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Research Article
Antioxidant Effect of Carnosine on Aluminum Oxide Nanoparticles (Al_2O_3-NPs)-induced Hepatotoxicity and Testicular Structure Alterations in Male Rats

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Abstract

Background and Objective: Aluminum and its compounds like aluminum oxide (Al_2O_3) are common contaminants of water and food as well as medications and cosmetics. Carnosine (β-alanyl-L-histidine) is an endogenous dipeptide made up of amino acids, beta-alanine and histidine. The current study aimed to elucidate oxidative injury of nanoalumina (nanoparticles of aluminium oxide, Al_2O_3-NPs) with a diameter <14 nm (9.43±1.10 nm) and the ameliorative role of carnosine. This study investigated the protective effects of carnosine against Al_2O_3-NPs induced hepatotoxicity and testicular injury. **Materials and Methods:** Male rats were divided into four groups and were treated intraperitoneally for 4 weeks: 1st group control group given physiological saline (1 mL kg^{-1}), 2nd group received aluminum oxide nanoparticles (Al_2O_3-NPs) in a dose (6.4 g kg^{-1}), 3rd group was given carnosine (Car) (100 mg kg^{-1}), 4th group received Al_2O_3-NPs+Car and was given the same dose of Al_2O_3-NPs and followed by Car. The characterization of Al_2O_3-NPs were characterized by TEM. At the end of the experiment, some biochemical parameters were measured as TNF-α, α-FP, IL-2, IL-6, antioxidant capacities markers CAT, SOD, GPx, MPo, XO and thiol level and reproductive indices as 8-OHDG with histological, comet assay and TEM examination of liver tissues. **Results:** Al_2O_3-NPs induced significant elevation in lipid peroxidation (LPO) level and induced alteration in biochemical parameters in liver and testis, Al_2O_3-NPs induced elevation in liver enzymes ALT and AST but using of carnosine significantly decreased liver enzymes as compared to Al_2O_3-NPs group. Carnosine exhibited lipid peroxidation and repaired the antioxidant defense deficits (glutathione reduced, superoxide dismutase and catalase) in liver and testis tissues due to aluminum oxide nanoparticles treatment. There was an increase in hepatic myeloperoxidase following aluminum oxide nanoparticles exposure that were markedly mitigated by carnosine. Additionally, carnosine improved aluminum oxide nanoparticles-afforded liver and testicular tissue damage significantly as confirmed by microscopic studies in both liver and testis in group treated with combination of carnosine and aluminum oxide nanoparticles in comparison with aluminum oxide nanoparticles treated group alone. **Conclusion:** Carnosine was observed to be a potential nominee to protect the liver and testis versus the harmful effect of aluminum oxide nanoparticles toxicity and using of carnosine reduces the oxidative stress and biochemical alterations induced by Al_2O_3-NPs treatment.

Key words: Al_2O_3 nanoparticles, carnosine, oxidative stress, antioxidant defense, testicular damage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nanoparticles, which have one dimension at least which isn't more than 100 nm. Nanoparticles products are rising in different sectors such as in electronics and biomedicine[1]. There were 54 nanoparticle products commercially in 2005, but they reach 1814 products in 2014[2].

Nanotechnology is one of the quick rising areas of progressing technology, thus being a source of prospect for medicine and pharmacy. It is approved that, exactly metal oxides designed as nanoparticles will be used to soak medical devices and clothes, to fight viruses and bacteria, in new drug delivery systems or cancer therapy[3].

For the medicinal science to completely profit from the new nanotechnology accomplishments, it is serious to determine the nanoparticles penetration mechanisms into the cells, their action inside the cells, the accumulation degree in various organs of living organisms. It has been cleared that numerous efforts at elucidating the biocompatibility of nano alumina for animals and humans[3].

Globally, nanoparticles are one of the ultimate leading nanomaterial products used in numerous fields because of its hopeful technological applications[4]. Aluminium (Al) and nanoalumina (Al_2O_3-NPs) are exceedingly used in drug delivery systems. They are also used in surface coatings, batteries, fuels, car finishing, sunglasses and the ceramic industry [5].

Furthermore, it was reported that Al_2O_3-NPs promoted the anticancer activities of immunotherapy[6]. Regrettably, slight studies have reported that Al_2O_3-NPs treatment may drive to various dangerous effects, such as genotoxicity[7], inflammatory response[8], carcinogenicity, cytotoxicity[9] and mitochondrial dysfunction[10].

The liver is an essential metabolic organ responsible for keeping homeostasis all over the body. It has several major functions such as metabolism, detoxification of xenobiotics, storage of glycogen and production of bile, cholesterol and proteins. As bisphenol A (BPA) is primarily metabolized by the liver through glucuronic acid conjugation, this organ is more vulnerable to lower doses of BPA than other organs[11].

Alpha-fetoprotein (α-FP) was promised to get together the different kinds of neoplasms with the presence of these tumor markers[12].

Carnosine (β-alanyl-L-histidine) is numerously dispensed in skeletal muscles. There are numerous carnosine biological effects as an immune-organization, anti-aging and anti-neurodegenerative disease, depend on its anti-oxidative capacities. Studies have described that the essential antioxidant properties of carnosine comprise chelating metal ions, scavenging hydrogen peroxide and radicals and prohibition of advanced glycation end-products formation[13,14].

Particularly, carnosine has great capability for irrigating acrolein, which is one of the ultimate toxic aldehydes[15]. However, implementations of carnosine as with anti-oxidative properties are finite. To enhance the anti-oxidative capacity of carnosine, there was a study investigating the carnosine effects against cyclophosphamide (CTX) induced oxidative DNA injury.

Carnosine (Car), the endogenous dipeptide, involved in many biological processes, mainly due to its antioxidant effect[16]. Previous investigations showed that Car significantly protected against oxidative stress of various organs, including the liver. It protected against rat liver injury induced by CCl_4, diethylnitrosamine[17], lipopolysaccharide and ethanol[18], thioacetamide[19] and ischemia-reperfusion[20]. The Car also significantly ameliorated hepatic damage caused by acetaminophen[21], cadmium[22], high-saturated fat diet[23] and titanium dioxide[24] in mice. Another study showed that Car suppressed the invasion and metastasis of SK-Hep-1 cells[25].

A lot of efforts need to be done before the proposition of carnosine in alleviation of nanoalumina toxicity (Al_2O_3-NPs). There was a study examining the mode of action of carnosine with antioxidant enzymes, such as SOD, MPO, GPx, CAT and xanthine oxidase[26], which are involved in testis and liver disturbances due to Al_2O_3-NPs effect. The assembly between oxidative DNA injury and Al_2O_3-NPs exposure has not been investigated before so, the 8-OHDG was measured as DNA oxidation marker. The aims of the study were to inspect the Al_2O_3-NPs effects with carnosine exposure on liver and testis inflammation and the histopathological alterations examination.

MATERIALS AND METHODS

Chemicals: Al_2O_3-NPs and carnosine, 2′7-Di-chlorofluorescein diacetate were purchased from Sigma-Aldrich. Nanoalumina was 99.98% in purity, CAS number 1344-28-1, melting point 2.040°C and density 4.0 g cm^{-3}). Nanoalumina was in ultrasonicated form.

Characterization of nanoparticles: The surface morphology and composition of Al_2O_3-NPs particles were analyzed by Transmission Electron Microscopy (TEM) analysis. Al_2O_3-NPs particles were prepared on carbon-coated copper TEM grids. TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder.

Animals and experimental design: The experiment was carried out by using 40 male albino rats weighing 180-190 g were obtained from the animal house of Faculty of Pharmacy, Zagazig University, Zagazig, Egypt (November, 2017). The time of treatment was between 9 and 10 am daily for 4 weeks. The animals were treated in animal house of Faculty of Pharmacy, Zagazig University. They were maintained an air-conditioned room. The animals were adapted for 2 weeks prior to their use in experiments and were following the Ethical Committee of Faculty of Pharmacy, Zagazig University (ECAHZU).

Animals were divided into four groups, each of 10 rats each and were treated intraperitoneally for 4 weeks: Control group given physiological saline (1 mL kg^{-1}), aluminum oxide nanoparticles (Al_2O_3-NP) group received Al_2O_3-NP (6.4 g kg^{-1}) (equal 50% of LD_{50}) in physiological saline (Morsy *et al.*[27]). Carnosine (Car) group was given 100 mg kg^{-1} of carnosine (Deng *et al.*[28]) at 9.00 am every day. Finally, Al_2O_3-NP+Car group was given the same dose of Al_2O_3-NP and directed by Car.

Blood collection: Blood samples of the fasted rats were taken from the retro-orbital plexus immediately under ether anesthesia. Then, blood was centrifuged at 5000 rpm for 10 min and serum was collected for various biochemical analyses.

Cytokines and tumor biomarkers: Serum IL-2 and IL-6 were determined using Abcam's rat (ELISA) kits. The values were estimated as ng mL^{-1}.

The levels of α-TNF were estimated by an ELISA assay (ELISA kit, USA). Serum α-FP was estimated by Enzyme Linked Fluorescent Assay (AFP kits, France). α-FP and α-TNF were estimated as IU mL^{-1} and pg mL^{-1}, consequently.

Assay of DNA damage and testis injury: The levels of 8-OHDG were determined using ELISA kit (Eastbiopharm, China).

Preparation of tissue homogenates: Prior to dissection, liver tissues were immersed with a 50 mM sodium phosphate buffer saline (pH 7.4) and 0.1 M (EDTA) to remove red blood cells. The supernatant was relocated into Eppendorf and kept in a deep freezer till used for estimation of antioxidant enzymes and oxidative stress markers (non-enzymes).

Determination of oxidative stress: The MPO is a peroxidase enzyme that was estimated by using fluorometric assay kit. The values were measured by a unit of nmol min^{-1} mL^{-1} [29,30].

Determination of antioxidant enzymes: Xanthine oxidase (XO) was estimated and the absorbance was measured at 650 nm. SOD was estimated as by Marklund and Marklund[31]. It's activity is measured as nmol g^{-1} tissue. CAT was estimated as Aebi[32] and measured by U g^{-1} tissue. GPx was estimated as by Hafeman *et al.*[33] in mol g^{-1}.

Evaluation of non-enzymatic antioxidant: Thiol level was determined as by Hu[34] and results are expressed in mM g^{-1}.

Histological evaluation: For histological examination, the liver portion was fixed in 10% neutral buffered formalin and other processing described by Gabe[35].

Single cell gel electrophoresis (SCGE) (comet assay): Testis of groups were placed in a petri dish with an ice solution (Ca^{2+}-, Mg^{2+}-free HBSS with 20 mM EDTA and 10% DMSO). The cell viability of examining organs was determined by analyzing the images of comet after electrophoresis[36].

Statistical analysis: Data were expressed as mean values ± SE. Statistical analysis was performed using two-way analysis of variance (ANOVA) to assess significant differences among treatment groups. For each significant effect of treatment, the *post hoc* Tukey's test was used for comparisons. The criterion for statistical significance was set at $p<0.05$. All statistical analysis were performed using SPSS statistical version 20 software package (SPSS Inc., USA).

RESULTS

TEM of Al_2O_3-NPs particles: The observed micrograph showed synthesized Al_2O_3-NPs nanoparticles in the form of aggregates and agglomerates (Fig. 1).

Immunological, tumor and DND biomarkers: Figure 2 showed that carnosine or/and Al_2O_3-NPs affect on IL-2 and

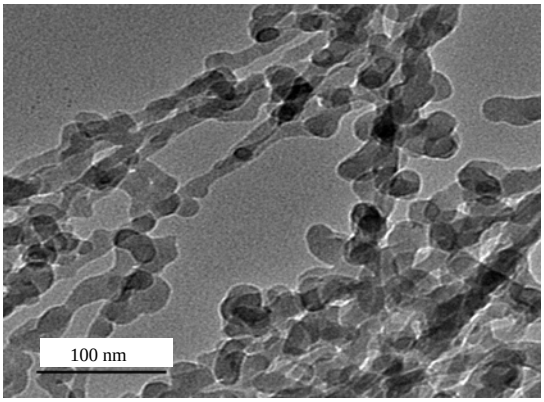

Fig. 1: TEM images of Al_2O_3

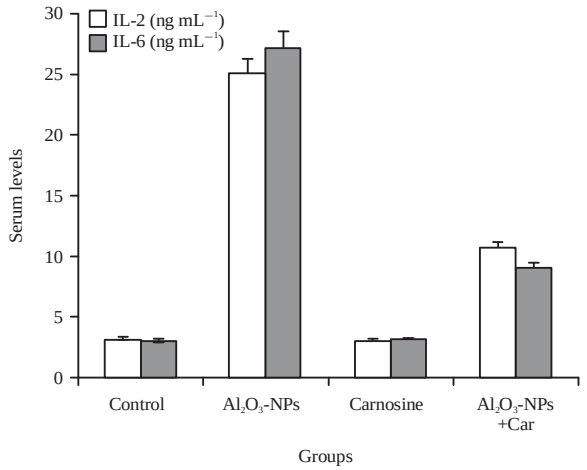

Fig. 2: Serum IL-2 and IL-6 levels of rats exposed to AL_2O_3-NPs (6.4 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group

AL_2O_3-NPs group was significantly higher ($p<0.05$) than the other treatments. Carnosine group was significantly lower ($p<0.05$) than the other treatments

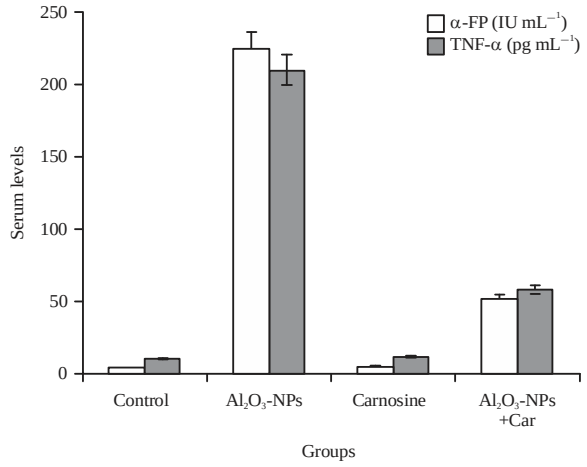

Fig. 3: Serum α-FP and TNF levels of rats exposed to AL_2O_3-NPs (6 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group

AL_2O_3-NPs group was significantly higher ($p<0.05$) than the other treatments. Carnosine group was significantly lower ($p<0.05$) than the other treatments

IL-6 levels. Al_2O_3-NPs treated group elevated the IL-2 and IL-6 levels in comparable to control group. While, the rats treated with carnosine in combination with Al_2O_3-NPs declined IL-2 and IL-6 by 70.13 and 65.30% in comparable to Al_2O_3-NPs-treated group.

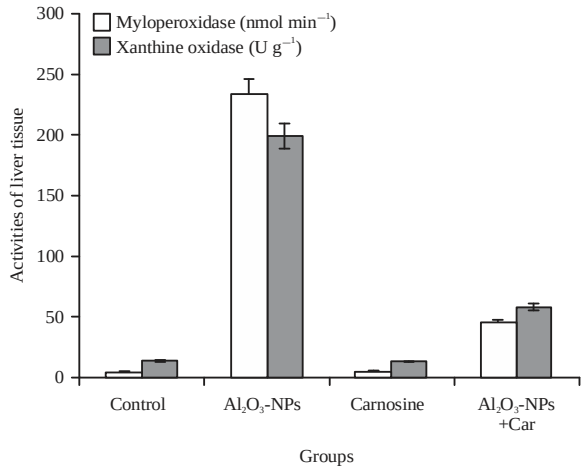

Fig. 4: Activity of MPO and XO of liver tissues of rats exposed to AL_2O_3-NPs (6.4 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group

AL_2O_3-NPs group was significantly higher ($p<0.05$) than the other treatments. Carnosine group was significantly lower ($p<0.05$) than the other treatments

The Al_2O_3-NPs elevated α-FP and α-TNF levels in comparable to control group. While, carnosine combined with Al_2O_3-NPs declined their levels in comparable to Al_2O_3-NPs group (Fig. 3).

The Al_2O_3-NPs elevated the activities of MPO and XO in comparable to control group, respectively (Fig. 4). The concurrent administration of carnosine and Al_2O_3-NPs decreased the activity of MPO and XO as compared to Al_2O_3-NPs group.

Testis oxidative damage marker: The serum 8-OHDG level increased by 2-fold in the Al_2O_3-NPs group in comparable with control group (Fig. 5). However, the group treated with carnosine combined with Al_2O_3-NPs had declined 8-OHDG level in comparable to Al_2O_3-NPs group.

Oxidative/antioxidant parameters: The lipid peroxidation (LPO) level was elevated by 89.25 fold in Al_2O_3-NPs group in comparable to control group (Fig. 6). Carnosine and Al_2O_3-NPs treated group had declined the LPO by 71.51% in comparable to Al_2O_3-NPs group.

In Fig. 7, obtained results cleared that Al_2O_3-NPs treatment elicited a remarkable decline in SOD activity of liver tissues. Carnosine administration did not afford any alterations in hepatic SOD activity in comparable to control group. Additionally, a remarkable

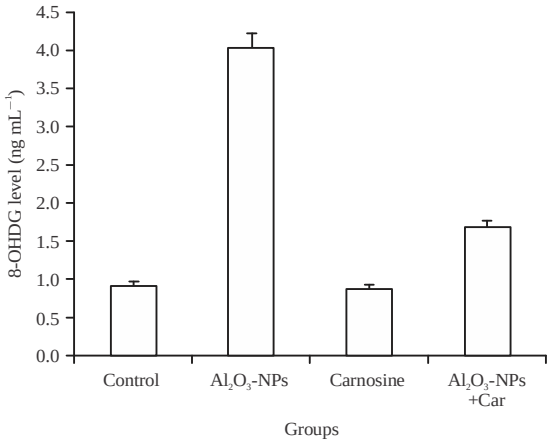

Fig. 5: 8-OHDG level of rats exposed to AL_2O_3-NPs (6.4 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group.

AL_2O_3-NPs group was significantly higher ($p<0.05$) than the other treatments. Carnosine group was significantly lower ($p<0.05$) than the other treatments

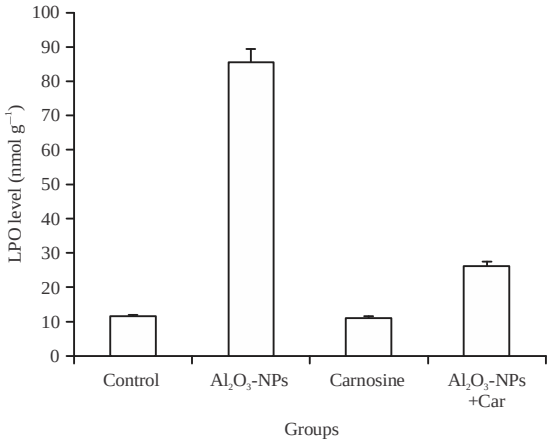

Fig. 6: Liver LPO level of rats exposed to Al_2O_3-NPs (6.4 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group

AL_2O_3-NPs group was significantly higher ($p<0.05$) than the other treatments. Carnosine group was significantly lower ($p<0.05$) than the other treatments

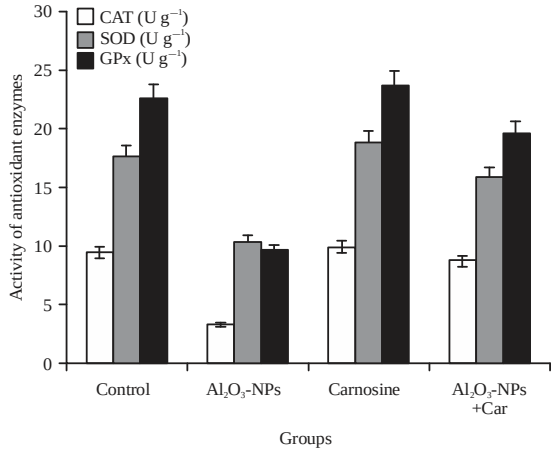

Fig. 7: Activities of some antioxidant enzymes of liver tissues of rats exposed to AL_2O_3-NPs (6.4 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group

Carnosine group was significantly higher ($p<0.05$) than the other treatments. AL_2O_3-NPs group was significantly lower ($p<0.05$) than the other treatments

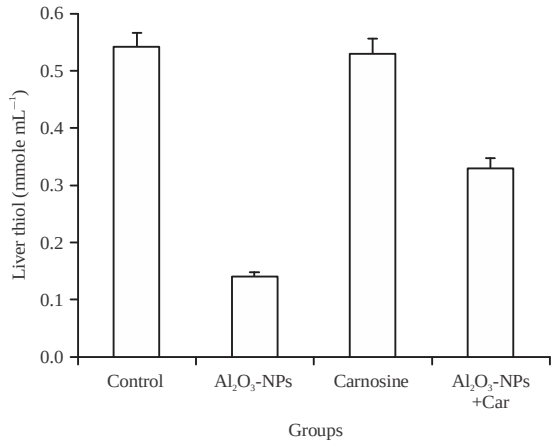

Fig. 8: Liver thiol of rats exposed to Al_2O_3-NPs (6.4 g kg^{-1} b.wt) or/and carnosine (100 mg kg^{-1} b.wt) for 4 weeks. Values are expressed as Means±SE, n = 10 for each treatment group.

Carnosine group was significantly higher ($p<0.05$) than the other treatments. AL_2O_3-NPs group was significantly lower ($p<0.05$) than the other treatments

recovery depending on SOD activity was spotted due to a combination of carnosine with Al_2O_3-NPs.

The CAT activity declined after Al_2O_3-NPs administration (Fig. 7). The treatment with carnosine combined with Al_2O_3-NPs markedly elevated hepatic CAT activity in comparable to Al_2O_3-NPs group.

The GPx activity was markedly declined in hepatic tissues of rats treated with Al_2O_3-NPs by 57.77% in comparable to the control group (Fig. 7). Carnosine treatment did not induce any remarkable alterations in enzyme activity, while the presence of carnosine with Al_2O_3-NPs diminished the noticed modification in hepatic enzymatic activity elicited by Al_2O_3-NPs.

Combination of carnosine with Al_2O_3-NPs treated rats ameliorated the level of thiol by 21.81 fold in comparable to Al_2O_3-NPs group (Fig. 8).

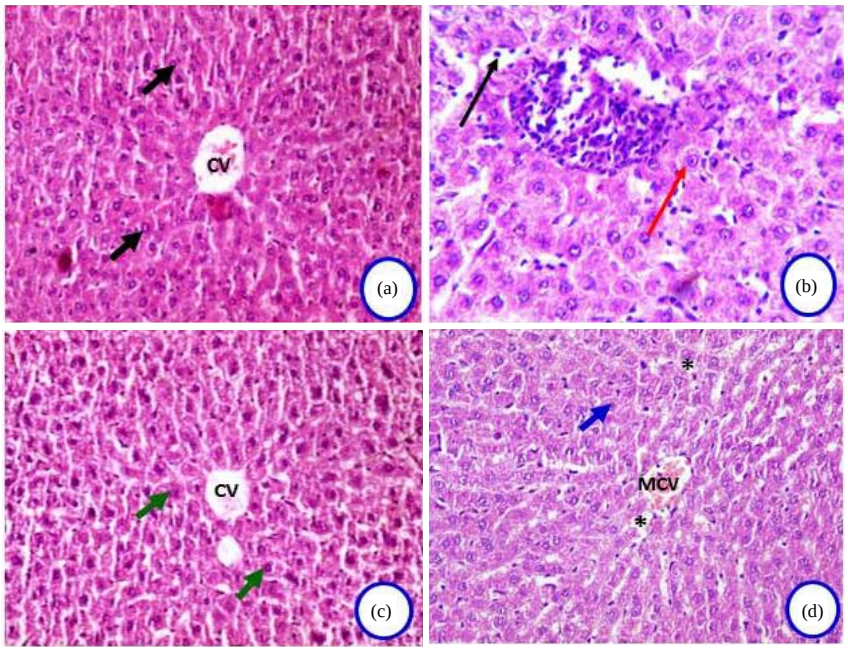

Fig. 9(a-d): Representative light micrographs of (a) Liver of control rats showing normal central vein (CV) with normal hepatocytes (black arrow) (400X) (b) Group treated with Al_2O_3-NPs showing focal coagulative necrosis (black arrow) replaced by mononuclear cell aggregation with proliferation of the Von Kuppfer cells (red arrow) (400X), (c) Group treated with carnosine showing normal hepatic structures (green arrow) with normal central vein (CV) and (d) Group treated with combination of Al_2O_3-NPs and Car showing almost restoration of hepatic cell structures (blue arrow) with mild congested vein (MCV) and appearance of little hydropic changes(*)(400X)
All the sections have been stained with H and E

Histological changes: The histological examination of liver sections of a control group and sections of rats treated with carnosine (Fig. 9a and c) showed the appearance of normal vintage hepatic lobules. Hepatocytes were polyhedral shaped, with boundaries defined pettishly. They had an acidophilic cytoplasm and central rounded nuclei with 1-2 nucleoli. Sinusoids were filled with flattened endothelial cells and Kupffer cells. Group treated with Al_2O_3-NPs showing a highly congested central vein (HCV) with the appearance of focal inflammation (FI) (Fig. 9b). Group treated with carnosine showing normal hepatic structures with normal central vein (CV) (Fig. 9c). Group treated with a combination of Al_2O_3-NPs and carnosine showing almost restoration of hepatic cell structures with mild congested vein (MCV) and appearance of little hydropic changes(*) (Fig. 9d). Sections stained with hematoxylin and eosin (H and E).

The histological appearance of the testicular tissues of the control group was normal in appearance (Fig.10a). Sections when stained with H and E showed cells of spermatogonia, spermatocytes and rounded spermatids, bundled with multi-layers in seminiferous tubules of the control group. Carnosine-treated rat was also normal seminiferous tubules with normal spermatogenesis (Fig. 10b). It was observed that there was a marked increase in necrotic and degenerative changes in germinal cells of rats that received Al_2O_3-NPs (Fig. 10c). The well-defined germinal cells with the reduction in necrosis, edema and congestion were found in the group that received Al_2O_3-NPs and carnosine (Fig.10d).

Comet assay: Comet of testis cells of the control group which showed intact nuclei and normal round cell without a tail (Fig.11a). Al_2O_3-NPs which showed a higher degree of damage with the appearance of more than one apoptotic cells with a large tail and small head and the relaxed loops of damaged DNA extend to the anode to form a comet-shaped structure (Fig.11b). The carnosine-treated group showed intact nuclei with undamaged DNA (Fig. 11c). Al_2O_3-NPs+carnosine showed amelioration of the cells as recorded fewer parameters in the tail length and percentage of damaged DNA and tail with more percent of intact cells with undamaged DNA and fewer numbers of comet cell (Fig. 11d).

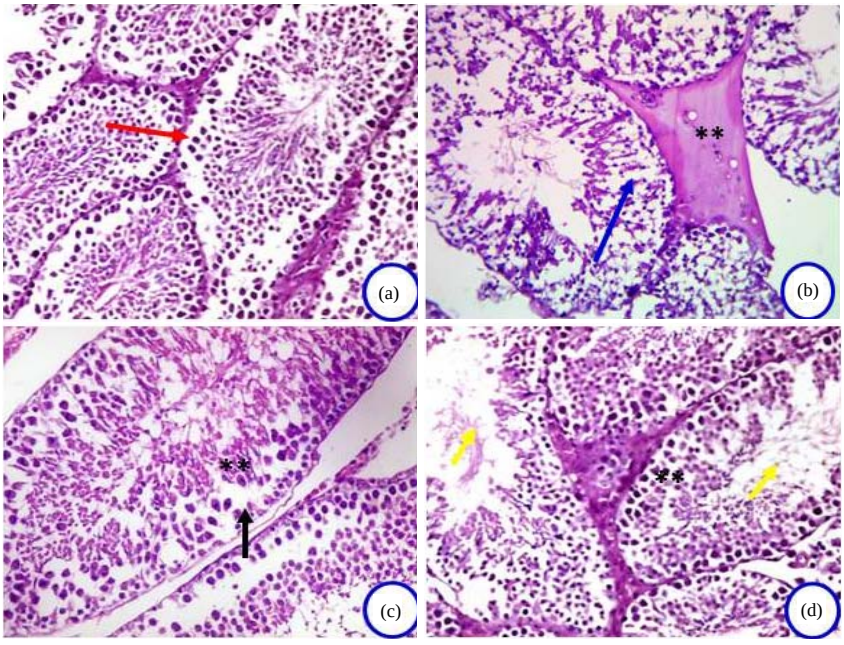

Fig.10(a-d): Representative light micrographs of (a) Testis of control rats showing normal histological structure (testis seminiferous tubules lined with stratified germinal epithelial cells (red arrow), spermatogonia primary spermatocytes, secondary spermatocytes and spermatids that rested on clear basement membranes) (400X), (b) Group treated with Al_2O_3-NPs showing necrotic germinal epithelium (blue arrow) with marked intertubular interstitial edema(**) (400X), (c) Group treated with carnosine showing normal seminiferous tubules (black arrow) with germinal layers and spermatozoa(**) (400X) and (d) Group treated with a combination of Al_2O_3-NPs and carnosine showing restoration of germinal layers(**) with spermatozoa (yellow arrow) (400X)

DISCUSSION

The current study inspected the conservative effects of carnosine on aluminum elicited oxidative injury in male albino rats' liver. During the phase of the study, rat did not display any relevant alterations marking that there was no toxicity due to carnosine giving and less significant oxidative stress in the group treated with a combination of Al_2O_3-NPs and carnosine.

Liver injury was elicited by Al_2O_3-NPs. The results showed an increment of oxidative damage level and decline in the antioxidant status in Al_2O_3-NPs group. Carnosine afforded reduction in oxidative damage markers, 8-OHDG, α-FP, interleukins and other enzymatic antioxidants were markedly elevated. Thus, this research clears that carnosine displays preservation against the liver injury by Al_2O_3-NPs.

The α-FP was elevated by Al_2O_3-NPs giving but reduced by combination f Al_2O_3-NPs and carnosine. The α-FP is a marker of tumor induction in hepatic cancer, however, Wilder *et al.*[37] found that the increased α-FP serum in lung cancer is likely to perform a metastasis.

Currently, 8-OHdG levels were elevated in Al_2O_3-NPs treated group which revealed the presence of DNA damage due to Al_2O_3-NPs. 8-OHdG represents oxidative stress marker, particularly to DNA, which is involved in diverse disease investigation[38].

In the current study, MPO was elevated in rats due to Al_2O_3-NPs while, decreasing in the group treated with a combination of Al_2O_3-NPs with carnosine. MPO employs H_2O_2 to manufacture hypochlorous acid (HClO) that interacts with proteins and unsaturated fatty acids[39].

The liver is an essential metabolic organ responsible for keeping homeostasis all over the body. As Al_2O_3-NPs is primarily metabolized by the liver through glucuronic acid conjugation, this organ is more vulnerable to lower doses of Al_2O_3-NPs than other organs[11].

Contrary to the obtained results, It was reported that nanoalumina does not afford apoptosis of BJ cells, although it can infiltrate into cells. As predicted, the increment in aluminium oxide nanoparticles concentration resulted in a remarkable elevation of aluminium content in the cells[40].

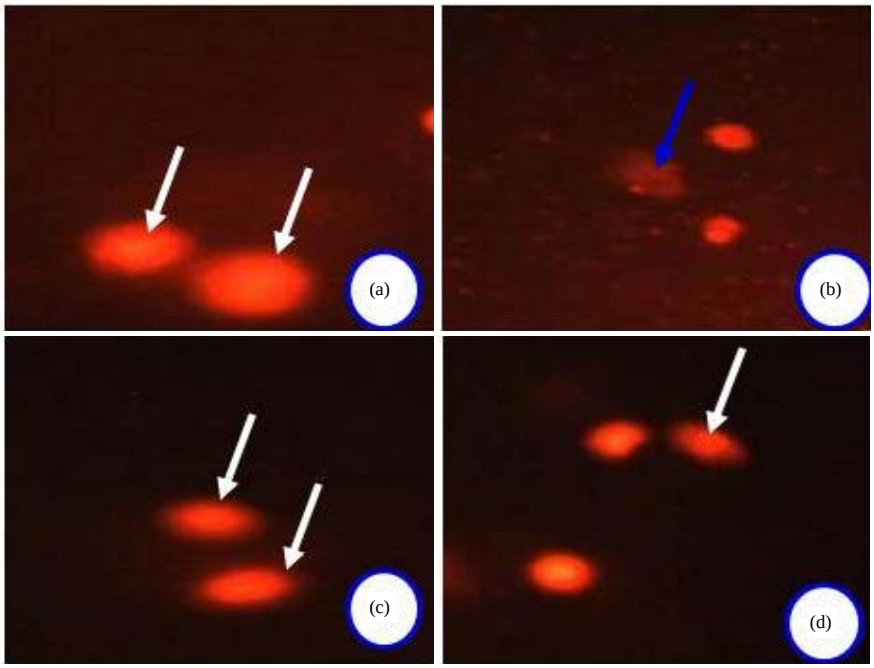

Fig.11(a-d): Comet images of (a) Cells derived from testis of the rat of the control group which showed intact nuclei and normal round cell without a tail (white arrow) (b) Al_2O_3-NPs which showed a higher degree of damage with the appearance of more than one apoptotic cells with a large tail and small head (blue arrow) and the relaxed loops of damaged DNA extend to the anode to form a comet-shaped structure (c) Carnosine-treated group showed intact nuclei (white arrow) with undamaged DNA and (d) Al_2O_3-NPs+carnosine showed amelioration of the cells as recorded fewer parameters in the tail length and % of damaged DNA and tail with more percent of intact cells with undamaged DNA and fewer numbers of comet cell (white arrow)

The current study cleared that co-giving of carnosine with Al_2O_3-NPs during treatment inhibited the oxidative injury effects by decreasing marker of lipid peroxidation, MDA and increasing antioxidant enzyme activities SOD, CAT and GPx. Similarly to results of Bommavaram *et al.*[41], who reported that BmGNPs prohibited the increment of TBARS content due to aluminum-administration. It is clear that natural products from natural extracts are with antioxidant characteristics can preserve tissues against free radicals injury[42].

It is apparent that aluminum is a pro-oxidant and enhances biological oxidation[43]. The current results reported that Al_2O_3-NPs induced significant reduction in CAT, GPx and SOD with elevating MDA level while, these levels were decreased greatly in group treated with combination of Al_2O_3-NPs and carnosine. Aluminum increases lipid peroxidation and cause depletion in levels of GPx and CAT. The reduced antioxidant against oxidant ratio plays a conclusive role in triggering oxidative damage[43].

Evidences have shown that an uncontrolled inflammatory cascade and generation of Reactive Oxygen Species (ROS) are involved in hepatic tissue remodelling and fibrosis[44]. On these bases, hepatic fibrosis are a likely consequence of Al_2O_3-NPs -induced liver injury. Therefore, this study aimed to explore the hepatotoxic effect of Al_2O_3-NPs after long-term exposure in male rats and to underline molecular mechanisms and the possible ameliorative effect of Res.

Accumulation of reactive oxygen species in the Al_2O_3-NPs group leads to lipid peroxidation and this consequence resulted in oxidative injury. The increment in MDA of the liver noticed in the current study may reveal that the liver disability to trigger ROS produced by Al_2O_3-NPs and/or antioxidant enzyme inactivation caused by the excess triggering of hepatic reactive oxygen species. Moreover, they obtained results elucidated that Al_2O_3-NPs giving to rats results in an increment of lung NO levels. Lipid peroxidation can be used as an indicator of oxidative stress and alteration of cell membrane permeability[42].

Cells utilize diverse antioxidant mechanisms to decrease ROS excess levels. In this study, elevated oxidative stress can demonstrate the relevant decrement of oxidative stress

hepatic enzyme markers as GPx, SOD and CAT[42], that offered lung free radicals scavenging exposed to Al_2O_3-NPs.

The GSH preserves the cell from oxidative injury by deteriorating peroxide radicals (OH·). However, antioxidant enzymes react against radicals and H_2O_2, while GPx has a scavenging effect against peroxyl radicals[45]. Low levels of SOD and CAT in BPA group could increase-oxidative stress and may drive to liver injury.

The histopathological study showed relevant morphological alteration in the liver of Al_2O_3-NPs -group and confirmed the biochemical alterations. This may be due to the liberation of α-TNF, types of IL and free radicals and therefore, inducing liver structure alterations and damage of the liver.

The obtained results are greatly reinforced by Deng *et al.*[28], who reported that carnosine treatment (100 and 200 mg kg^{-1}) markedly prohibited the liberation of reactive oxygen species and (8-oxo-dG),and declined bone marrow chromosomal aberrations in animals treated with CTX (20 mg kg^{-1}). Carnosine obviously mitigated CTX that afforded bone marrow cells arrest. These results together propose that carnosine can preserve bone marrow cells from CTX-elicited DNA damage through its antioxidant capacity.

The anti-oxidative capacities of carnosine have been catching attention. The carnosine giving spectacularly declined reactive oxygen species and 8-oxo-dG levels in bone marrow of CTX-group and this advocate the current finding. Though, a preceding study demonstrated that different types of antioxidants failed to minimize the oxidative DNA injury of 8-oxo-dG[46]. Diverse studies have reported that carnosine has the ability to react with α,β-unsaturated aldehydes and this is convenient with the current results.

Carnosine consist of an amino group of the β-alanyl residue and the imidazole ring of L-histidine and it also has been revealed that it acts synergistically when bound as a dipeptide[47].

The decrement of 8-OHDG, a biomarker of oxidative DNA damage, in the Al_2O_3-NPs+carnosine group compared to the Al_2O_3-NPs group, also proved confirmation for the antioxidant and free radical-scavenging activities of carnosine. The obtained finding are harmonious with the current studies which found that some active compounds have been playing an efficient role in diminishing oxidative DNA damage *in vivo* and *in vitro*[48].

Furthermore, the NO high concentration in inflammation and the prevention of iNOS might be an essential anti-inflammatory mechanism which may assigned to carnosine[49].

In the present study, GPx, CAT, SOD and G6PD, which altered after exposure to nicotine, were markedly elevated by carnosine administration. Preceding studies had also shown that carnosine has antioxidant activities[50].

CONCLUSION

Al_2O_3-NPs administration caused a remarkable increment in lipid peroxidation and markedly reduced hepatic antioxidant enzymes, the biochemical mediators induced liver disorders (IL-6, IL-2, α-FP and α-TNF), also induced histopathological alterations. The administration of carnosine combined with Al_2O_3-NPs ameliorated liver and testis injury, which was related to enhance all the biochemical parameters which revealed the capability of carnosine to preserve liver functions and reproductive capacities.

SIGNIFICANCE STATEMENT

This study discovers the hepatoprotective and testicular protective effects of carnosine against oxidative stress induced by nanoaluminia Al_2O_3-NPs particles. The current study reported a novel mechanism of ameliorative effect of carnosine against especially testicular alterations induced by Al_2O_3-NPs with oxidative stress and hepatotoxicity. Administration of carnosine with Al_2O_3-NPs decreased TNF-α and α-FP and elevated antioxidant enzyme biomarkers as SOD, CAT, GPx and decreased lipid peroxidation marker MDA with decreasing 8-OHDG. Therefore, these results clearly demonstrated that carnosine supplementation protected against Al_2O_3-NPs alteration injuries and also improved their reproductive capacities with enhancing hepatic function biomarkers which improved the protective role for carnosine against hepatotoxicity and reproductive toxicity induced by nanoaluminia Al_2O_3-NPs particles.

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