Effect of L Carnitine against Mercuric Chloride-Induced Nephrotoxicity

Ali M Gado*[1], Abdel Nasser I Adam[2], Meshaal R. Alanazi [3], Majed M. Alqahtani [3], Fahad I. Alanazi[3], Faheid A. Almutairyi [3] and Khalid A. Almutairi [3]

Toxicology and Pharmacology[1], Physiology[2] and Clinical Pharmacy[3] Departments
College of pharmacy, Riyadh colleges of Dentistry and pharmacy, Riyadh, Kingdom of Saudi
daligado@yahoo.com

Abstract: The effects of L-carnitine (CAR) against nephrotoxicity of mercury, an oxidative-stress inducing substance, in rats were investigated. A single dose of mercuric chloride (5 mg/kg intra peritoneal injection) induced renal toxicity, manifested biochemically by significant increase in serum creatinine and blood urea nitrogen (BUN). Pretreatment of rats with CAR (200 mg/kg/day, ip), starting 5 days before mercuric chloride injection and continuous during the experimental period, resulted in a complete reversal of Hg-induced increase in creatinine and BUN to control values. Moreover, histopathological examination of kidney tissues confirmed the biochemical data, wherein pretreatment of CAR prevents Hg-induced degenerative changes of kidney tissues. These results indicate that AG is an efficient cytoprotective agent against Hg-induced nephrotoxicity.
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I. Introduction

Mercury is a hazardous environmental and industrial pollutant which induces severe alterations in the body tissues of both humans and animals[1,2]. The toxicity of mercury depends on the forms of the mercury compounds (elemental, inorganic and organic). Inorganic mercury accumulates predominantly in the kidneys causing acute renal failure.[3,4] The uptake, accumulation and toxicity of inorganic mercury in the kidney have been related to its binding to endogenous thiol-containing molecules.[5] Thiol-containing enzymes have been recognized as the targets of inorganic mercury.[5,6] Moreover, binding of mercuric ions to thiol groups may cause decreased glutathione levels, leading to increases in levels of reactive oxygen species (ROS), such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, which provoke lipid, protein, DNA and RNA oxidation.[7,8] Considering that oxidative stress and endogenous thiol depletion are involved in inorganic mercury toxicity, it has been suggested that antioxidants could contribute to the treatment of mercury poisoning.[9,10] In this way, melatonin, curcumin and vitamin E have been found to play a protective effect against mercuric chloride ($HgCl_2$) induced acute renal toxicity.[2,11-13] Similarly, a number of plant extracts with antioxidant properties have been shown to inhibit $HgCl_2$ induced renal toxicity.[14-16]

L-Carnitine (γ-trimethylamino-β-hydroxybutyrate) is synthesized in vivo from methionine and lysine. [17] It is assumed that in normal circumstances, the biosynthesis of L-carnitine is sufficient to meet metabolic requirements, though in several disease situations (apart from primary carnitine deficiency) oral L-carnitine supplements may be necessary as therapy .[18] The primary function of L-carnitine is to act as a carrier for translocation of long-chain fatty acids from the cytosol into mitochondria for β-oxidation, hence sustaining the supply of energy .[19] However, besides this well-known effect, there is growing evidence that L-carnitine also plays a role in other physiological processes in humans and animals. Indeed, L-carnitine act as very potent reactive oxygen species scavengers [20,21] and are known to have immunomodulatory properties in mammalian as well as avian species .[22]

L-carnitine has been known as a glucocorticoid mimicker because it activates the intracellular glucocorticoid receptor and modulates the expression of glucocorticoid-dependent genes during inflammation .[23-25] Glucocorticoids have a suppressive effect on the synthesis of pro-inflammatory cytokines by macrophages, and this effect was mimicked by L-carnitine. [22]

To the best of our knowledge, there are no studies concerning the nephroprotective effect of CAR against mercury intoxication. Therefore, the present study was carried out to investigate

(1) The adverse effect of acute mercury intoxication on the kidneys based on serum biochemical parameters, histo-pathological alterations and

(2) The possible mitigating effect of CAR against acute mercury intoxication in rats.

2. Materials and Methods

Chemicals

Mercury (Hg) in the form of mercuric chloride was purchased from CHEMA TEC CO. Alexandria, Egypt. L-carnitine was purchased from Sigma-Tau Pharmaceuticals, Pomezia, Italy. All other chemicals were of the highest grade commercially available.

Animals:

Male Swiss albino rats weighing 150-200 g were used in all experiments and obtained from animal house of College of Pharmacy, King Saud University. Animals were maintained under standard conditions of temperature & humidity with regular light/dark cycle and allowed free access to food (Purina Chow) and water. All animal experiments were conducted according to the regulations of the Committee on Bioethics for Animal Experiments of Riyadh colleges of dentistry and pharmacy

Animal Treatment:

The animals were divided at random into four groups of 5 animals each. The first group (control) received vehicles used for Hg (physiological saline solution, i.p). The second group, was injected with CAR (200 mg/kg i.p) for 10 days. [21] The third group was injected with mercury chloride (HgCl2) (5 mg kg-i p).[22] The fourth group, injected CAR (200 mg/kg i.p) for five days then injected with HgCl2 (5 mg kg- i p) and continued on CAR daily till the end of the experiment for one week. Then, blood samples were taken by cardiac puncture, under light ether anesthesia, into non-heparinized tubes. Serum was separated by centrifugation for 5 min at 1000 xg and stored at -20°C until analysis. Animals were sacrificed by cervical dislocation and the kidneys were quickly isolated, washed with saline, blotted dry on filter paper, weighed, and then 10% (weight/volume) homogenate of the left kidney was made in ice cold saline.

Measurement of serum biochemical parameters:

Serum creatinine and blood urea nitrogen concentrations were determined colorimetrically as described respectively, using commercially available diagnostic kits (bioMérieux-RCS Lyon-France).[26,27]

Histopathology

Histopathological examination was performed on the animals of each group. Right kidney samples were taken. The tissues were fixed for at least 48 hours in 10% formalin in phosphate buffer (pH 7). The samples were then embedded in paraffin wax, cut into 5 µm sections, and stained with hematoxylin-eosin. The slides were coded and were examined by histopathologist who was unaware about the treated groups.

Statistical Analysis

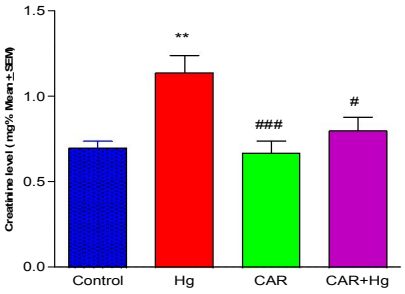
Data are expressed as mean ± standard error. Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test to judge the difference between various groups. Significance was accepted at $P < 0.05$.

3. Results:

Effects of CAR on Hg-induced changes in serum biochemical parameters:

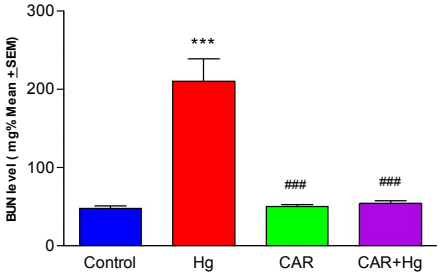
Serum creatinine and blood urea nitrogen (BUN) were significantly increased after injection of HG as compared with the control group ($P<0.001$) (Figs 1,2) . Pretreatment of animals with CAR (200 mg kg-1 day-i p.o) five days before and concomitantly with Hg markedly reduce significantly the rise in the level of BUN and creatinine.

Fig (1) Effects of CAR on elevated leve of serum creatinine induced by Hg



CAR (200 mg/kg/day ip) was given for 5 days before and concomitant with Hg
* Significantly different from control group #Significantly different from Hg
p. o per oral # P<0.05 ** P<0.01 ### P<0.001

Fig (2) Effects of CAR on elevated level of blood urea nitrogen induced by Hg



CAR (200 mg/kg/day ip) was given for 5 days before and concomitant with Hg
* Significantly different from control group # Significantly different from Hg
p.o per oral *** P<0.001 ### P<0.001

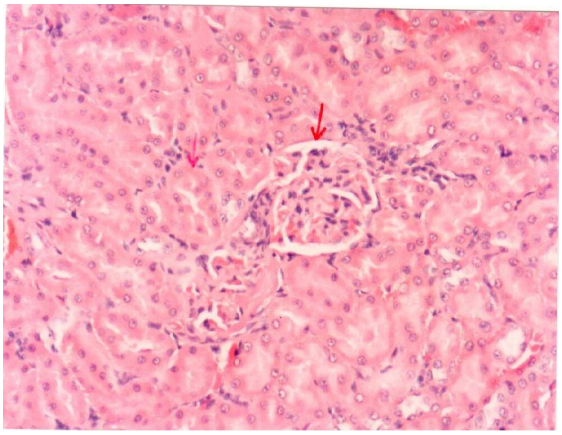


Fig. H 1: A photomicrograph of renal cortex of a control rat. The arrow is showing parenchyma with normal glomeruli and tubules (H&E...x200)

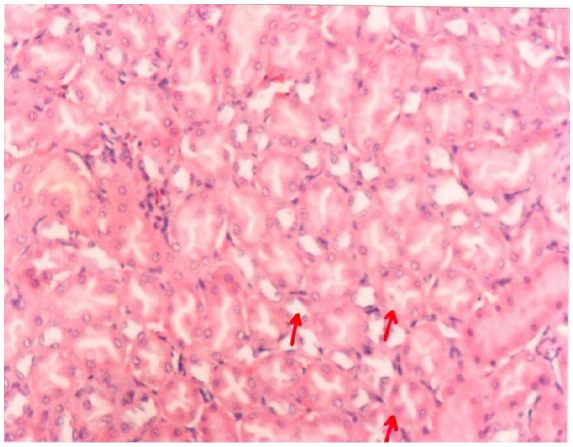


Fig. H2: A photomicrograph of kidney of CAR - treated rat. The arrow is showing cortical tubules and peritubular capillaries with no pathogenic changes (H&E...x200).

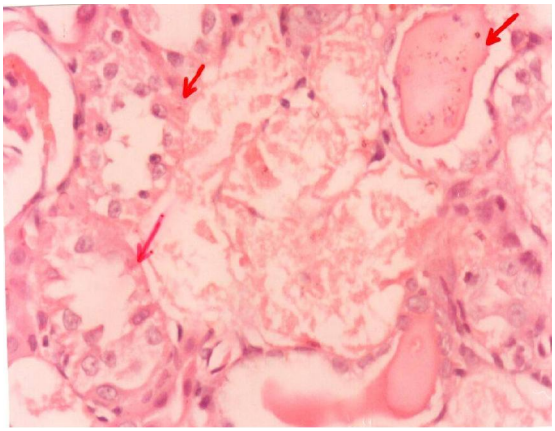


Fig. H3: A photomicrograph of kidney of Hg-treated rat. The arrow is showing necrotic changes of the renal tubular cells and some tubules contain casts (H&E...x400)

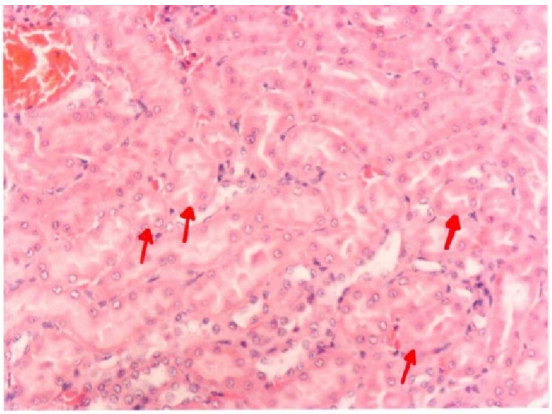


Fig. H4: A photomicrograph of kidney of CAR and Hg treated rat. The arrow is showing insignificant tubular epithelial changes in the form of cloudy swelling. (H&E...x200)

Kidney Pathology

Pathological examination of the kidneys of control, and CAR groups showed normal morphology of the renal parenchyma with well-defined glomeruli and tubules with non-significant changes (Figs. H1 and 2). However, animals treated with Hg showed clear signs of glomerular and tubular necrosis, interstitial nephritis and desquamation of the tubular epithelial cells in the renal cortex (Fig.H3). Interestingly, kidney specimens from rats treated with CAR and Hg revealed significant improvement in glomeruli and renal tubules, evidenced by less vacuolization and more preservation of tubular histology (Fig.H4).

4. Discussion

Mercuric ion, one of strongest thiol-binding agents, increases the intracellular levels of reactive oxygen species and induces oxidative stress resulting in tissue damage.[28-30] Toxicity of mercury is associated with superoxide radical generation and glutathione reduction.[31, 32]

Many studies demonstrated that the treatment of rats with $HgCl_2$, revealed a significant enhancement in TBARS levels indicative of the generation of lipid peroxides. Enhanced lipid peroxidation levels were also reported in mercury toxicity.[33,13] Mercuric chloride is known to increase the production of many reactive oxygen species (ROS) such as superoxide and H_20_2, which cause lipid peroxidation subsequently oxidative tissue damage.[34-36]

Endogenous glutathione has specific role in protecting the body from mercury toxicity due to its function as a carrier of mercury and its antioxidant properties. GSH binds with mercury, forms a complex that prevents mercury from binding to cellular proteins and subsequently causing damage to both enzymes and tissue.[37] Mercury poisoning leads to reduction of intracellular glutathione content and decrease the antioxidant potential of the cells. The present study revealed that mercury-treated rats showed a significant depletion of serum GSH levels. Agarwal *et al.* reported a significant reduction of GSH levels in liver, kidney and brain tissues.[12,13]

Alterations observed in the activity of Enhanced creatinine and BUN levels of mercury-exposed animals indicate indicate nephrotoxicity was also reported.[38] Histopathological alterations in kidney tissues after mercury exposure were revealed.[39-44]

Pretreatment with CAR attenuated the Hg-induced oxidative damage. The kidneys are the primary target organ for accumulation and toxicity of inorganic mercury.[5] In fact, during as little as 1 hour, 50% of an administered dose of inorganic mercury is present in the kidney.[45] Within the kidney, the majority of mercuric ions were detected in the cortex and outer stripe of the outer medulla. This finding was expected considering that the proximal tubule, which spans these

two renal zones, is the primary site of accumulation of mercuric ions.[5] The histopathological findings in the kidney tissue of Hg-treated rats include severe diffuse acute necrosis of tubular epithelium, fragmentation and shedding of tubular epithelium in the Iumina of the renal tubules and interstitial edema as a result of tubular leakage. The interaction of mercury with protein thiol groups is thought to play an important role in nephrotoxicity induced by mercury at cellular level.[5]

The results of this study indicate CAR improved Hg-induced nephrotoxicity which manifested by decease in both serum creatinine and urea levels and minimize the intensity of the renal lesions. The nephroprotective effect of CAR against many nephrotoxic agents was reported by several reports.[18,21, 46-51] The anti-oxidation induced by CAR might be one of the most likely mechanism contributing to its beneficial effect against renal injury. It could be suggested that CAR scavenges Hg free-radical generation and, in turn, inhibits lipid peroxidation–induced injury in renal tissues, which has been suggested to protect renal structure and function. Therefore, the protective effect is provided by CAR on renal tissue through antioxidants as well as by scavenging free radicals *in vivo*.

Conclusion

In summary our data indicate that Hg-induced nephrotoxicity is related to lipid peroxidation. Co-administration CAR provided protection against HG-induced nephrotoxicity possibly by inhibiting the free radical mediated process. These protective effects of CAR on renal injury-induced by Hg might have a considerable impact on developing clinically feasible strategies to treat patients with toxin induced renal failure.

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