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Does L-Carnitine Prevent Cadmium-Induced Damage in Gastrointestinal Contractility and Histological Changes in Prepubertal Rat

¿La L-Carnitina Previene el Daño Inducido por Cadmio en la Contractilidad Gastrointestinal y los Cambios Histológicos en Ratas Prepúberes?

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SUMMARY: Cadmium (Cd) is the industrial and environmental toxic heavy metal which is found in air, water and soil. Cd, adversely affects many organs in humans such as kidney, intestine, liver, testis and lungs. L-carnitine (LC) is an important agent that plays essential role in energy metabolism. In our study, we aimed to work out whether LC application has any protective effect on intestinal contractility and morphologic damage of prepubertal rat duodenum on Cd-induced toxicity. Twenty eight prepubertal female Wistar rats were divided into four groups. The first group is control (C), second group; Cd group; Cadmium chloride was given 2 mg/kg 28 days with a one-day break by i.p. The third group; Cd+LC, which cadmium chloride was given 2 mg/kg i.p. and LC was given orally by gastric lavage. The LC dose was given as 75 mg/kg. The fourth group; LC, which only LC was given orally. The intestinal segments were isolated and suspended in tissue bath. Contractile responses were induced by acetylcholine (ACh) and relaxation was achieved with phenylephrine. Also the segments were examined for histological changes by light microscopy. Ach-induced contractions were higher in Cd+LC, LC, and control group compared to the Cd group in duodenal segments. The phenylephrine-induced relaxations were lower in Cd groups as compared with Control, Cd+LC and LC group in duodenal segments. In Cd group intestinal morphology was observed to be severely damaged whereas in Cd+LC group the damage was noticeably lower. Cd administration caused severe cellular damage and decreased gastrointestinal motility. Treatment with the LC has affected the gastrointestinal contractility and reduced the damage in intestinal morphology, which occured after Cd application.

KEY WORDS: Cadmium; L-Carnitine; Gastrointestinal contractility; Histological changes.

INTRODUCTION

Cadmium (Cd) is one of the most toxic and widely distributed heavy metals in the environment. Cd is usually present as inorganic salt. Cigarette smoking and ingestion of food contaminated with Cd is a major source of Cd exposure. Fish, meat, grain, cereal products, potatoes, leafy and root vegetable contains high levels of Cd. Since it is taken with food and water, it makes the gastrointestinal system the target organ of Cadmium where it can exert toxicities. Chemotactic cytokine MIP-2 and neutrophile infiltration is responsible for intestinal toxicity in response to a single oral administration of Cd to mice. The local effects of Cd intake were evaluated in the homogenates of duodenum, the intestinal region most reactive to Cd (Zhao *et al*., 2006).

L-carnitine is derived from endogen biosynthesis and dietary sources in human body. LC is a powerful antioxidant agent that plays a valuable role in energy metabolism. It was shown that LC was found to be an effective antioxidant when compared to other standard antioxidant compounds. It plays important physiological roletransferring the long-chain fatty acids across the inner mitochondrial membrane for b oxidations and ATP production in peripheral tissues (Gülçin, 2006). Reactive Oxygen Species (ROS) continuously produced during normal physiological events. They can easily initiate the peroxidation of membrane lipids. Antioxidants can protect the human body from free radicals and ROS effects.

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It was shown that dietary carnitine deficiency causes gastrointestinal contractility disorder and gastrointestinal discomfort and dysfunctions in hemodialysis patients (Irıe *et al*., 2017). Weaver *et al*. (1992) showed that dietary carnitine deficiency in infancy may cause smooth muscle dysmotility.

For the general population food and drinking water are the main sources of Cd exposure. For this reason the gastrointestinal tract is the first organ that is susceptible to Cd contamination. Although LC is co-factor in beta oxidation, there are many unknown effects on many organs and yet to be discovered function in physiology. However, the exact mechanism of the induction of intestinal inflammatory response to Cd largely unknown. There are not enough studies in cadmium-induced damage about intestinal function, gastrointestinal contractility and histological changes and the repairing effects of LC. Therefore, in this study, we aimed to investigate the effects of cadmium toxicity on intestinal contractility and morphologic damage of prepubertal rat duodenum and if there is any protective effects of LC application on cadmium toxicity. Also further studies are necessary to provide more information focusing on Cd toxicity and the effects of L-C on gastrointestinal contractility and histological changes

MATERIAL AND METHOD

This study has received the approval by the local ethics committee of Near East University (Date 18.11.2021, reference number, 2021-143). Healthy 21 days old prepubertal female Wistar rats (65-70 g) were housed under standard conditions. They were maintained using a 12 hr light/dark cycle and provided with commercially available rat chow and tap water ad libitum. Rats were randomly divided into four experimental groups. The first group was the control group (C), second experimental group was Cd group, which received $CdCl_2$ 2 mg/kg 28 days with a one-day break, i.p. (Karami *et al*., 2022), the third experimental group, was Cd+LC group, in addition to given Cd the animals received LC 75mg/kg by orally gastric lavage for 28 days and the fourth group, LC group, which received only LC by orally gastric lavage for 28 days.

At the end of 28 days the animals were anaesthetized with ketamine 90 mg/kg and ksilasine 10mg/kg i.p. Each of the duodenal tissue segments (0.3-0.5 mm long pieces) were surgically removed and then placed in petri dish containing Krebs solutions. Than the strips were suspended in tissue baths containing 20 ml of the Krebs-Hense-leit solution (mM NaCl 118.9, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 11), and a 95% O_2, 5% CO_2 mixture at 37 ºC, pH 7.4, was passed into the solutions. The segments were brought into equilibrium for 60 min under an optimal resting tension of 1g. After equilibration, the duodenum segments were contracted with ACh and relaxed with phenylephrine .The Ach ($3x10^{-7}$ M) and phenylephrine ($3x10^{-7}$ M) doses were considered as maximal doses, after the cumulative addition of ACh and phenylephrine into the control group. The acetylcholine (Sigma A6625), phenylephrine (Sigma P6126) and L-C (Carnitine; Santa Farma, Istanbul) and Cd (Sigma-Aldrich, USA) were obtained from Sigma chemical. Contraction and relaxation responses were recorded in the organ bath (May-ITBS 08).

Histological preparation: Duodenal segments were first fixed in 10% formalin and were embedded in paraffin, sectioned (5 μm) and stained with hematoxylin and eosin (H&E) for morphological analysis in light microscopy (Leica LAS EZ Version 3.00). Villous lengths were evaluated for each group in duodenum in four different area. Villous lengths were recorded using fiji/imagej program by measuring 5 different areas in x10 objectives from each group and evaluated statistically.

Cd injury was scored in each animal under light microscopy. The scores obtained from each rat were summed and averaged, thus obtaining a single mean duodenum score for each rat. Histological evaluation of intestinal tissues injury was performed based on the method described by Yulug *et al*. (2015). The injury was evaluated according to the following criteria: Grade 0- There was no specific pathological changes like villous epithelial degeneration, congestion-hemorrhage and increase in inflammatory cell. Grade 1- mild mucosal damage: degeneration of villous epithelium, otherwise normal structure. Grade 2- moderate damage: loss of villous height and epithelial degeneration with evidence of congestion, hemorrhage, and inflammation in the mucosa. Grade 3-severe damage: loss of a large number of villous, with the damage in the mucosa.

Statistical Analysis. All of the contraction, relaxation and histological results were statistically evaluated by the Graphpad Prism 8.3.1 program. One-Way Analysis of Variance test was used to statistically compare the differences among the groups, with multiple comparison using Tukey's test. Values was accepted statistically significant when $p<0,05$.

RESULTS

Muscle Function: The effects of LC on the cadmium-induced damage on gastrointestinal contractility and histological changes on duodenal segments were examined

in our experiments. The effects of LC on Ach-induced contraction in duodenal segments in all experimental groups are respectively exhibited in Fig. 1.

In Fig.1, it can be observed that ACh-induced contractions in Cd group significantly decreased as compared to Control ($p<0,05$), Cd+LC ($p<0,002$) and LC ($p<0,002$) groups in duodenum.

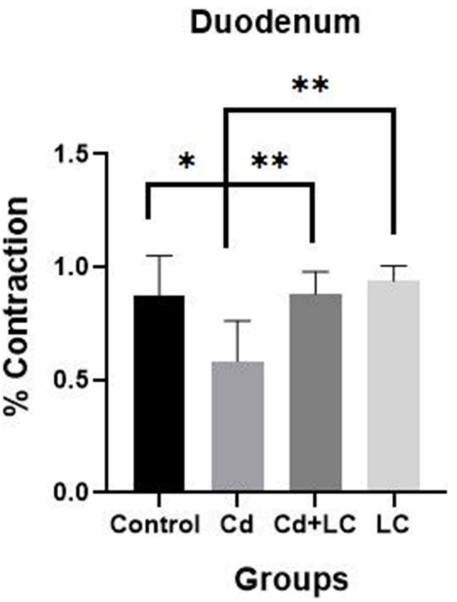

Fig. 1. The ACh-induced contractions on isolated duodenum in control and experimental groups. ***p<0,001, **p<0,002, *p<0,05

Although there was a decrease in phenylephrine-induced relaxation in the Cd group compared to the Control, Cd+LC and LC group, these data were not statistically significant in duodenal segments as seen in Fig. 2.

Histological Results: Fig. 3A, shows that the villous length and epithelial tissue in duodenum were normal in structure in control and L-C groups. No histological damage was observed in any rat in the control and L-C groups. In the Cd group; in duodenum, intestinal tissue morphologies were severely damaged and the villous epithelial cells were degenerate, exhibiting congestion, hemorrhage, and inflammatory cell infiltration which were seen in Fig. 3B. In the Cd+LC group, it was observed that, loss of villous height, epithelial cell degeneration, congestion and inflammatory cell infiltration reduced when compared to the Cd group (Fig. 3C).

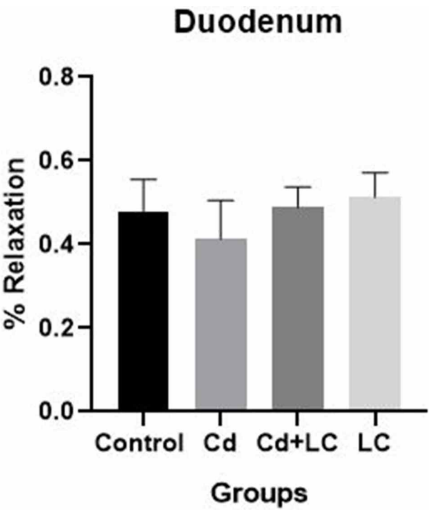

Fig. 2. Relaxations effects of phenylephrine on the control and experimental groups on isolated duodenum.

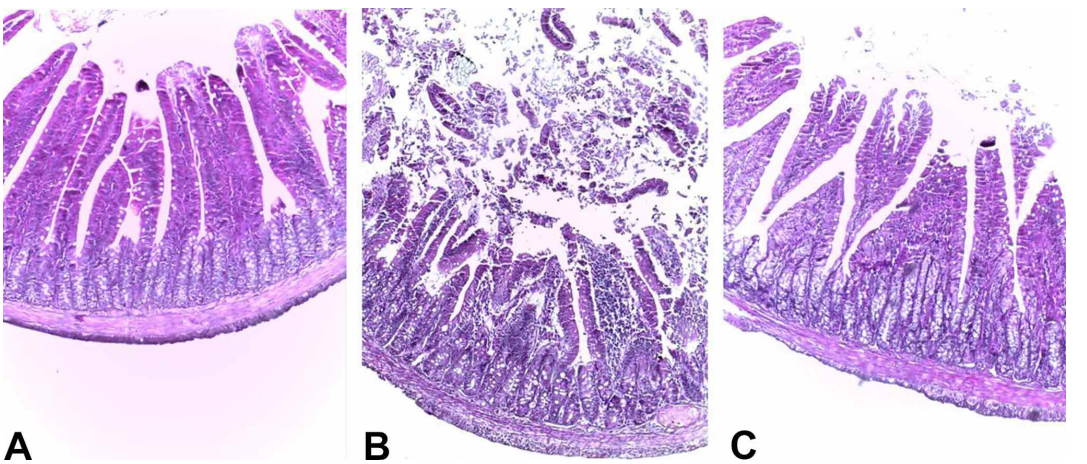

Fig. 3. Light micrographs of rat duodenum section in experimental groups. A. Control group of duodenum, the villous lengths are normal and there was not any degenerative changes, B. Cd group of duodenum, The villi structures were disrupted, and there was degeneration in epithelial tissue, C. IR+L-C group; the villous degeneration was lower than in the Cd group. Hematoxylin and eosin (x10).

The most extensive changes in morphology were detected in the Cd group, which was statistically different from the other groups; control or Cd+L-C and L-C groups. In histological score, no statistically significant difference was observed in congestion and inflammatory cell infiltration between all groups. Villous lengths were significantly lower in Cd group compared to the control group in the duodenum ($p<0,002$). In Cd +LC group villous lengths were significantly higher when compared to the Cd ($p<0,05$). In Cd group epithelial damages are significantly higher when compared to the control and LC groups (respectively $p<0,001$, $p<0,001$). In Cd+LC group damage was significantly lower compared to the Cd group ($p<0,05$) (Fig. 4A-D).

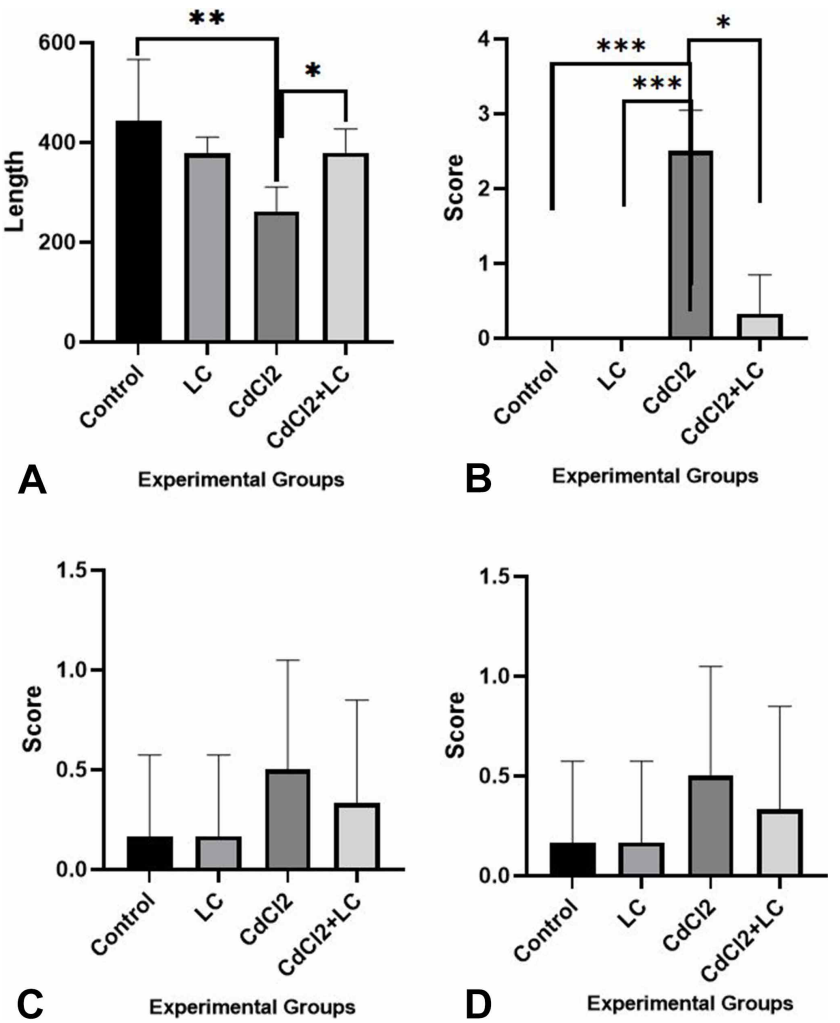

Fig. 4. A. Effect of L-Carnitine on the villus length in the control and experimental groups on duodenum. ***$p<0,001$, **$p<0,002$, *$p<0,05$. B. Effect of L-Carnitine on the epithelial tissue degeneration in the control and experimental groups on duodenum. ***$p<0,001$, **$p<0,002$, *$p<0,05$. C. Effect of L-Carnitine on the inflammatory cell infiltration in the control and experimental groups on duodenum. D. Effect of L-Carnitine on the congestion in the control and experimental groups on duodenum.

DISCUSSION

Our study demonstrated that subchronic Cd exposure for 28 days significantly decreased gastrointestinal contractility and caused histological damage. Treatment with the LC (for 28 days) cause partly repair on the cadmium-induced damage on the gastrointestinal dysmotility and histological damage in the duodenal segments. Cadmium is a natural element which is found as a mineral in combination with oxygen, chlorine or sulfur (Cuypers *et al*., 2010). At the cellular level, Cd induced oxidative stress in many organisms (Thévenod *et al*., 2009), which results in physiological damage in different organs (Nawrot *et al*., 2008; Järup & Akesson, 2009). It was shown that the effects of Cd on oxidative capacity are dual. First, Cd induce oxidative stress via the inhibitions of antioxidants, and second it also activates several antioxidative components as a result of disturbed redox balance and a consecutively induced signal transduction cascade (Cuypers *et al*., 2010). Since cadmium generally taken orally, gastrointestinal tract is the first organ to be directly contacted and affected. For this reason we aimed to investigate how the Cd toxicity affects the gastrointestinal contractility and histological changes with subchronic (28 days) i.p. intake of 2 mg/kg.

Our results showed that in the Cd-treated group, the contractile responses induced by ACh were significantly decreased in duodenal segments. Since the absorption of Cd mainly occurs in the small intestine (Zhai *et al*., 2016), it is expected to be effective on duodenal contractility as in our findings. It was shown that chronic administration by drinking water (one and two months) of Cd reduces the contractile response to ACh in duodenal segments (Koç *et al*., 2008). Our histological results showed that Cd caused substantial tissue damage in duodenum and LC has similarly helped limit the extent of tissue damage through its protective or healing effect and thus LC helped to prevent and reduce epithelial damage. The study of Bayazıt *et al*. (2002) which their results indicated that Cd exposure impairs

neurogenic and myogenic contractile activity in rat detrussor muscle was similar to our findings. It was shown that Cd intake causes degenerative changes such as damaged mitochondria, indentation of nuclear membrane. In morphometric data it was indicated that reduction in principles cells number and mildly dilated rough endoplasmic reticulum. Also, when only LC was given to the animal, their histological results similar to those of control on hippocampal structure (Amer *et al*., 2020).

Many studies have been shown that Cd can induce microvessel injury which is attributed to oxidative stress (Shukla *et al*., 1996; Wang & Du, 2013). Cell membrane damage like other organelles destruction are followed by an increased in the sodium permeability, which exceeds the capacity of ion pump to extrude sodium (Afifi & Embaby, 2016). Accumulation of sodium in the cell leads to an increased water content thus leading swelling.

Our findings are similar to Ninkov *et al*. (2015) results which they showed that the ingested Cd is retained in GUT mucosa, resulted in intestine tissue damage and inflammation. Our results showed that histological examination of Cd treated group has shown the presence of degenerative changes in the epithelial cells, the irregularity of villous length, epithelial cell damage, congestion and some groups there was increase in inflammatory cells.

It was shown that Cd exposure causes significant damage to the gut barrier, including the toxicity of enterocyte, induction of inflammatory response, death of epithelial cells and damage to the tight junctions in the intestine (Blais *et al*., 1999). However in the literature, contrary to our findings, it was shown that when cadmium selenite administrated for a period of 28 days in rats, there was no histopathological findings related cadmium selenite injury expect for one case of focal hepatic inflammations in high dose (1000 mg/kg) (Kim *et al*., 2009).

Although the toxicity caused by Cd is still being studied intensively, it mainly causes apoptosis when given at low or medium dose concentrations (Sancho *et al*., 2006). Also, exposure to Cd causes necrotic cell death, characterized by cell membrane disintegration followed by dissemination of intracellular contents (Sancho *et al*., 2006). There were many factors that affect cellular injury induced by Cd such as dose, route of exposure and duration of exposure.
It was shown that L-carnitine prevents oxidative stress and regulates nitric oxide, the cellular respiration (Brown *et al*., 1999) and the activity of enzymes involved in defense against oxidative damage (Kremser *et al*., 1995). The possible antioxidant effects of LC was shown in different in vitro antioxidant assays (Gülçin, 2006). Oral supplementation of L-carnitine to the patients receiving hemodialysis improved their gastrointestinal disorders (Irıe *et al*., 2017).

Our morphometric results showed that Cd decreased the measured parameters when compared to those of control group. The administration LC in association with Cd improved the measured parameters that approached the normal values of the control group. Although, in our study, we indicated that there were alterations in the intestinal motility and histological damage of intestine on Cd administration and the repairing effects of LC, the exact mechanisms responsible for these changes remain unclear. Many experiments claimed that the oxidant stress increased, possibly due to an elevated reactive oxygen species production or due to a decrease in the function of natural antioxidant pathways.

In conclusion, this study has demonstrated that treatment with LC can relatively relieve Cd-induced toxicity in gastrointestinal dysmotility and histological damage. Our findings suggesting that Cd plays a role on intestinal contractility and histological disorders and may have clinical implications for people who expose to cadmium.

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RESUMEN: El cadmio (Cd) es el metal pesado tóxico industrial y ambiental que se encuentra en el aire, el agua y el suelo. El Cd afecta negativamente a muchos órganos humanos, como los riñones, los intestinos, el hígado, los testículos y los pulmones. La L-carnitina (LC) es un agente importante que juega un rol esencial en el metabolismo energético. El objetivo de este estudio fue determinar si la aplicación de LC tiene algún efecto protector sobre la contractilidad intestinal y el daño morfológico del duodeno de rata prepuberal sobre la toxicidad inducida por Cd. Veintiocho ratas Wistar hembras prepúberes se dividieron en cuatro grupos. El primer grupo control (C), segundo grupo; grupo cd; Se administró cloruro de cadmio 2 mg/kg durante 28 días con un descanso de un día por vía i.p. El tercer grupo; Cd+LC, al que se administró cloruro de cadmio 2 mg/kg i.p. y LC se administró por vía oral mediante lavado gástrico. La dosis de LC se administró como 75 mg/kg. El cuarto grupo; LC, al cual solo LC se administraba por vía oral. Los segmentos intestinales fueron aislados y suspendieron en baño de tejido. Las respuestas contráctiles fueron inducidas por acetilcolina (ACh) y la relajación se logró con fenilefrina. También se examinaron los segmentos en busca de cambios histológicos mediante microscopía óptica. Las contracciones inducidas por Ach fueron mayores en Cd+LC, LC y el grupo control en comparación con el

grupo Cd en los segmentos duodenales. Las relajaciones inducidas por fenilefrina fueron menores en los grupos Cd en comparación con el grupo Control, Cd+LC y LC en los segmentos duodenales. En el grupo Cd se observó que la morfología intestinal estaba severamente dañada mientras que en el grupo Cd+LC el daño fue notablemente menor. La administración de Cd causó daño celular severo y disminución de la motilidad gastrointestinal. El tratamiento con LC afectó la contractilidad gastrointestinal y redujo el daño en la morfología intestinal, que ocurría después de la aplicación de Cd.

PALABRAS CLAVE: Cadmio; L-carnitina; Contractilidad gastrointestinal; Cambios histológicos.

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