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ORIGINAL ARTICLE

Ameliorative effects of L-carnitine on rats raised on a diet supplemented with lead acetate

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Abstract Lead intoxication has been a major health hazard in humans. It affects people at all ages. Its toxicity is associated with various organs of the body and affects different metabolic pathways. Based on histological data, L-carnitine reduced the severity of tissue damage produced as a result of exposure of rats to lead acetate. The main objective of this study was to evaluate the underlying mechanism of protection offered by L-carnitine against lead acetate intoxication using male Sprague–Dawley rats.

Forty male Sprague–Dawley rats were randomly divided into four groups with ten rats in each. The first group (G1) served as the control group and animals received standard diet only. The second group (G2) received lead acetate in their diet. The third group (G3) was the L-carnitine treated group and received the normal standard diet supplemented with L-carnitine. While the fourth group (G4) had a diet supplemented with both lead acetate and L-carnitine. At the end of each experiment, blood (serum and whole blood) were collected from each animal and analyzed for the following parameters: serum testosterone levels, serum nitric oxide and serum malondialdehyde. This is in addition to looking at the enzymatic activities of two important enzymes (superoxide dismutase

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and catalase) and on (glutathione reductase) which are indicative of the antioxidant activities in the whole blood. The results indicated that L-carnitine will counteract the undesirable effects of lead intoxication. It exerted its antioxidant potential by reducing the production of ROS and scavenging free radicals by maintaining and protecting the level of the of antioxidant enzymes SOD, CAT and glutathione peroxidase.

Conclusion: L-Carnitine may play an important role in reversing the undesirable effects of lead intoxication. Future studies should be conducted to see whether such an effect is applicable in humans exposed to lead poising.

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1. Introduction

Lead toxicity has been recognized as a major environmental health hazard worldwide affecting both humans and animals at all ages especially young children in humans for a long time (Lalith Kumar and Muralidhara, 2014). Lead does not have any beneficial effects to humans, and its presence at high concentrations produce very undesirable toxic consequences to humans affecting all the body organs (Ibrahim et al., 2012; Markowitz, 2011; Mcguigan, 2012). The proposed mechanisms for lead toxicity involve fundamental biochemical processes including its ability to inhibit metabolic activities, mimic the action of calcium (which can affect calcium-dependent processes in the body) and interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups (ASTDR, 2007).

Lead toxicity results in reducing the levels of antioxidants in the blood as well certain enzymes like catalase and superoxide dismutase (Bennet et al., 2007; Singh et al., 2013). It also decreases the blood concentration of nitric oxide due to its interference with calcium dependent enzyme like the nitric oxide synthase and thus causes hypertension in animal models (Nava-Ruiz et al., 2012; Vaziri, 2008). Lead toxicity results in an elevation in malondialdehyde (MDA) levels (Dogru et al., 2007; Sharma et al., 2014). The catalase and superoxide dismutase (SOD) activities and MDA levels were significantly higher in animals drinking water contaminated with lead acetate as compared to the control (Soltaninejad et al., 2003). Plasma luteinizing hormone and follicle stimulating hormone concentrations were lower in rats exposed to lead resulting in a considerable decline in fertility status among those rats (Batra et al., 2004; Mendiola et al., 2011). Lead toxicity induced a significant decrease in plasma sex steroid testosterone in males and 17 beta-estradiol in animals (Ronis et al., 1998; Pillai et al., 2010). The effects of lead on the biosynthesis of steroid hormones by Leydig cells in rats were studied and the testosterone production was dropped by Leydig cells in rats exposed to lead (Huang et al., 2002).

Levo-carnitine (L-carnitine) is water soluble antioxidant located on the mitochondrial membrane and is found in all mammals (Brass, 2000; Dunning and Robker, 2012). L-Carnitine is derived from different types of foods (75%) and is synthesized by the body using various essential amino acids (25%) like Lysine and Methionine (İzgüt-Uysal et al., 2001). L-carnitine has very negligible health effects (Liu et al., 2015), and posses high antioxidant properties. As such, it is used in the prevention and treatment of oxidative stress and related health problems (Xiang et al., 2013; Ahmed et al., 2014). It helps in facilitating the transport of long chain fatty acids, through the mitochondrial membrane, used for the production of energy. It exerts its antioxidant potential by reducing the production of reactive oxygen species (ROS) and scavenging free radicals (İzgüt-Uysal et al., 2003; Li et al., 2012) by maintaining and protecting the level of the of antioxidant enzymes (SOD, CAT and glutathione peroxidase) (Gómez-Amores et al., 2007).

Histological data were obtained from a study on the protective effects of L-carnitine in Female Wistar-albino rats exposed to lead acetate (PbAc). It was found that animals exposed to PbAc caused a significant decrease in HB, HCT and RBC, a significant increase in WBC, AST, ALT and creatinine as compared to controls. The administration of L-carnitine did not reverse the effects on HB and HCT levels, while it corrected the decrease in RBC and the increase in WBC, AST, ALT and creatinine. These data showed that L-carnitine may reduce the severity of tissue damage caused by PbAc (Ozsoy et al., 2011).

The main objective of this study was to evaluate the underlying mechanism of the ameliorative effects of L-carnitine, using male Sprague–Dawley rats exposed to lead acetate.

2. Materials and methods

2.1. Experimental design

Animals: 12–16 weeks old 40 male Sprague–Dawley rats with an average body weight 140–190 g were used. Rats were obtained from laboratory animal research center, Faculty of veterinary medicine, Mansoura University, Mansoura, Egypt. Animals were housed in separate metal cages. The animals were left for 14 days for acclimatization before the beginning of the experiment. Clean drinking water was provided ad-libitum. Rats were kept at constant environmental and nutritional conditions (12-h light: 12-h dark and 23 + 2 °C, 60–65% humidity) for the duration of the experiment.

2.2. Compounds used

Lead acetate was obtained from El-Naser company, Egypt. L-carnitine was obtained from Mepaco company, Cairo, Egypt. Diet: The rats were put on a diet containing nutrients at these concentrations: protein (150 g/kg), Fat (50 g/kg) with a total caloric value of 3800–4100Kcal/kg.

The first group (G1) served as the control group and animals received standard diet only. The second group (2) received lead acetate in their diet at a concentration of 24 mg

/kg body weight daily for one month according to Polak et al. (1996).

The third group (G3) was the L-carnitine treated group and received the normal diet supplemented with L-carnitine at a dose of 100 mg/kg body weight daily for one month, While the fourth group (G4) consumed a diet supplemented with both lead acetate (dose of 24 mg/kg body weight and an L-carnitine (dose of 100 mg/kg body weight) daily for one month (Ozsoy et al., 2011).

Ethical approval: The protocol met the approval of the Institutional Animal Care and Use Committee at Mansoura University. The experiments were performed in strict compliance with the ethical guidelines for humane treatment of animals as set by the Mansoura University guidelines for the protection of animals used for experimental purposes.

2.3. Collection of blood samples

At the end of each experiment, animals were anesthetized and then sacrificed by decapitation in a very humane manner. Blood samples were then collected and divided into two parts: the first part was dispensed into heparinized tubes to prevent coagulation and used for the determination of reduced glutathione (GSH) (Beutler et al., 1963), Catalase activity (Fossati et al., 1980), and superoxide dismutase (SOD) activity (Nishikimi et al., 1972). The second part of blood sample was collected in sterile vial and centrifuged at 3000 rpm for 10 min for collection serum used for biochemical analysis of serum levels of Malondialdehyde (MDA) (Ohkawa et al., 1979), serum nitric oxide (NO) (Montogommery and Dymock, 1961) and serum testosterone level (Bayer, 1968).

2.4. Statistical analysis

The mean values and standard errors were calculated for the obtained data, and the significances for all means have been carried out by applying *F*-test using SPSS computer program. The values have been calculated according to Snedecor and Cochran (1989).

3. Results

3.1. Serum malondialdehyde (MDA) level

The mean value of MDA content in serum in-group (2) was (0.2 ± 0.01 μmol/L) which was higher than that of the control group (0.1601 ± 0.00023 μmol/L). The mean value of MDA content in serum in group (3) was (0.284 ± 0.00403 μmol/L) is lower than that of normal control animal (0.1601 ± 0.00023 μmol/L). The mean concentration of MDA content in serum in group (4) was (0.1746 ± 0.00174 μmol/L and was lower ($p \leqslant 0.05$) than that of group (2) as shown in Table 1.

Table 1 Effect of L-carnitine on serum MDA level in rats exposed to lead acetate toxicity (μmol/L).

Groups	Range		Mean ± SEM
	Minimum	Maximum	
Group1	0.159	0.162	0.1601 ± 0.0002333[a]
Group2	0.16	0.21	0.2 ± 0.00666[b]
Group3	0.01	0.041	0.0284 ± 0.00403[c]
Group4	0.169	0.183	0.1746 ± 0.00174[d]

Means with the same letter in each column are not significantly differed ($P > 0.05$).
Means with different letter in each column are significantly differed ($P \leqslant 0.05$).
SEM = Standard error of mean.
F-value = 368.69.

3.2. Serum nitric oxide (NO) level

The mean value of NO content in serum in-group (2) was (0.0588 ± 0.00444 μmol/L) which was lower than that of control group (0.4472 ± 0.00849 μmol/L). The mean value of NO content in serum in group (3) was (0.1104 ± 0.0004 μmol/L) and is lower than that of normal control animal (0.4472 ± 0.00849 μmol/L) as shown in Table 2. In group (4), the mean value for NO was (0.0464 ± 0.00535 μmol/L) and was lower than that of control as shown in Table 2.

3.3. Whole blood reduced glutathione (GSH)

The mean value of whole blood GSH content in group (2) was (0.3219 ± 0.00606 μmol/L) which was lower than that of normal control group (0.5275 ± 0.00357 μmol/L). The mean value of whole blood GSH content in group (3) was (0.3953 ± 0.00158 μmol/L) which is lower than that of normal control rats (0.5275 ± 0.00357 μmol/L) but higher than that of lead acetate treated rats (0.3219 ± 0.00606 μmol/L). In group (4) the mean value of whole blood GSH was (0.4366 ± 0.00229 μmol/L) which was lower than that of normal control rats (0.5275 ± 0.00357 μmol/L) but higher than that carnitine treated rats (0.3953 ± 0.00158 μmol/L) and lead acetate treated rats (0.3219 ± 0.00606 μmol/L) as shown in Table 3.

3.4. Whole blood catalase activity

The mean value of whole blood catalase content in group (2) was (0.2564 ± 0.0100 μmol/L) which was lower than that of the control group (0.8138 ± 0.0350 μmol/L). The mean value of whole blood catalase content in group (3) was

Table 2 Effect of L-carnitine on serum nitric oxide level in rats exposed to lead acetate toxicity (μmol/L).

Groups	Range		Mean ± SEM
	Minimum	Maximum	
Group1	0.41	0.479	0.4472 ± 0.00849[a]
Group2	0.045	0.08	0.0588 ± 0.00444[b]
Group3	0.11	0.114	0.1104 ± 0.0004[c]
Group4	0.025	0.065	0.0464 ± 0.00535[db]

Means with the same letter in each column are not significantly differed ($P > 0.05$).
Means with different letter in each column are significantly differed ($P \leqslant 0.05$).
SEM = Standard error of mean.
F-value = 1192.71.

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Table 3 Effect of L-carnitine on blood GSH level in rats exposed to lead acetate toxicity (mg/100 ml).

Groups	Range		Mean ± SEM
	Minimum	Maximum	
Group1	0.439	0.661	0.5275 ± 0.0357[a]
Group2	0.309	0.35	0.3219 ± 0.00606[b]
Group3	0.389	0.402	0.3953 ± 0.00158[c]
Group4	0.428	0.45	0.4366 ± 0.00229[cd]

Means with the same letter in each column are not significantly differed ($P > 0.05$).
Means with different letter in each column are significantly differed ($P \leqslant 0.05$).
SEM = Standard error of mean.
F-value = 22.19.

(0.4439 ± 0.00805 μmol/L which was higher than that of group (2) (0.2564 ± 0.0100 μmol/L) as shown in Table 4. In group (4) the mean value of whole blood catalase content was (0.3228 ± 0.0153 μmol/L which is higher than that of group (2) (0.2564 ± 0.0100 μmol/L) as shown in Table 4.

3.5. Whole blood superoxide dismutase activity (SOD)

In the present study the mean value of whole blood SOD content in group (2) was (0.2629 ± 0.0174 μmol/L) which was lower than that of normal control rats (0.3105 ± 0.0211 μmol/L). The mean value of whole blood SOD content in group (3) was (0.3105 ± 0.0154 μmol/L) which was higher than that of group (2) (0.2432 ± 0.0107 μmol/L) as shown in Table 5. In group (4) the mean value of whole blood SOD content was (0.2989 ± 0.0117 μmol/L) which is higher than that of group (2) (0.2432 ± 0.0107 μmol/L) and significantly lower than that of group (3) (0.3105 ± 0.0154 μmol/L) as shown in Table 5.

3.6. Serum testosterone

The mean value of testosterone levels in serum from group (2) treated rats was (0.664 ± 0.0397 μmol/L) which was lower than that of normal control rats (1.562 ± 0.0674 μmol/L). The mean value of testosterone content in serum in group (3) treated rats was (3.85 ± 0.302 μmol/L) which was higher than that of normal control rats (1.562 ± 0.0674 μmol/L) as shown in Table 6. In group (4) the mean value of testosterone in serum (5.598 ± 0.429 μmol/L) was higher than that of normal control group (1.562 ± 0.0674 μmol/L) as shown in Table 6.

Table 4 Effect of L-carnitine on erythrocytes catalase activity in rats exposed to lead acetate toxicity (μgm/ml).

Groups	Range		Mean ± SEM
	Minimum	Maximum	
Group1	0.74	1.02	0.8138 ± 0.0350[a]
Group2	0.23	0.312	0.2564 ± 0.0100[b]
Group3	0.41	0.47	0.4439 ± 0.00805[c]
Group4	0.237	0.43	0.3228 ± 0.0153[d]

Means with the same letter in each column are not significantly differed ($P > 0.05$).
Means with different letter in each column are significantly differed ($P \leqslant 0.05$).
SEM = Standard error of mean.
F-value = 152.115.

Table 5 Effect of L-carnitine on erythrocytes SOD activity in rats exposed to lead acetate toxicity (μgm/ml).

Groups	Range		Mean ± SEM
	Minimum	Maximum	
Group1	0.25	0.482	0.3756 ± 0.0211[a]
Group2	0.22	0.32	0.2432 ± 0.0107[b]
Group3	0.32	0.43	0.3105 ± 0.0154[c]
Group4	0.2	0.32	0.2989 ± 0.0117[d]

Means with the same letter in each column are not significantly differed ($P > 0.05$).
Means with different letter in each column are significantly differed ($P \leqslant 0.05$).
SEM = Standard error of mean.
F-value = 12.555.

4. Discussion

The results are consistent with what has been reported indicating that dietary supplementation of L-carnitine reduced malondialdehyde levels and provided a protective effect in rats exposed to lead (Löster and Böhm, 2001; Geng et al., 2004; Shokrzadeh et al., 2013). L-Carnitine attenuated free radical induced oxidative stress under various pathological conditions (Gülçin, 2006). It was hypothesized that L-carnitine may reduce intermittent hypoxia induced by oxidative stress and, thereby, improving the skeletal muscle performance, resulting in delaying muscle fatigue mediated by the reduction of free radical induced oxidative damage due to the antioxidant and anti-free radical activity of L-carnitine (Bin and Hussain, 2012).

The results concerning the inhibition of NO production in rats exposed to low levels of lead have been in concurrence

Table 6 Effect of L-carnitine on serum testosterone level in rats exposed to lead acetate toxicity (n mol/L).

Groups	Range		Mean ± SEM
	Minimum	Maximum	
Group1	1.36	1.86	1.562 ± 0.0674[a]
Group2	0.55	0.84	0.664 ± 0.0397[b]
Group3	3	5.47	3.85 ± 0.302[c]
Group4	3.18	6.52	5.598 ± 0.429[d]

Means with the same letter in each column are not significantly differed ($P > 0.05$).
Means with different letter in each column are significantly differed ($P \leqslant 0.05$).
SEM = Standard error of mean.
F-value = 70.72.

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with what has been reported earlier which indicated that such an effect resulted in increases in vascular resistance, decreases in renal blood flow and glomerular function and an enhancement of oxidative stress. It was suggested that lead-induced hypertension might be related to a decrease in NO and consequent vasoconstriction, rather than to a decrease in renal blood flow or to decreases in renal sodium (Dursun et al., 2005; Sun et al., 2005; Nascimento et al., 2014). It was reported that nitric oxide synthase activity in the hippocampus, cerebellum and cerebral cortex were inhibited by exposure to low levels of lead and the level of inhibition was time as well as concentration dependent (Dong et al., 2003). The data indicated that the antioxidant activity of L-carnitine and its derivatives resulted in decreased endothelial NO synthase (ecNo) gene expression. This corroborates what was reported by others where a direct stimulatory effect of L-carnitine on gene and protein expression of oxidative stress related markers like hemeoxygenase-1 (HO-1) and ecNO was reported. Hemeoxygenase-1 and ecNO are known antioxidants with both anti-proliferative and anti-inflammatory potential. Thus, their increased expression would be expected to provide a protective effect from oxidative stress related damage (Calò et al., 2006; Saeidnia and Abdollahi, 2013). L-carnitine kept NO in a reduced form which may have therapeutic potential (Dunlap et al., 2008). It was published that the prolonged critical illness, maintaining normoglycemia, and not glycemia-independent action of insulin prevented excessive systemic NO release on day 3 and appeared to preserve local endothelial function. Factors contributing to this finding may comprise direct endothelial damage, direct effects on the enzyme activity, decreased substrate availability or less NO-induced inhibition (Ellger et al., 2008).

The data showed that lead resulted in significantly decreasing the levels of reduced glutathione. These findings were in agreement with published work that showed that lead significantly decreased reduced glutathione (GSH)/oxidative glutathione (GSSG) and protein sulfhydryl groups (PSH)/glutathione–protein mixed disulfide (GSSP) ratio as well as glutathione reductase activities in a concentration-dependent manner (Chen et al., 2004; Suresh et al., 2011). It was reported that lead induced cell death involved in GSH deprivation with a significant decrease in the level of GSH, a critical intracellular antioxidant, observed at all the lead concentrations. These results suggested that the neurotoxic effect of lead may be mediated by apoptosis and prostaglandin E2 release, which could be potentially detrimental to neuronal survival. In another study, it was indicated that a reduction in the concentration of GSH in liver and kidney tissues after lead exposure resulted in a decrease in the liver and kidney concentrations of GSH due to oxidative action caused by lead (Jurczuk et al., 2006).

Acetyl L-carnitine protected against oxidative stress and was proposed as a therapeutic agent for several neurodegenerative disorders. Accordingly, it was suggested that treatment of astrocytes by acetyl-L-carnitine which induces hemeoxygenase-1 resulting in generating the vasoactive molecule carbon monoxide and the potent antioxidant bilirubin, which may explain the protective system potentially active against brain oxidative injury (Cao et al., 2014).

The data indicated that the catalase activity declined after an initial compensatory rise due to oral exposure of lead. Lead reduced the erythrocyte thiol content and antioxidant defense indicating possible role of free radicals in pathogenesis lead toxicity (López et al., 2007; Kasperczyk et al., 2014).

Published data indicated that the administration of L-carnitine resulted in elevation of antioxidant enzymes like catalase, SOD, and GSH (Ucüncü et al., 2006; Hisatomi et al., 2008). They reported that L-carnitine had an antioxidant potential, antioxidant defense enzyme in the cauda epididymis (Izgüt-Uysal et al., 2001). In another study, L-carnitine treatment increased catalase activity in both blood and gastric mucosa due to its ability to scavenge oxygen free radicals in mammalian tissues (Gómez-Amores et al., 2006; Li et al., 2014). Data reported in the literature indicated that propionyl L-carnitine (PLC) protected cells from toxic oxygen reactive free radical species and enhanced the catalase activity. The antioxidant capacity of PLC in spontaneously hypertensive rats and its beneficial use protecting tissues from hypertension-accompanying oxidative damage (Gómez-Amores et al., 2006). Other studies also reported that the antioxidant properties of L-carnitine proponyl and L-carnitine on spontaneously hypertensive animals prevented endothelial dysfunction through their antioxidant activities (Sleem et al., 2014).

The results indicated that lead resulted in a decrease in SOD activity, while an increase in this enzyme's activity was noted in rats fed with L-carnitine in their diet. It is well known that SOD represents the first line of defense against oxygen toxicity. It catalyzes the dismutation of superoxide anion producing hydrogen peroxide. These data are consistent with what has been reported in the literature whereby a significant decrease in SOD activity was noted in adult rats that received lead as lead acetate solution and that Lead induced oxidative stress in a dose dependent manner (Annabi Berrahal et al., 2007). Other studies confirmed those findings and reported a significant decrease in the activities of antioxidant enzymes such as erythrocyte-SOD and erythrocyte-catalase in battery manufacturing workers of Western Maharashtra (India) who were occupationally exposed to lead over a long period of time (Patil et al., 2006). A decrease in erythrocyte-SOD and erythrocyte-catalase activities had an adverse effect on heme biosynthesis and imbalance of pro-oxidant resulting in an increase in lipid peroxidation. L-Carnitine increased SOD levels and other antioxidant enzymes. These results were supported by those of others (Tan et al., 2008) who reported that the change in the antioxidant potential of retinal pigment epithelium cells was induced by an increase in SOD and GSH. It was indicated that L-carnitine enhanced T-cell proliferative responses, and may have a vital role in improving functions immune system cells particularly the lymphocytes due to its antioxidant activities. Also, L-carnitine had a radioprotective role in addition to its antioxidant abilities (Thangasamy et al., 2008). Studies on L-carnitine supplementation either individually or in combination with vitamin E reversed brain and retinal damage caused by radiation via increasing the activity of SOD and catalase enzymes in the brain (Sezen et al., 2008). L-Carnitine and alpha-lipoic acid seemed to have protective effects against oxidative damage in adjuvant arthritis model (Cabral et al., 2014).

The results indicated that lead acetate reduced the testosterone levels, while L-carnitine had an opposite effect. Published work on lead showed that it influenced Leydig cell

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steroidogenesis, which resulted in a reduction of testosterone levels resulting in a low sperm counts in both human beings and animals. Lead acetate significantly inhibited human chorionic gonadotropin (hCG) and dibutyryl cAMP (dbcAMP) stimulated progesterone production from 20 to 35% in MA-10 cells (Liu et al., 2003). A study on testosterone levels in serum of aged rats, showed a significant decrease in those levels in relation to age ($P \leqslant 0.05$). However, L-carnitine and L-arginine reversed this effect and returned testosterone levels near to the levels of that of the control in the young rats (Masi et al., 2003; El-Sayed et al., 2005). It was also found that acetyl L-carnitine protects against the decreases in dopamine and testosterone that normally occur after exposure to both acute and chronic stresses and decreases other markers of stress. These results are supported by Rani and Panneerselvam (2002) who found that neuro-protective effect on the brain in old rats was achieved by the elevation of antioxidants with L-carnitine. L-Carnitine has also antioxidant properties that protect sperm membranes against toxic reactive species and may preserve sperm membranes in roosters, thereby extending the life span of sperm and thus increasing male fertility (Neuman et al., 2002). It was reported that the level of free L-carnitine in seminal plasma, significantly correlated with sperm concentration, motility, and viability. As a result, L-carnitine levels can be taken as a biochemical index used for guidance for clinical treatment of male infertility as well as for studying the mechanisms of male reproduction (Banihani et al., 2014; Manee-In et al., 2014; Pons-Rejraji et al., 2014).

5. Conclusion

Based on the above, it would be obvious to conclude that L-carnitine has a protective power to counteract the effect of lead acetate in rats. Future study should focus on the use of L-carnitine in workers who are exposed to lead toxicity.

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