

Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

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To cite this article: A. Ozcan , E. Atakisi , M. Karapehlivan , O. Atakisi & M. Citil (2007) Effect of L-Carnitine on Oxidative Damage to Liver, Kidney and Spleen Induced by Phenylhydrazine in Mice, Journal of Applied Animal Research, 32:1, 97-100, DOI: 10.1080/09712119.2007.9706855

To link to this article: https://doi.org/10.1080/09712119.2007.9706855

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Published online: 14 Nov 2011.

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J. Appl. Anim. Res. 32 (2007) : 97-100

Effect of L-Carnitine on Oxidative Damage to Liver, Kidney and Spleen Induced by Phenylhydrazine in Mice[1]

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(Received August 3, 2006; accepted March 18, 2007)

Abstract

Ozcan, A., Atakisi, E., Karapehlivan, M., Atakisi, O. and Citil, M. 2007. Effect of L-carnitine on oxidative damage to liver, kidney and spleen induced by phenylhydrazine in mice. J. Appl. Anim. Res., 32: 97-100.

To investigate the ameliorative effect of L- carnitine on phenylhydrazine induced oxidative damage to liver, kidney and spleen, twenty-eight Swiss albino mice were divided into four groups and injected 0.9% NaCl (control), 40 mg/kg/day phenylhydrazine, phenylhydrazine+L-carnitine or 1000 mg/kg/day L-carnitine. Malondialdehyde (MDA) level was found to be significantly (P<0.001) lower in phenylhydrazine+L-carnitine group compared to the phenylhydrazine group in all tissues. Reduced kidney glutathione (GSH) level in phenylhydrazine given group was restored to normal by L-carnitine. However, increased uric acid level by phenylhydrazine was not decreased by L-carnitine administration. It appears that L-carnitine may prevent tissue damage and lipid peroxidation induced by phenylhydrazine.

Key words: Phenylhydrazine, L-carnitine, mice, reduced glutathione, malondialdehyde, uric acid.

Introduction

Hydrazine derivatives are widely used in industry as fuels, pesticides and explosives as well as in medicine for the treatment of tuberculosis, hypertension (Valenzuela and Guerra, 1985), antidepressant and antineoplastic agents. (Morris *et al.*, 1994). Interaction of phenylhydrazine with hemoglobin generates hydrogen peroxide and destroys the hemoglobin pigment through the formation of oxidized derivatives and free radicals of hydrazine. It is capable of inhibiting the function of liver microsomal cytocrome P_{-450} via the formation of free radicals. The formation of free radicals, during the microsomal oxidation of hydrazines, leads to the hypothesis that such free radicals are involved in the hepatotoxicity of hydrazine derivatives (Valenzuela and Guerra, 1985).

L-carnitine is essential for the normal oxidation of fatty acids by mitochondria (Seim and Löster, 1996). It decreases lipid peroxidation via transporting fatty acids into mitocondria for β-oxidation to generate adenosine triphosphate (ATP) energy

[1]The research work has been carried out with the approval of the Institutional Ethics Committee of Kafkas University, Faculty of Veterinary Medicine.

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J. Appl. Anim. Res. 0971-2119/2007/$5.00 © GSP, India.

(Neuman *et al.,* 2002). Phenylhydrazine causes haemolysis, leading to free iron increases in the blood and tissues (Karbownik *et al.,* 2000). This free iron is able to activate reactive oxygen species (ROS). L-carnitine has iron-chelating properties, which may partially prevent the generation of ROS (Neuman *et al.,* 2002).

This study aimed to search the oxidative damages probably occuring in the tissues of the liver, kidney and spleen after acute phenylhydrazine administration in mice and to evaluate the effect of L-carnitine on this damage.

Materials and Methods

Twenty-eight healthy Swiss albino mice, weighing 25-30 g, were randomized into four groups (control, phenylhydrazine, phenylhydrazine+L-carnitine and L-carnitine administrated). They were housed in stainless steel cages, in a well-ventilated room at 22-25C and 55% relative humidity with a 12 h light/dark cycle. They were allowed access to tap water and standard feed *ad libitum*. Phenylhydrazine and L-carnitine were obtained from Sigma-Aldrich Chemie, Germany. All mice were given intra peritoneal injection for 7 d 0.5 ml normal saline (control) or 40 mg/kg/day phenylhydrazine in normal saline or 40 mg/kg/d phenyl hydrazine in normal saline and 1000 mg/kg/day L-carnitine or 1000 mg/kg/day L-carnitine.

There after all mice were decapitated under ether anaesthesia and liver, kidney and spleen were excised immediately. The tissues were rinsed with ice-cold physiological serum solution followed by homogenization in phosphate buffer (prepared with 0.1 M KCl) in an ice bath. The homogenates were centrifuged at 1000xg for 15 min. Supernatants were acquired for biochemical analysis (Stocks *et al.,* 1974).

Uric acid level was measured by a commercial kit obtained from bioMérieux, France. GSH level was determined by the method of Beutler (Beutler *et al.,* 1963). MDA level was measured by method of Yoshoiko *et al.* (1979).

Data were analyzed for ANOVA and Duncan's test using SPSS 10.0 Windows programme.

Results and Discussion

Phenylhydrazine administered group showed increased MDA in all tissues suggesting that it may have caused oxidative stress in these tissues. The levels were brought down to some extent when L-carnitine was administered with phenylhydrazine (Table 1) indicating ameliorative effect of L-carnitine on oxidative damage caused by phenylhydrazine. MDA has been used as an indicator of lipid peroxidation in plasma.

Ferrali *et al.* (1997) reported that the MDA level was higher in phenylhydrazine given rat liver when compared to the control group, allegedly due to the increased content of free, redox-active iron. Karbownik *et al.* (2000) reported MDA level of liver and kidney homogenates were higher in a phenylhydrazine given group when compared to the control group.

Neuman *et al.* (2002) have reported that feeding young and aging White Leghorn roosters with dietary L-carnitine for 5 weeks improved sperm concentration and reduced sperm lipid peroxidation. Luo *et al.* (1999) reported that exogenous L-carnitine could attenuate the doxorubicin-induced lipid peroxidation in the heart tissue of the rat. The benefits of L-carnitine are postulated to be due to it's role in energy metabolism and antioxidant effects.

GSH was decreased in kidney of phenylhydrazine treated mice. It was almost normal when phenylhydrazine was given with L-carnitine and even increased when L-carnitine was given alone. However, GSH was increased in liver of phenylhydrazine treated mice. This high GSH level may show

Table 1
Glutathione (GSH), malondialdehyde (MDA) and uric acid levels (mean ± SE) in the liver, kidney and spleen of mice (n=7)

Biochemical parameters (g-wet tissue)	Control	Phenylhydrazine	Phenylhydrazine +L-carnitine	L-carnitine	P values
GSH_{liver} (mg)	0.10±0.018^{c}	0.25±0.017^{b}	0.27±0.013^{ab}	0.31±0.016^{a}	<0.001
GSH_{kidney} (mg)	0.24±0.035^{b}	0.08±0.029^{c}	0.23±0.014^{b}	0.32±0.024^{a}	<0.001
GSH_{spleen} (mg)	0.13±0.020	0.10±0.021	0.11±0.011	0.12±0.018	>0.05
MDA_{liver} (µmol)	0.32±0.020^{c}	0.79±0.040^{a}	0.61±0.046^{b}	0.27±0.021^{c}	<0.001
MDA_{kidney} (µmol)	0.33±0.018^{c}	0.44±0.015^{a}	0.39±0.018^{b}	0.35±0.011^{bc}	<0.01
MDA_{spleen} (µmol)	0.18±0.013^{c}	0.37±0.034^{a}	0.27±0.020^{b}	0.22±0.034^{bc}	<0.01
Uric $asit_{liver}$ (mg)	0.19±0.013^{c}	0.65±0.058^{a}	0.69±0.048^{a}	0.44±0.024^{b}	<0.001
Uric $asit_{kidney}$ (mg)	0.47±0.013^{b}	0.84±0.061^{a}	0.76±0.054^{a}	0.31±0.022^{c}	<0.001
Uric $asit_{spleen}$ (mg)	0.19±0.034^{b}	0.27±0.015^{a}	0.35±0.031^{a}	0.18±0.026^{b}	<0.01

^{a,b,c}Means with no common superscript differ (P<0.05).

that increased level of GSH synthesis in liver could be in response to the body's defence mechanism against toxication

GSH provides removal of H_2O_2. Decrease in GSH content results in free radical generation and the subsequent damage to mitochondria. L-carnitine has several effects on energy metabolism and cell structure. It increases GSH synthesis. Aging (Rani and Panneerselvam, 2001; 2002) and methamphetamine (Virmani *et al.*, 2002) studies demonstrated that L-carnitine reduced lipid peroxidation and increased GSH level, suggesting it's protective effect.

Uric acid provides an excellent example of the adaptation of the organism to oxidative stress. Urate, the physiological state of uric acid, reacts with hydroxyl radicals producing a stable urate radical that can be regenerated by ascorbate to it's prior state, urate. Urate also protects protein from nitration; it can chelate metal ions, such as copper and iron and prevent them from participating in redox cycling (Kohen *et al.*, 2002). Loots *et al.* (2004) suggested that a neurotoxic material was impared by acethyl-l-carnitine. In contrast to the findings of Loots *et al.* (2004), L-carnitine did not have any effect on increased uric acid level in phenylhydrazine given group in our study. The increases in the uric acid levels in the analysed tissues of the phenylhydrazine given group can be explained by it's synthesis against the oxidative stress in the organism.

To conclude L-carnitine may prevent tissue damage and lipid peroxidation induced by phenylhydrazine in mice.

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ए. ओज्कान, ई. अताकिसी, एम. कारापेहलिवान, ओ. अताकिसी, एम. सितिल। चूहों में फिनाइलहाइड्राजिन से प्रेरित यकृत, वृक और तिल्ली के आक्सीकृत क्षय पर एल-कार्निटीन का प्रभाव।

यकृत, वृक और तिल्ली के फिनाइलहाइड्राजिन प्रेरित आक्सीकृत क्षय पर एल-कार्निटीन के सुधारक प्रभाव को जानने के लिए 28 स्विस एल्बिनो चूहों को चार वर्गों में वितरित किया गया और नियामक में 0.9% सोडियम क्लोराइड, दूसरे में 40 मिग्रा/किग्रा/दिन की दर से फिनाइलहाइड्राजिन, तीसरे में दूसरे के साथ एल-कार्निटीन और चौथे में 1000 मिग्रा/दिन की दर से एल-कार्निटीन के इंजेक्शन लगाए गये। वर्ग 2 की तुलना में वर्ग 3 के चूहों के सभी ऊत्तकों में मैलोनडाइएल्डिहाइड का स्तर कम था। वर्ग 2 के चूहों में एल-कार्निटीन देने से वृकीय ग्लुटाथायान का स्तर घट कर सामान्य हो गया। परन्तु, इसमें फिनाइलहाइड्राजिन के कारण बढ़े हुए यूरिक अम्ल में कमी नहीं हुई। ऐसा लगता है कि एल-कार्निटीन से ऊत्तक क्षय और वसा का अति आक्सीकरण बचाया जा सकता है।