.*, 2011). (Akpinar *et al.*, 2007)** demonstrated that, rats given alpha lipoic acid while being exposed to stress were protected from lipid peroxidation by the antioxidant properties of alpha lipoic acid. The obtained results for cadmium intoxicated rats get in accordance with (Kowalczyk et al., 2002) which observed that, long-term intoxication with cadmium chloride elevated serum concentrations. These results may be related to that of Cd inhibits the

activity of majority of enzymes involved in AOS **(Casalino *et al*., 2002)**. Cadmium intoxicated rats showed significant decrease in serum GSH concentrations. These results came in accordance with the recorded data of **(Renugadevi and Prabu, 2009)** who found that, reduced glutathione level was depressed and its dependent enzymes in Cd-intoxicated rats.
Lipoic acid (LA) has the ability to generate endogenous antioxidants, such as GSH **(Biewenga *et al*., 1997)**.

REFERENCES

Akpinar, D.; Yargiçoğlu, P., Derin, N.; Alicigüzel, Y. and Ağar, A. (2007): The effect of Lipoic Acid on Antioxidant Status and Lipid Peroxidation in Rats Exposed to Chronic Restraint Stres. Physiol. Res. 57: 893-901.

Beutler, E.; Duron, O. and Kelly, M. B. (1963): Pharmacologic effect of selenium and antioxidants on liver and kidney of cadmium intoxicated rats, J. Lab Clin Med.**,** 61,882.

Biewenga G.; Haenen G. and Bast A., (1997). The pharmacology of antioxidant lipoic acid. Gen Pharmacol. 29: 315-31.

Casalino, E., Calzaretti, G., Sblano, C., and Landriscina, C., (2002). Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 179: 37-50.

Chen F., Ding M., Castranova V., and Shi X. L., (2001). Carcinogenic metals and NF-kappa B activation. Mol Cell Biochem 2001; 222: 159-71

Debosree A., Ghosh M., Firdaus S. B., Mitra E., Dey M., and Adhyay D. B., (2012). Protective effect of aqueous leaf extract of Murraya Koenigi against lead induced oxidative stress in rat liver, heart and kidney: a dose response study, Asian of Pharm. and Clinical Res., Vol 5, Suppl 4.

Flora S. J., Mittal M., and Mehta A., (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J. Med. Res., 128: 501–523.

Gary Kobs, (1998). Isolation of RNA from Plant, Yeast and Bacteria. Promega Notes Number 68, p. 28.

Gaurav, D.; Preet, S. and Dua, K. K. (2011). Protective influence of dietary nutrients on antioxidant defense system in the blood of rats treated with cadmium. Adv. Applied Sci. Res., 2: 69-78.

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg P., Reich, A. and Groneberg D. A. (2006). The toxicity of cadmium and resulting hazards for human health, J. Occup. Environ. Med., 22: 1-6.

Gruzman, A., Hidmi, A., Katzhendler, J., Haj-Yehie, A., and Sasson, S. (2004). Synthesis and characterization of new and potent alpha-lipoic acid derivatives. Bioorg. Med. Chem. 12 (5): 1183-1190.

Haleagrahara N, Jackie T, Chakravarthi S, Kulur AB. (2011). Protective eff ect of alphalipoic acid against lead acetate-induced oxidative stress in the bone marrow of rats. Internat J Pharmacol 7: 217–227.

Jin Y.H., Clark A.B., and Slebos R.J., (2003). Cadmium is a mutagen that acts by inhibiting mismatch repair. Nat Genet., 34(3):326-329.

Lakshmi, G. D.; Kumar, P. R.; Bharavi, K.; Annapurna, P.; Kumar, C. S. and Rao, G. (2012). Protective effect of Tribulus terrestris linn on liver and kidney in cadmium intoxicated rats. Indian J. Exp. Biol., 50:141-146.

Leonard S. S., Harris G. K., and Shi X. L., (2004). Metal-induced oxidative stress and signal transduction. Free Rad. Biol. Med., 37: 1921-42.

Liu MC, Liu X.Q., Wang W, Shen X.F., Che H.L., Guo Y.Y. (2012). Involvement of microglia activation in the lead induced long-term potentiation impairment. PLoS ONE 2012;7: e43924.

Mahaffey K (1999). Nutriet- led interaction. In: lead toxicity (Singhal and Thomas j, eds.) pp. 425-460. Urban and Schwarenbeg, Baltimore-Munich.

Majid S, (2010). Experimental studies on effect of Metals presence in Industrial Wastewater on Biological Treatment, International journal of environmental sciences; Vol. 1, No 4.

Mesbah, L.; Soraya, B.; Narimane, S. and Jean, P.F. (2004): protective effect of flavonides against the toxicity of vinblastine cyclophosphamide. Haematol., 7: 59-67.

Omobowale, T.O., Oyagbemi, A.A., Akinrinde, A.S., Saba, A.B., Daramola, O.T., Ogunpolu, B.S., Olopade, J.O., (2014). Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats, Environmental Toxicology and Pharmacology, http://dx.doi.org/10.1016/j.etap.2014.03.002.

Ou. P. M., Tritschler. H.J, Wolff S.P., (1995). Thioctic (lipoic) acid: a therapeutic metal chelating antioxidant? Biochem. Pharmacol. 50 (1995) 123–126.

Paglia, D.E. and Valentine, W.N. (1967): Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med **70**: 158-169.

Paoletti, F. and Macali, A. (1990): Determination of superoxide disumutase activity by purely chemical system based on NAD(P)H oxidation. Meth.Enzymol., 186: 209–220.

Pearson, E.S. and Hartley, H.O., (1951). Charts of the power function for analysis of variance tests, derived from the non-central F-distribution. Biometrika; 38:112-130.

Renugadevi, J., Prabu, S.M., (2009). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp. Toxicol Pathol doi:10.1016/j.etp.03.010.

Sabine M., and Wendy G., (2009). Human Health Effects of Heavy Metals, Kansas State University. 104 Ward Hall. Manhattan KS 66506. 785-532-6519. www.engg.ksu.edu/CHSR.

Self W. T., Tsai L., and Stadman, T. C., (2000). Synthesis and characterization of selenotrisulfide-derivatives of lipoic acid and lipoamide. Proc. Natl. Acad. Sci., 97:12481-12486.

Van Vleet J.F., Boon D., Ferrans V.J., (1981). Induction of lesions of selenium-vitamin E deficiency in weanling swine fed silver, cobalt, tellurium, zinc, cadmium, and vanadium. Am. J. Vet. Res., 42, 789-799.

Xu, J.B.; Yuan, X.F. and Lang, P.Z. (1997): Determination of catalase activity and catalase inhibition by ultraviolet spectrophtometry. Chin. Environ. Chem., 16: 73-76.

التغيرات البيوكيميائية علي مضادات التأكسد الموجودة في الدم بعد التعرض لكلا من الرصاص والكادميوم

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يعتبر الرصاص والكادميوم من العناصر الثقيلة السامة التي تسبب اثار صحية سيئة للانسان والحيوان. وقد اجريت الدراسة الحالية علي عدد ٥٠ فأر من الفئران البيضاء الذكور لمتابعة الاثار السامة لعنصري الرصاص والكادميوم ومدي تأثيرهل علي مضادات التاكسد الموجودة في الدم، وتم تقسيم هذه الفئران الي خمسة مجموعات كل مجموعة تحتوي ١٠ فئران، المجموعة الاولي "الضابطة" لم تعطي أي ادوية، والمجموعة الثانية "المسممة بالرصاص" تم تجريعهم بالرصاص يوميا عن طريق الفم بجرعة ٢٥ ملليجرام لكل كيلو جرام من وزن الجسم. والمجموعة الثالثة "المسممة بالكادميوم" تم تجريعهم بالكادميوم يوميا عن طريق الفم بجرعة ٥ ملليجرام لكل كيلو جرام من وزن الجسم والمجموعة الرابعة "المسممة بالرصاص مع العلاج بمضاد التأكسد" وتم تجريعهم الرصاص بنفس الجرعة وتقديم مضاد الأكسدة "حمض الثايوكتك" بجرعة قدرها ٥٤ ملليجرام لكل كيلو جرام من وزن الجسم لمدة عشر اسابيع. والمجموعة الخامسة "المسممة بالكادميوم مع العلاج بمضاد التأكسد" وتم تجريعهم الرصاص بنفس الجرعة وتقديم مضاد الأكسدة "حمض الثايوكتك" ايضا بنفس الجرعة السابقة لمدة عشر اسابيع.

وقد أظهرت النتائج: مدى التأثير السام القوي علي نسب مضادات التأكسد نتيجة التعرض المستمر للكادميوم والرصاص وظهر ذلك في المجموعة المسممة بالرصاص بوجود ارتفاع ملحوظ في نسبة المالونداي الدهيد وبتقديم حمض الفا ليبوك كعلاج حدث انخفاض ملحوظ في مستوي هذا الانزيم، علي الجانب الاخر كان هناك نقص ملحوظ في انزيم الكتاليز والسوبر اكسيد ديسميوتاز والجلوتاثيون المتـأكسد والمختزل وبتقديم حمض الفا ليبوك حدث زيادة في هذه المعاملات، اما في حالة المجموعة المسممة بالكادميوم كان هناك ارتفاع في نسبة الماونداي الدهيد والسوبر اكسيد دسميوتاز وقل بتقديم حمض الفاليبوك علي الجانب الاخر حدث نزول في انزيم الكتاليز والسوبر اكسيد ديسميوتاز والجلوتاثيون المتـأكسد والمختزل وبتقديم حمض الفا ليبوك حدث زيادة في هذه المعاملات وأظهرت النتائج الفارق الملحوظ في تحسن هذه الخلايا في المجموعات " الرابعة والخامسة" التي تجرعت مضاد التأكسد "حمض الثايوكتك" ، ولذلك نوصي بإستخدامه في هذه الحالات ودراسة متراكباته مع مواد علاجية اخري للحصول علي أفضل النتائج المرجوة .