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Original Article

Effect of Levo-Carnosine on Biomarkers of Oxidative Stress and Hepatotoxicity in Cisplatin-Treated Male Sprague Dawley Rats

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ABSTRACT

Objective: To determine the effect of Levo-Carnosine on biomarkers of oxidative stress and hepatocellular damage in cisplatin-treated rats.

Study Design: Laboratory based experimental study.

Place and Duration of Study: Army Medical College, Rawalpindi Pakistan from Jul 2015 to Jun 2016.

Methodology: This study was performed on ninety healthy male Sprague Dawley rats. Rats were separated into three groups (n=30 each), i.e., Group-I (control), Group-II (Cisplatin) and Group-III (Carnosine-Cisplatin combined) randomly. An Intraperitoneal injection of Cisplatin 2mg/kg body weight twice a week for one month was given to the rats of Group-II. In contrast, Group-III was administered a similar dose of Cisplatin as administered to Group-II plus intraperitoneal Levo-Carnosine 10mg/kg body weight twice a week for one month. After blood sampling, rats were dissected, and liver tissue samples were obtained for quantitative analysis of total glutathione from liver tissue homogenate.

Results: Levo-carnosine supplementation in Group-III showed a significant (p<0.001) decrease in mean serum MDA (4.0±0.30 µmol/L), ALT (52.3±4.3 IU/L) and AST (188.2±12.43 IU/L) levels as compared to Group-II (MDA 8.1±0.51 µmol/L, ALT 87.17±6.47 IU/L and AST 357.7±19.37 IU/L) whereas a significant (p<0.001) increase in plasma Albumin (3.9±0.46 g/100 ml) and Glutathione levels (3.86±0.44 µmol/L) in the liver homogenate as compared to Group-II (albumin 3.01±0.51 g/100 ml, glutathione 2.01±0.31 µmol/L).

Conclusion: Levo-carnosine supplementation significantly attenuated Cisplatin-induced hepatotoxicity by decreasing oxidative stress.

Keywords: Hepatotoxicity, Levo-carnosine, Oxidative stress.

How to Cite This Article: *Ali S, Rasul A, Latif N, Aleem SB, Zia R, Khan B. Effect of Levo-Carnosine on Biomarkers of Oxidative Stress and Hepatotoxicity in Cisplatin-Treated Male Sprague Dawley Rats. Pak Armed Forces Med J 2022; 72(4): 1334-1338. DOI: https://doi.org/10.51253/pafmj.v72i4.7815*

INTRODUCTION

In the living systems, oxygen undergoes metabolism to produce energy. Apart from energy production, oxygen metabolism also produces a small number of reactive oxygen species (ROS), which includes hydroxyl radical (OH), superoxide anion (O2–), peroxyl (ROO–), and hydrogen peroxide (H2O2), and hypochlorous acid (HClO).[1] Oxidative stress reflects either an overwhelming production of ROS or reduced natural antioxidants of the body.[2] Oxidative stress severely affects cellular homeostasis and interrupts cell proliferation, differentiation, and survival.[3] Cisplatin is a widely used antineoplastic drug for treating various malignancies in humans. The drug targets deoxyribonucleic acid (DNA), and its antitumor action is attributed to the hampering of DNA synthesis in rapidly dividing cancerous cells.[4] The clinical use of Cisplatin is also associated with the toxic insult to the normal tissues due to the development of oxidative stress. The drug binds with mitochondrial DNA (mDNA) and interferes with the production of electron transport chain proteins, resulting in premature leakage of electrons and increased formation of ROS.[5] This effect is more pronounced in highly metabolic cells of the liver, which makes this organ vulnerable to the toxic insult of cisplatin due to oxidative stress.[6]

Levo-carnosine is a naturally occurring cytoplasmic dipeptide (β-alanyl-l-histidine) predominantly found in muscles (skeletal and cardiac), cerebral tissue, liver, kidney, olfactory bulb and lens of various species of vertebrates, including human.[7] It is derived either from dietary sources or synthesized endogenously. Meat, fish and dairy products are the major dietary sources of carnosine. Levo-carnosine has effective antioxidant properties attributed to its strong capability to scavenge ROS and inactivate reactive aldehydes.[8] Due to its water solubility, Levo-carnosine provides a strong antioxidant defence system in the cytosolic environment of the cell, which contains high concentrations of oxidation mediators like transition metals and ROS.[9] Levo-carnosine stabilizes the structural and functional integrity of cell membrane by preventing lipid peroxidation of membrane lipids. In

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Received: 09 Dec 2021; revision received: 24 Mar 2022; accepted: 01 Apr 2022

addition to the quenching of already formed ROS, Levo-carnosine also increases levels of natural antioxidants of the body such as glutathione by forming adducts with reactive aldehydes and sparing glutathione from being consumed in neutralizing reactive aldehydes.[10]

Considering the above, this study was designed to ascertain the role of Carnosine supplementation in reducing oxidative stress and hepatotoxicity in laboratory animals treated with a Cisplatin chemotherapeutic regimen.

METHODOLOGY

This Laboratory based experimental study was conducted at Army Medical College, Rawalpindi, from July 2015 to June 2016. IERB permission was obtained from the Ethical Review Committee of the institute (ERC/ID/152). The study was conducted on ninety male Rats (Sprague Dawley) procured from the Animal House of the National Institute of Health (NIH) Islamabad.

Inclusion Criteria: Healthy rats with an average weight of approximately 250 ± 50 grams and an age range extending between 80-90 days were included in the study.

Exclusion Criteria: Rats suffering from liver disease (confirmed by serum ALT and AST levels at the start of the study by rat tail vein sampling) were excluded from the study.

The animals were kept in a separate room where temperature, ventilation and daily photoperiod of light and dark cycles were maintained per standard protocol.[11] Rats were separated into three groups (n=30 each), i.e. Group-I (control), Group-II (Cisplatin) and Group-III (Carnosine-Cisplatin combined) randomly. All groups were fed with standard chow and water as per recommended guidelines. Intraperitoneal injection of Cisplatin 2 mg/kg body weight twice a week for one month was given to the rats of group II. In contrast, Group III was administered a similar dose of Cisplatin as administered to group II plus intraperitoneal Levo-carnosine 10 mg/kg body weight twice a week for one month.

At the study duration, rats were anaesthetized and euthanized by dislocation of the cervical column. Blood samples were collected by intracardiac puncture and centrifuged in a cold centrifuge machine for 15 minutes at 4oC and 4000 rpm. Separated serum was used for quantitative analysis of MDA by ELISA on Stat Fax® 2100 Microplate Reader using TBARS assay kit, and bioassays of serum, ALT, AST and albumin levels on Vitalab Selectra E (automated clinical chemistry analyzer) using commercial kits. Following blood sampling, abdominal dissection of rats was carried out by midline incision. The liver of the animals was removed and washed thoroughly with icecold 1x phosphate buffered saline (PBS). To prevent coagulation of the organ, heparin (0.16 mg/ml) was added to the washing solution. The liver was allowed to dry, and then 10% (w/v) homogenate was prepared using a glass rod in the ice-cold medium in which pH 7.4 was maintained by adding 50 mmol Tris-HCl. Cold centrifugation of homogenate was done then the quantitative estimation of total glutathione was carried out from obtained sample by ELISA on Stat Fax® 2100 Microplate Reader using OxiSelect™ Total Glutathione (GSSG/GSH) assay kit.

Data analysis was carried out through IBM Statistical Package for the Social Sciences (SPSS) version 23.00. Mean±SD was calculated for numerical variables. The difference among groups was determined by applying a One-way analysis of variance (ANOVA), whereas a pairwise comparison of groups was made by application of the Post hoc Tukey test. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

The study was carried out on an animal model comprised of ninety male Sprague Dawley rats. The mean age of the rats in Group-I was 82.00±3.05 days, Group-II 81.03±3.19 days, and Group-III was 82.07±2.41 days. The age difference was statistically insigni-ficant (p=0.309) among the groups. The mean weight of the rats in Group-I was 268±7g, Group-II 272±6.01 g and Group-III was 271.07±7.11g. The difference in weight was statistically insignificant (p=0.06) among the groups.

A comparison of mean serum MDA, ALT, AST, Albumin and liver tissue total glutathione levels (Table-I) revealed a significant difference (p<0.001) among all three groups. The serum levels of MDA (8.1±0.51 µmol/L), (ALT 87.17±6.47 IU/L), and AST (357.7±19.37 IU/L) were found to be high (p<0.001), whereas, levels of albumin in the serum (3.01±0.51 g/100 ml) and Glutathione in the liver tissue (2.01±0.31 µmol/L) were significantly decreased (p<0.001) in the Cisplatin Group in comparison to the other two groups. Levo-carnosine supplementation showed a significant decrease (p<0.001) in serum MDA (4.0±0.30 µmol/L), ALT (ALT 52.3±4.3 IU/L) and AST

(188.2±12.43 IU/L) levels whereas, a significant increase (p<0.001) in serum albumin (3.9±0.46 g/100 ml) and total glutathione levels (3.86±0.44 µmol/L) of liver tissue homogenate in Group-III as compared to Group-II.

Comparison of parameters between Group-III and Group-I revealed that although the Levo-carnosine supplementation has significantly decreased serum MDA, ALT, and AST levels and significantly increased serum albumin and total glutathione levels of liver tissue homogenate in Group-III as compared to Group -II, these levels were still not consistent with the Group-I and showing a significant difference of p-value. Pairwise comparison between the two groups was presented in Table-II.

Table-I: Comparison of Serum Malondialdehyde Level, ALT, AST, Albumin, and Liver tissue total Glutathione levels among all three groups (n=90)

Variables	Group I (Control) (n=30)	Group II (Cisplatin) (n=30)	Group III (Carnosine-Cisplatin combined (n=30)	p-value
Serum Malondialdehyde Level (µmol/L)	3.40±0.40	8.10± 0.51	4.00± 0.30	<0.001*
Liver tissue Glutathione (µmol/L)	4.28±0.39	2.01±0.31	3.86± 0.44	<0.001*
Serum ALT (IU/L)	35.83±4.72	87.17±6.47	52.30±4.30	<0.001*
Serum AST (IU/L)	124.20±12.75	357.7±19.37	188.20±12.43	<0.001*
Serum Albumin (g/100 ml)	4.21±0.31	3.01±0.51	3.90±0.46	<0.001*

**p-value significant*

Table-II: Pairwise comparison of groups for Serum Malondialdehyde Level, ALT, AST, Albumin, and Liver Total Glutathione Levels.

Variables	Group I v/s Group II	Group II v/s Group III	Group I v/s Group III
Serum Malondialdehyde	<0.001	<0.001	<0.001
Liver tissue Glutathione	<0.001	<0.001	<0.001
Serum ALT	<0.001	<0.001	<0.001
Serum AST	<0.001	<0.001	<0.001
Serum Albumin	<0.001	<0.001	0.020

DISCUSSION

Oxidative stress has been coupled with more than 200 different diseases, including atherosclerosis, diabetic complications, hepatocellular injuries, renal tubular epithelial cells injuries, rheumatoid arthritis and myelosuppression. Oxidative stress-induced injury to the normal tissues is one of the frequently detected adverse outcomes associated with the Cisplatin chemotherapy regimen.[11,12] ROS-mediated cellular insult is attributed to the lipid peroxidation of polyunsaturated fatty acids of cell membranes leading to the impairment of normal cellular functioning. Increased lipid peroxidation products (serum MDA, 8-isoprostane, etc.) in the body are useful biochemical markers of oxidative stress.[13]

In the present study, we determined Levo-Carnosine's antioxidant effect on oxidative stress and hepatotoxicity biomarkers. In our study, Group-II showed significantly increased serum MDA levels compared to Group-I. Our results reflect Cisplatin-induced lipid peroxidation of membrane phospholipids and subsequent MDA formation. Elsayed *et al*. studied the Cisplatin-induced effect on serum MDA levels of control and Cisplatin admini-stered Group of rats.[14] Their work revealed signifi-cantly increased MDA levels in drug administered Groups which was consistent with our study. Levo-Carnosine co-administration with Cisplatin in Group-III of the present study has significantly lowered levels of serum MDA as compared to Group-II. Our results showed that Carnosine administration significantly ameliorated ROS-mediated lipid peroxidation of membrane phospholipids by reducing ROS formation and effectively removing already formed ROS, which reduced serum MDA levels. It is documented that Carnosine supplementation slowed the ROS-mediated lipid peroxidation in oxidative stress. Decreased syn-thesis of ROS, neutralization of already formed ROS, and restoration of antioxidant reserves contribute to the lowering of oxidative stress by carnosine.[15]

Xie *et al*. studied the antioxidant role of Carnosine administration in rats with intracerebral haemorrhage brain tissue. Their study revealed that Carnosine administration has significantly (p<0.001) attenuated cerebral oedema, disruption of the blood-brain barrier (BBB) and oxidative stress by decreasing the production of ROS. In addition, the authors have mentioned significantly decreased MDA levels and increased glutathione levels in Carnosine-administered rats, which were consistent with our study.[16] In another study, Aydin *et al*. mentioned the role of Carnosine

treatment on D-Galactose-induced oxidative stress in male rats. The authors showed a significant reduction in advanced Glycation end products (AGE) formation and MDA levels in the Carnosine-administered rats.[17] Their results were similar to ours, but in contrast to our study, they administered intraperitoneal Carnosine 250 mg/kg/daily (5 days/ week) for two months.

Oxidative stress weakens the antioxidant defence mechanism of the body by reducing natural antioxidants. This effect is reflected in the liver by decreased content of natural antioxidants, especially glutathione and its associated enzymes. Glutathione forms a major antioxidant defence system of the cell, which effectively scavenges ROS (OH- and O2–). It also reduces oxidative stress by serving as a cofactor in several antioxidant enzymes, such as glutathione peroxidase, which is an important mediator of H2O2 and lipid peroxidase neutralization in the body. Despite its exclusive synthesis in the cytosol, glutathione is transported in the mitochondria, which acts as an important regulator of redox balance, thus protecting the hepatocytes and other cells of the body from oxidative damage.[18] In the present study, we found significantly low ($p < 0.001$) glutathione levels in the liver of Group-II compared to Group-I. This finding was similar to that of Pınar *et al.* who evaluated the Alpha-lipoic acid on Cisplatin-induced hepatotoxicity in rats. They have observed significant histological damage to the liver, which was revealed by dilatation of perivenule sinusoid, congestion of central vein, inflammation of liver parenchyma and nuclear shrinkage and dissolution in hepatocytes along with a significant decrease in catalase and glutathione peroxidase levels in the Group which received cisplatin.[19] Levo-carnosine co-administration in the present study has significantly ($p <0.001$) increased glutathione levels in the liver of Group-III. The chemical structure of Carnosine offers preferred sites which readily react with lipid peroxidation products such as 4-hydroxynonenal (4-HNE) and MDA.[8] By decreasing free circulating reactive aldehydes, Carnosine spares GSH and therefore increases its level in the tissues. Aydin *et al*. studied the effect of Carnosine on oxidation and Glycation products in serum and liver of Streptozotocin-induced diabetic rats.

Their results showed that Carnosine administration had ameliorated hepatic dysfunction by decreasing the accumulation of oxidation and Glycation products in rats.[20] In another study, Kalaz *et al*. evaluated the role of Carnosine and Taurine supple mentation on D-galactose mediated oxidative stress and liver tissue damage. Their study revealed signifi-cantly raised total glutathione levels in the Group which received Carnosine along with D-Galactose as compared to the Group which received only D-Galactose. The result of their study was in accordance with the present study, but the Carnosine administration protocol was different from our study as the administered intraperitoneal Carnosine 250 mg/kg/daily; 5 days/week) for two months.[21]

Cisplatin-induced damage to the liver was manifested significantly in Group-II of this study. In this case, these cytoplasmic enzymes are raised in the serum due to structural damage to the hepatocytes caused by lipid peroxidation. Apart from elevated levels of ALT and AST, hepatotoxicity is also associated with increased serum calcium and decreased serum albumin levels. El-Gizawy *et al*. evaluated cisplatin-induced nephrotoxicity and hepatotoxicity in rats and the effect of Curcumin nanoparticles on these toxicities.[22] The treatment protocol of their study to produce hepatotoxicity was different (single dose Cisplatin 12 mg/kg body weight) from the current study; however, the results of their study are analogous to the present study.

In the present study, serum ALT and AST levels were significantly ($p<0.001$) decreased in Group III compared to group II. Our findings agreed with observations documented by Artun *et al.* who conducted a study to evaluate the effect of Carnosine pretreatment on oxidative stress and hepatotoxicity in binge ethanol administered rats. The authors mentioned significantly decreased oxida-tive stress markers and liver enzymes in the Carno-sine-treated Group.[23] This study showed that Levo-Carnosine co-administration with Cisplatin signifi-cantly ameliorates Cisplatin-induced oxidative stress and hepatotoxicity in laboratory animals.

LIMITATIONS OF STUDY

In this study, we could not measure the concentration of raised reactive oxygen species as it was beyond the scope of this study.

ACKNOWLEDGEMENTS

The authors are highly obliged to Dr Hussain Ali, Veterinarian, National Institute of Health, Islamabad, for his unconditional assistance in the animal handling phase of the research.

CONCLUSION

Levo-carnosine supplementation significantly attenuated cisplatin-induced hepatotoxicity by decreasing oxidative stress.

Conflict of Interest: None.

Author Contribution

SA: Design of the study, biochem evalution, result compilation, AR: Biochem evalution, data collection, NL: Data collection, result compilation, SBA: Statistical analysis, result compilation, RZ:, BK: Statistical analysis, discussion writing.

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