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Prophylactic administration of carnosine and melatonin abates the incidence of renal toxicity induced by an over dose of titanium dioxide nanoparticles

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Abstract

The alleviative effects of two antioxidants, carnosine (Car) and melatonin (Mel), against titanium dioxide nanoparticles (TiO_2-NPs) toxicity-induced oxidative and inflammatory renal damage were examined in rats. Administration of these antioxidants along with TiO_2-NPs effectively reduced serum urea, uric acid, creatinine, glucose, tumor necrosis factor-α, interleukin-6, C-reactive protein, immunoglobulin G, vascular endothelial growth factor, and nitric oxide, as well as a significant amelioration of the decrease in glutathione levels in renal tissue was observed, compared to those in rats treated with TiO_2-NPs alone. The renoprotective properties of the antioxidants were confirmed by reduced intensity of renal damage as demonstrated by histological findings. In conclusion, Car and Mel play protective roles against TiO_2-NPs-induced renal inflammation and oxidative injury, likely due to their antioxidant and anti-inflammatory properties.

KEYWORDS

carnosine, creatinine, GSH, melatonin, VEGF

1 | INTRODUCTION

Titanium dioxide nanoparticles (TiO_2-NPs) are commonly used in the decontamination of water and air and as a coloring agent in cosmetic, pharmaceutical, and paint formulations.[1] However, several reports have revealed the toxicological effects of these nanoparticles on organisms. Numerous studies have unequivocally shown that TiO_2-NPs accumulate in the hepatic, renal, pulmonary, and cardiac tissues of animals,[2,3] leading to severe inflammatory damage reflected by biomarkers in the serum and disturbed balances of blood sugar and lipids.[3] Additionally, Gui et al.[4] reported that TiO_2-NPs accumulates in renal tissue, resulting in nephric inflammation, cell necrosis, and damage. TiO_2-NPs exposure activates nucleic factor-κB by triggering the expression of tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, transforming growth factor-β (TGF-β) and interferon-γ. Nephrotoxicity-like pathological changes have been observed in the kidneys after exposure to TiO_2-NPs.[2]

Alterations in antioxidant defense systems coupled with increased oxidative stress may increase susceptibility to tissue damage. Thus, antioxidants play important protective roles against oxidative damage. Carnosine (Car), a naturally occurring dipeptide (β-alanyl-L-histidine), is found in long-lived tissues.[5] Car prevents inflammation, scavenges free radicals,[6] and inhibits protein glycosylation.[7] Additionally, Car suppresses acidification of intracellular environments and maintains muscle activity.[8] It also decreases lipid peroxidation by scavenging free radicals and chelating metals.[9] Further, Car is involved in blood glucose regulation[10] and protects against gentamicin-induced

nephrotoxicity.[11] It protects against ferritin-mediated and hydrogen peroxide mediated DNA damage.[12]

Melatonin(Mel; *N*-acetyl-5-methoxytryptamine) is a powerful anti-inflammatory, antiapoptotic, free radical scavenger, and effective antioxidant;[13–16] it modulates the inflammatory process in the liver and pancreas.[16,17] It protects against inflammation and oxidative damage-induced renal impairment.[18–20] The anti-inflammatory activity of Mel was determined through its inhibition of NF-κB,[21,22] which is a major transcription factors essential for optimum transcription of many pro-inflammatory markers, such as TNF-α, IL-1β, IL-6, and others. Mel also protects against oxidative DNA damage.[23]

The present study aimed to verify the protective efficacy of Car and Mel against inflammatory and oxidative stress-induced renal damage in response to TiO_2-NPs toxicity.

2 | MATERIALS AND METHODS

Fifty male Wistar rats (150–170 g) were obtained. All animal experiments conformed to the guidelines established by the Experimental Animal Laboratory and were approved by the Animal Care and Use Committee of the College of Pharmacy, King Saud University. The animals were housed under standard temperature and humidity conditions and provided with a rat pellet diet and water ad libitum. The rats were fasted overnight before treatment. After a 1-week acclimation, the control group was treated with only 1% carboxymethyl cellulose (CMC) solution. Three experimental groups of 10 rats each were orally administered TiO_2-NPs (600 mg/kg body weight/day)[24] as follows: TiO_2-NPs group received TiO_2-NPs alone, TiO_2-NPs and Car group received TiO_2-NPs and 200 mg/kg Car,[24] and TiO_2-NPs and Mel group received TiO_2-NPs and 100 mg/kg Mel.[24] TiO_2-NPs were administered in 1% CMC. Car and Mel were suspended in CMC and orally administered daily for 3 weeks simultaneously with and after TiO_2-NPs administration. After 3 weeks, serum was separated from blood samples by centrifugation at 3000 rpm. Afterward, the rats were sacrificed, and kidney samples were collected, homogenized in phosphate buffer saline, and stored at −80°C. Another kidney samples were stored in formalin.

2.1 | Biochemical serum analysis

Urea, uric acid, creatinine, and glucose levels were estimated using kits (Diamond Diagnostics, Holliston, MA). TNF-α levels were quantified using a commercial ELISA kit (Endogen, Woburn, MA). IL-6 levels were quantified by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). Immunoglobulin G (IgG) levels were measured by a sandwich ELISA (Sigma-Aldrich, St. Louis, MO). C-reactive protein (CRP) levels were measured by latex-enhanced immunonephelometry with a Behring BN II Nephelometer (Siemens AG, Berlin, Germany). Vascular endothelial growth factor (VEGF) levels were determined by quantitative colorimetric sandwich ELISA at 492 nm (R&D Systems).[25] Nitrite (NO) concentration was assayed by the Griess test.[26]

2.2 | Biochemical assay of kidney tissue

Reduced glutathione (GSH) content was measured according to a previously reported method.[27]

2.3 | Histopathological observation

Small sections of kidney tissue were fixed in 4% formalin, and then stained with hematoxylin–eosin as well as Masson's trichrome.

2.4 | Statistical analysis

The results are expressed as the mean ± SD. Significant differences were analyzed by using one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA.

3 | RESULTS

Figure 1 illustrates that TiO_2-NPs significantly elevated kidney function biomarkers in the serum (urea, creatinine, and uric acid) over levels observed in the control group ($P < 0.001$). Administration of Car and Mel significantly declined the levels of these biomarkers compared with levels in the TiO_2-NPs group ($P < 0.001$). TiO_2-NPs

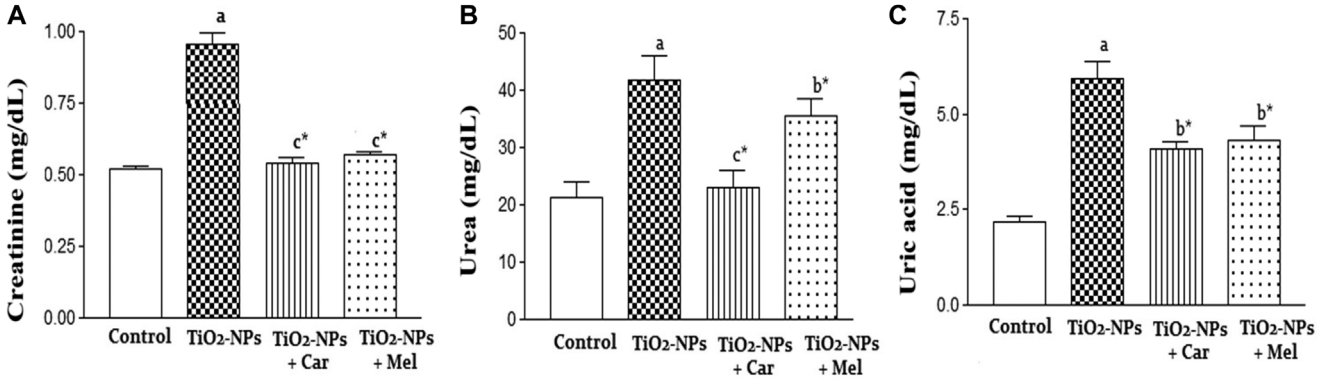

FIGURE 1 Effects of Car and Mel treatments on kidney function biomarkers in TiO_2-NP-intoxicated rats. (A) Creatinine, (B) urea, and (C) uric acid. Values are expressed as mean ± SD of 10 rats. $^aP \leq 0.001$, $^bP \leq 0.01$, $^cP \leq 0.05$ compared with the control group, $^*P \leq 0.001$ compared with the TiO_2-NPs administered group.

TABLE 1 Effect of Car and Mel treatments on serum biochemical markers in TiO_2-NPs-intoxicated rats

Parameter	Normal	TiO_2-NPs group	TiO_2-NPs and Car group	TiO_2-NPs and Mel group
NO (μmol/mL)	31.8 ± 3.31	80.21 ± 4.24[a]	50.88 ± 2.63[b]	52.62 ± 1.33[b,*]
VEGF(pg/mL)	180.8 ± 4.2	250 ± 4.4[a]	200 ± 3.7[b,*]	189 ± 2.5[b,*]
GSH (μmol/g)	1.38 ± 0.01	0.74 ± 0.082[a]	1.12 ± 0.1[b,*]	1.13 ± 0.1[b,*]

Values are expressed as mean ± SD of 10 rats. [a]$P \leq 0.001$, [b]$P \leq 0.01$ compared with the control group; [*]$P \leq 0.001$ compared with the TiO_2-NPs group.

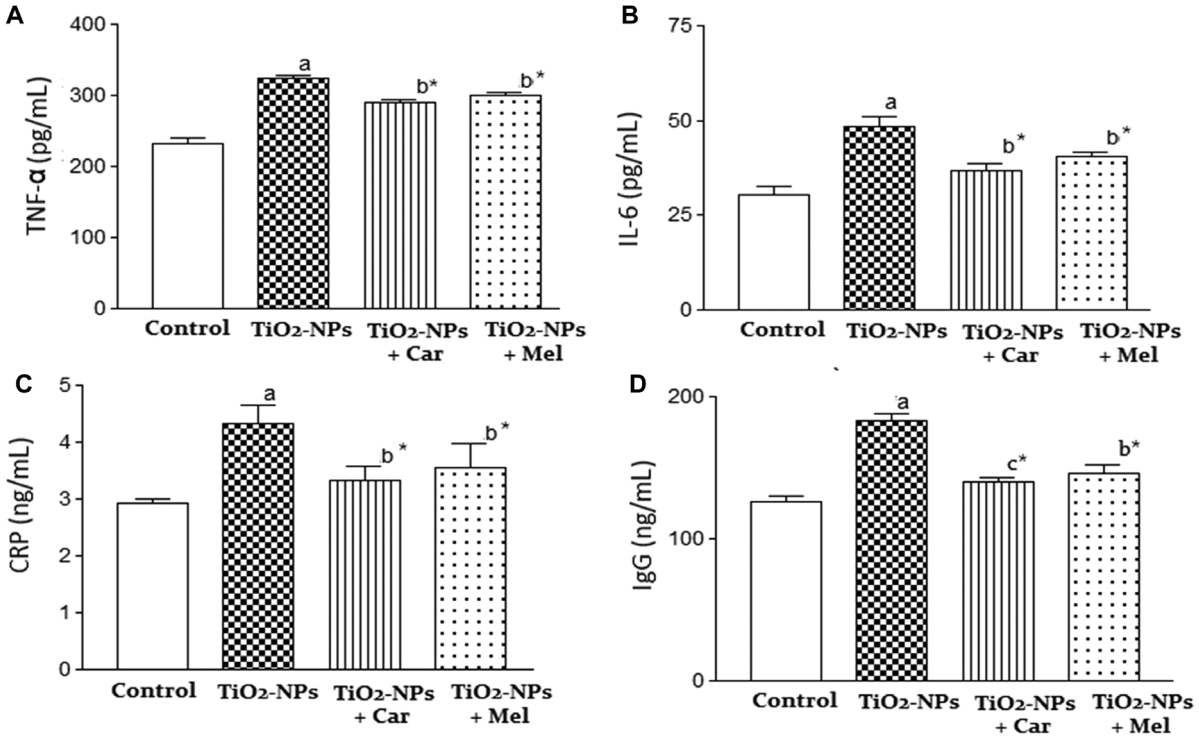

FIGURE 2 Effects of Car and Mel treatments on serum immuno-inflammatory biomarkers in TiO_2-NP-intoxicated rats. (A) TNF-α, (B) IL-6, (C) CRP, and (D) IgG. Values are expressed as mean ± SD of 10 rats. [a]$P \leq 0.001$, [b]$P \leq 0.01$, [c]$P \leq 0.05$ compared with the control group, [*]$P \leq 0.001$ compared with the TiO_2-NPs group.

ingestion significantly increased serum VEGF and nitric oxide (NO) levels and decreased renal GSH content (Table 1) compared with corresponding values in the control group ($P < 0.001$). Additionally, the levels of serum inflammatory markers (TNF-α, IL-6, CRP, and IgG) were significantly elevated in the three experimental groups compared to those in the control group ($P < 0.001$) (Figure 2). Co-administration of Car or Mel with TiO_2-NPs significantly reduced the levels of these kidney function, biochemical, and inflammatory markers compared to their levels in the TiO_2-NPs group ($P < 0.001$). The previous data were supported by histopathological examination of renal tissues, which revealed that rats treated with TiO_2-NPs alone exhibited massive atrophy and fragmentation of numerous glomeruli along with epithelial desquamation and necrosis in many renal tubules (Figure 3). An increase in collagen fibers was also observed in the interstitial tissue (Figure 4). Co-administration of either Car or Mel along with TiO_2-NPs greatly improved the histomorphological architecture of rat kidneys, as demonstrated by normal features of the glomeruli and most renal tubules in addition to a smaller increase in collagen fibers present in the interstitial tissue.

4 | DISCUSSION

In this study, TiO_2-NPs induced nephrotoxicity via enhancement of inflammatory cytokines, as demonstrated by significantly increased serum levels of urea, uric acid, creatinine, TNF-α, IL-6, IgG, and CRP. These results are consistent with those of Zhao et al.[28] and Gui et al.,[4] who reported the severe inflammatory response and impairment of function and in kidneys of animals exposed to TiO_2-NPs. Another study suggested that increased antibody production was initiated by TNF-α during inflammation.[29]

Car and Mel significantly alleviated TiO_2-NP-induced nephrotoxicity and effectively downregulated inflammatory markers, implying their protective anti-inflammatory action.[11,30] The beneficial anti-inflammatory effects of the two agents have been previously documented.[6,16,17] Consistently with a report by Liu et al.,[3] the present study demonstrated that TiO_2-NP-induced elevation of glucose and CRP levels are closely associated with insulin resistance and complications such as fatty liver and hyperglycemia;[31] additionally, such elevation plays a major role in the activation of pro-inflammatory pathways in various cell types.[32] Co-administration of

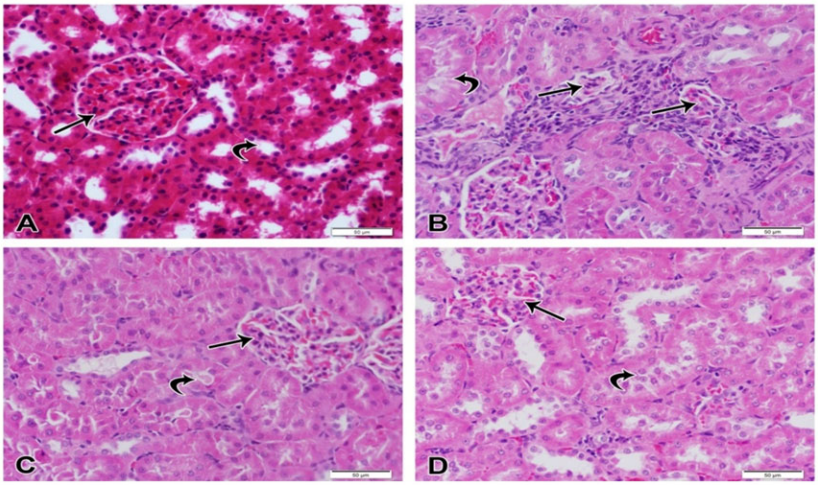

FIGURE 3 Photomicrograph of kidney sections stained with hematoxylin and eosin, scale bar = 50 showing the effects of Car and Mel treatments on kidneys of TiO_2-NP-intoxicated rats. (A) Kidney section of the control group rats showing normal architecture of renal corpuscles and tubules. (B) Kidney section of TiO_2-NPs group rats showing massive atrophy and fragmentation of numerous glomeruli (arrows) as well as epithelial desquamation and necrosis in many of the renal tubules (curved arrows). (C) Kidney section of TiO_2-NPs and Car group rats showing fewer atrophied glomeruli (curved arrows). (D) Kidney section of TiO_2-NPs and Mel group rats showing normal renal corpuscles. Kidney sections of rats receiving TiO_2-NPs and either Car (c) or Mel (d) showing normal architecture.

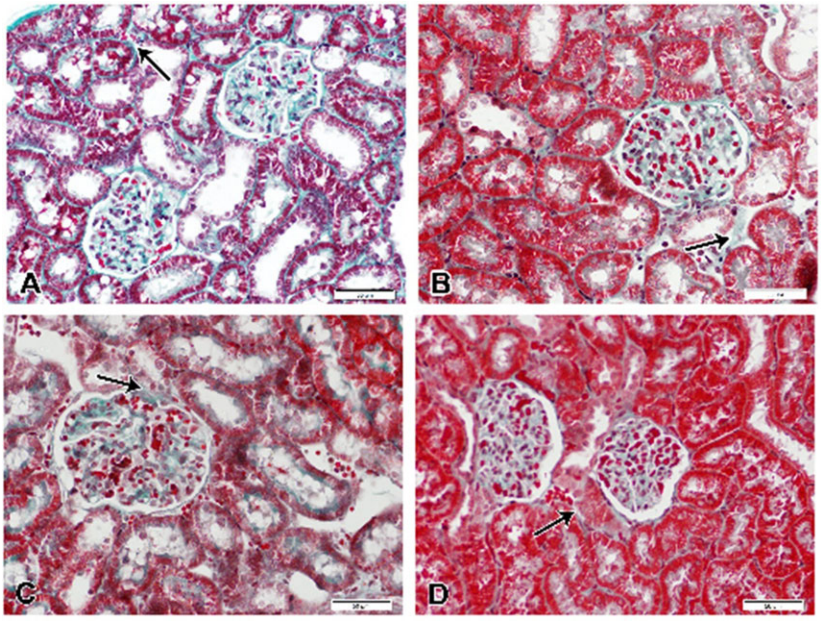

FIGURE 4 Photomicrograph of kidney sections stained with Masson's trichrome, scale bar = 50. (A) Kidney section of the control group rats showing normal distribution of collagen fibers in the interstitial tissue (arrow). (B) Kidney section of TiO_2-NPs group rats showing increased collagen fibers in the interstitial tissue (arrow). (C and D) Kidney sections of TiO_2-NPs and Car (C) or Mel (D) group rats showing normal distribution of collagen fibers in the interstitial tissue (arrow).

Car or Mel along with TiO_2-NPs successfully downregulated the elevation of glucose, likely due to the ability of Car and Mel to inhibit CRP production.[10,33] The present study demonstrated that TiO_2-NPs enhance the production of NO and the expression of VEGF. High levels of NO indicate inflammatory stimuli and increased expression of cytokines, which result in inflammatory tissue injury, and overproduction of reactive oxygen species (ROS) stimulates VEGF-synthesizing immune and inflammatory cells.[34,35] Car and Mel markedly reduced NO and VEGF levels in TiO_2-NPs-intoxicated rats, which is consistent with the results of other studies demonstrating the pivotal roles of Car and Mel in the inhibition of ischemia, ROS, NO, and cytokine production, all of which contribute to overexpression of VEGF.[36,37]

In this study, high doses of TiO_2-NPs caused GSH depletion in renal tissue. A similar effect has been documented in other studies reporting that exposure of cultured BEAS-2B cells to nanoparticles led to cell death, ROS elevation, and a decline in GSH levels,[38] possibly attributable to increased lipid hydroperoxide.[39] Amelioration of

renal GSH levels in TiO_2-NPs-intoxicated rats by treatment with either Car or Mel suggests their potential antioxidant effects.[17,40] In the present study, TiO_2-NPs induced massive atrophy and fragmentation of numerous glomeruli as well as epithelial desquamation and necrosis in renal tubules. Moreover, TiO_2-NP treatment increased the number of collagen fibers in the interstitial tissue. Treatment with Car and Mel nearly restored kidney architecture.

In conclusion, ingestion of Car or Mel protected against inflammatory and oxidative renal damage induced by TiO_2-NP toxicity in rats. These findings suggest Car and Mel as candidate therapies for other nanoparticle toxicities.

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