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The Nephroprotective Role of Carnosine Against Ifosfamide-Induced Renal Injury and Electrolytes Imbalance is Mediated Via the Regulation of Mitochondrial Function and Alleviation of Oxidative Stress

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Key words

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ABSTRACT

Background Ifosfamide (IFO) is an alkylating agent administered against different types of malignancies. Several cases of renal injury and serum electrolytes disturbances have been reported in IFO-treated patients. Oxidative stress and mitochondrial dysfunction are suspected of being involved in the mechanism of IFO nephrotoxicity. Carnosine is a dipeptide which its antioxidant and mitochondria protecting properties have been mentioned in different experimental models. The current study aimed to evaluate the nephroprotective properties of carnosine against IFO-induced renal injury.

Methods Rats were treated with IFO (50 mg/kg, i.p) alone or in combination with carnosine. Serum and urine biomarkers of renal injury in addition to kidney markers of oxidative stress were evaluated. Moreover, kidney mitochondria were isolated, and some mitochondrial indices were assessed.

Results Elevated serum creatinine and BUN, hypokalemia, and hypophosphatemia, in addition, to an increase in urine glucose, protein, γ-GT, and alkaline phosphatase (ALP), were evident in IFO-treated animals. IFO also caused an increase in kidney reactive oxygen species (ROS) and lipid peroxidation (LPO). Renal GSH levels and antioxidant capacity were also depleted with IFO therapy. Mitochondrial dehydrogenase activity, GSH level, membrane potential, and ATP content were decreased while mitochondrial LPO and permeabilization were increased in IFO group. Carnosine (250 and 500 mg/kg, i.p) mitigated IFO-induced oxidative stress and mitochondrial impairment in renal tissue.

Conclusion Our data suggest mitochondrial dysfunction and oxidative stress as fundamental mechanisms of renal injury induced by IFO. On the other hand, carnosine supplementation protected kidneys against IFO-induced injury through regulating mitochondrial function and mitigating oxidative stress.

* The authors contributed equally to this work.

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Introduction

Several drugs which are widely administered in cancer chemotherapy regimens are associated with renal injury and serum electrolytes abnormalities [1]. Ifosfamide (IFO) is an alkylating agent administered against several types of malignancies especially in cyclophosphamide-resistant cases [2]. Adverse drug reactions including an increase in serum transaminase level, nausea, vomiting, and hemorrhagic cystitis are attributed to IFO therapy [2]. Several cases of renal injury and serum electrolytes abnormalities have been reported in IFO-treated patients as well as animal models of IFO-induced renal injury [3, 4]. IFO also might lead to acute renal failure [5, 6]. Although the precise mechanism(s) of renal injury induced by IFO is far from clear, some investigations mentioned the role of oxidative stress and its associated events in IFO nephrotoxicity [7, 8]. Hence, administration of protective agents with antioxidant capacity might mitigate IFO-induced renal injury.

The nephrotoxicity of many xenobiotics including several drugs is characterized by a generalized transport defect in the renal proximal tubules [9]. This adverse effect leads to impaired renal tubular transport of electrolytes [9]. As mentioned, IFO administration is associated with several cases of renal proximal tubule defect and disturbances in serum electrolytes [3, 4, 6].

Renal tissue contains numerous mitochondria which their proper function guarantees sufficient energy (ATP) needed for chemicals reabsorption process in the kidney tubules [10]. The nephrotoxicity of several xenobiotics might be mediated through affecting cellular mitochondrial function [11]. It has been found that IFO might affect renal tissue mitochondrial function [12]. Therefore, targeting cellular mitochondria could be a therapeutic point of intervention against IFO nephrotoxicity.

Carnosine (β-alanyl histidine; CAR) is a dipeptide widely investigated for its cytoprotective properties [13]. Some biological and pharmacological functions have been attributed to the CAR [13]. Mechanistically, CAR could scavenge reactive species, mitigate oxidative stress, and prevent the disruption of biological targets [13]. Several investigations also mentioned that mitochondria protecting properties of CAR could play a significant role in its cytoprotective mechanisms [14]. In the current study, CAR (250 and 500 mg/kg, i.p) was administered as a potential protective agent against IFO nephrotoxicity. The data obtained from this study might help to develop safe and clinically reliable therapeutic options against IFO-induced nephrotoxicity and electrolytes abnormalities.

Materials and Methods

Chemicals

2′,7′-Dichlorofluorescein diacetate, trichloroacetic acid, 3-(N-morpholino) propane sulfonic acid, 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, D-mannitol, 2, 4, 6-tripyridyl-s-triazine, rhodamine123, sucrose, and thiobarbituric acid were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). Ifosfamide was obtained from Baxter®. Kits for evaluating serum and urine biochemistry were obtained from Pars Azmun® (Tehran, Iran). Ethylenediaminetetraacetic acid, meta-phosphoric acid, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and n-butanol were obtained from Merck (Darmstadt, Germany).

Animals

Male Sprague-Dawley rats (n = 32, weighing 200–250 g) were obtained from the Laboratory Animal Breeding Center, Shiraz University of Medical Sciences, Shiraz, Iran. Rats were housed in a standard condition (Ambient temperature of 23 ± 1 °C with a ≈ 40 % of relative humidity and 12 h light/dark cycle). Animals had free access to tap water and a standard rodent's chow diet (Behparvar®, Tehran, Iran). All procedures involving laboratory animal use were in accordance with the guidelines for care and use of laboratory animals which was approved by an ethics committee in Shiraz University of Medical Sciences, Shiraz, Iran (95–01–36–13619).

Experimental setup

Animals were randomly allotted into four groups (n = 8/group). Rats were treated as follows: 1) Control (Vehicle-treated group), 2) IFO (50 mg/kg/day, i.p) for 5 consecutive days; 3) IFO (50 mg/kg/day, i.p) + Carnosine (250 mg/kg, i.p) for 5 consecutive days; 4) IFO (50 mg/kg/day, i.p) + Carnosine (500 mg/kg, i.p) for 5 consecutive days. Carnosine was administered 2 h after IFO each day. It has been previously reported that a dose of 50 mg/kg/day of IFO for 5 consecutive days caused marked renal injury in rats [7]. The therapeutic doses for IFO varies based on the type of malignances. However, in clinical situation IFO is administered up to 5000 mg/m^2/day which is equivalent to approximately 900 mg for an adult [2]. In the current study, we used the previous reported nephrotoxic dose of ifosfamide in rats (50 mg/kg/day) [7].

Specimen collection

At the end of experiments (At day 6th, 24 h after the final dose of IFO), urine samples (100 µL) were collected during animal handling. Samples were diluted to 400 µL (with normal saline 0.9 % W: v, 4 °C) and centrifuged (5 000 g, 5 min, 4 °C). The clear supernatant was collected and used for urinalysis. Then, animals were anesthetized (Thiopental 70 mg/kg, i.p) and their blood and kidney samples were collected. A Mindray BS-200® auto analyzer and standard kits (Pars Azmun®, Tehran, Iran) were used to assess biomarkers of kidney injury.

Histopathological assessment

For histopathological assessments of the kidney tissue, samples were fixed in 10 % formalin solution (10 % formaldehyde in distilled water, 0.4 % sodium phosphate monobasic, NaH_2PO_4, 0.64 % sodium phosphate dibasic, Na_2HPO_4, and; pH = 7.4). Then, paraffin-embedded sections (5 µm) of renal tissue were stained with hematoxylin and eosin (H&E). Renal histopathological alterations were scored as previously described based on a model of renal injury [15].

Kidney tissue reactive oxygen species (ROS) formation

Kidney samples (500 mg) were homogenized in 5 mL of ice-cooled Tris-HCl buffer (40 mM, pH = 7.4, 4 °C) (1:10 w/v). Then, 100 µL of tissue homogenate was mixed with 1 mL of Tris-HCl buffer (40 mM, pH = 7.4) and 5 µL of 2′, 7′-dichlorofluorescein diacetate (Final concentration 10 µM). The mixture was incubated in the dark (15 min, 37 °C). Finally, the fluorescence intensity of samples was assessed (FLUOstar Omega®, BMG Labtech, Germany, $\lambda_{excitation} = 485$ nm and $\lambda_{emission} = 525$ nm) [16].

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Renal tissue lipid peroxidation

Kidney tissue homogenate (1 mL, 10 % w- v in Tris-HCl 40 mM, 4 °C, pH = 7.4) was added to 3 mL of TBARS assay reaction mixture (Thiobarbituric acid, 0.375 % w: v, trichloroacetic acid 15 % w: v, and hydrochloric acid, pH = 2). Samples were mixed well and heated in a water bath (100 °C, 45 min). Then, 2 mL of n-butanol was added, vigorously mixed, and centrifuged (10 000 g, 10 min). Finally, the absorbance of the developed color in n-butanol (Upper phase) was measured (λ = 532 nm, EPOCH plate reader, Bio-Tek® Instruments, Highland Park, USA) [16].

Kidney and isolated mitochondria glutathione content

Kidney tissue and isolated mitochondria levels of reduced (GSH) and oxidized (GSSG) glutathione were measured by an HPLC method [17]. Briefly, deproteinized samples (TCA 50 %) were derivatized with iodoacetic acid and fluoro-2,4-dinitrobenzene and analyzed using an NH_2 column (250 mm × 4 mm ID, Bischoff chromatography, Leonberg, Germany). The flow rate was 1 mL/min and a UV detector (λ = 254 nm) was used [17]. In this method, the mobile phases consisted of buffer A (Water: Methanol; 1:4 v/v) and buffer B (Buffer A: Acetate buffer; 4:1 v/v) and a gradient method with a steady increase of buffer B to 95 % (in 20 min) [17]. Kidney tissue (200 mg) were homogenized in 5 mL of Tris-HCl buffer (40 mM; pH = 7.4; 4 °C). Then, 500 µL of trichloroacetic acid (TCA, 50 % w- v, 4 °C) was added to 2 mL of the tissue homogenate. Mitochondria samples (1 mL; 10 mg protein/mL) were also treated with 50 µL of TCA (50 % w- v). Samples were mixed well and incubated on ice (10 min). Then, samples were centrifuged (15 000 g, 15 min, 4 °C) and 1 mL of the supernatant was collected, and the NaOH: $NaHCO_3$ (2 M: 2 M) was added (≈ 300 µL) till the gas production was subsided. Afterward, 100 µL of iodoacetic acid (1.5 % w- v in water) was added, and samples were incubated in the dark (1 h, 4 °C). After the incubation period, 500 µL of 2, 4-dinitrofluorobenzene (DNFB; 1.5 % w- v in absolute ethanol) was added and incubated in the dark (25 °C, at least for 24 h). Finally, 25 µL of samples were injected into the previously described HPLC system [17, 18].

Ferric reducing antioxidant power (FRAP) of the kidney tissue

The working FRAP solution was freshly prepared by mixing 10 volumes of the acetate buffer (300 mmol/L, pH = 3.6), with 1 volume of ferric chloride (20 mmol/L in deionized water) and 1 volume of and TPTZ (10 mmol/L in 40 mmol/L HCl). Homogenized kidney tissue (100 µL) was added to 150 µL of deionized water and 1.5 mL of the mentioned FRAP solution. The reaction mixture was incubated at 37 °C (5 min, in the dark). Finally, samples were centrifuged (15 000 g, 2 min,4 °C) the absorbance of developed color was measured (λ = 595 nm, EPOCH plate reader, Bio-Tek® Instruments, Highland Park, USA) [16].

Kidney mitochondria isolation

Rat kidneys were washed and minced in an ice-cooled (4 °C) buffer medium containing 70 mM mannitol, 0.5 mM EGTA, 225 mM sucrose, 0.1 % w: v of bovine serum albumin, and 2 mM HEPES, pH = 7.4). Minced tissue was transported into the fresh isolation buffer (10:1 w- v Buffer: Tissue) and homogenized. Kidney mitochondria were isolated by differential centrifugation of the tissue homogenate. First, unbroken cells and nuclei were pelleted at 1 000 g for 20 min at 4 °C; second, the supernatant was centrifuged at 10 000 g for 20 min at 4 °C to pellet the mitochondria fraction (dark brown). This step was repeated three times using fresh isolation buffer medium to increase mitochondria yield [16].

Mitochondrial dehydrogenases activity

A colorimetric method using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was applied for the determination of mitochondrial dehydrogenases activity [16]. Briefly, mitochondrial suspension (1 mg protein/mL) was treated with 40 µL of the MTT (0.4 % w: v, 37 °C, 30 min, in the dark). Samples were centrifuged (12 000 g, 5 min) and the product of purple formazan crystals (Pellet) was dissolved dimethyl sulfoxide, and the optical density (OD) at λ = 570 nm was measured (EPOCH plate reader, BioTek®, Highland Park, USA) [16].

Mitochondrial depolarization

The uptake of the rhodamine 123 as a cationic fluorescent probe was used for the assessment of mitochondrial depolarization [19, 20]. Samples (0.5 mg protein/mL) were incubated with rhodamine 123 (10 µM final concentration) for 30 min (37 °C, with continuous shaking). Afterward, samples were centrifuged (15 000 g, 5 min, 4 °C) and the fluorescence intensity of the supernatant was measured (FLUOstar Omega® multifunctional microplate reader, BMG Labtech, Germany, $\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 525 nm) [21].

Mitochondrial swelling and permeabilization

Analysis of mitochondrial permeabilization and swelling was estimated by the light scattering method which monitors the changes in light absorbance at λ = 540 nm (Constant temperature 30 °C) [16]. Briefly, 100 µL of the mitochondrial suspension was added to a 96-well plate, and the absorbance of samples was monitored at λ = 540 nm during 30 min of incubation (EPOCH plate reader, BioTek® Instruments, Highland Park, USA) [16].

Isolated kidney mitochondria lipid peroxidation

Mitochondria samples were washed once to remove sucrose. For this purpose, mitochondria samples were added to 5 mL of ice-cooled (4 °C) MOPS-KCl buffer (100 mM KCl, 50 mM MOPS, pH = 7.4), and centrifuged (15 000 g, 20 min, 4 °C) [16]. The supernatant was discarded, and mitochondria pellet was re-suspended in MOPS–KCl buffer and used for TBARs assay. The mitochondrial suspension was added with twice its volume of TBARs assay mixture (Trichloroacetic acid 15 % w: v, thiobarbituric acid 0.375 % w- v, 0.24 N HCl, and 0.5 mM Trolox). Samples were heated for 15 min at 100 °C in a water bath. After centrifugation (15 000 g, 10 min), the absorbance of the supernatant was measured (λ = 532 nm, EPOCH® plate reader, BioTek® Instruments, Highland Park, USA) [16, 22].

Mitochondrial ATP level

A luciferase-luciferin-based kit (Enliten® from Promega, Madison, USA) was used to assess mitochondrial ATP content. Briefly, 500 µL of mitochondrial samples (1 mg protein/mL) were treated with

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200 µL of ice-cooled TCA solution (0.3 % w- v in double distilled water, 4 °C) and centrifuged (15,000 g, 20 min, 4 °C) [23]. Afterward, 100 µL of the supernatant was treated with 100 µL of ATP kit content, and the luminescence intensity of samples was measured at λ = 560 nm.

▶ **Table 1** Serum biochemical measurements in ifosfamide (IFO)-treated rats.

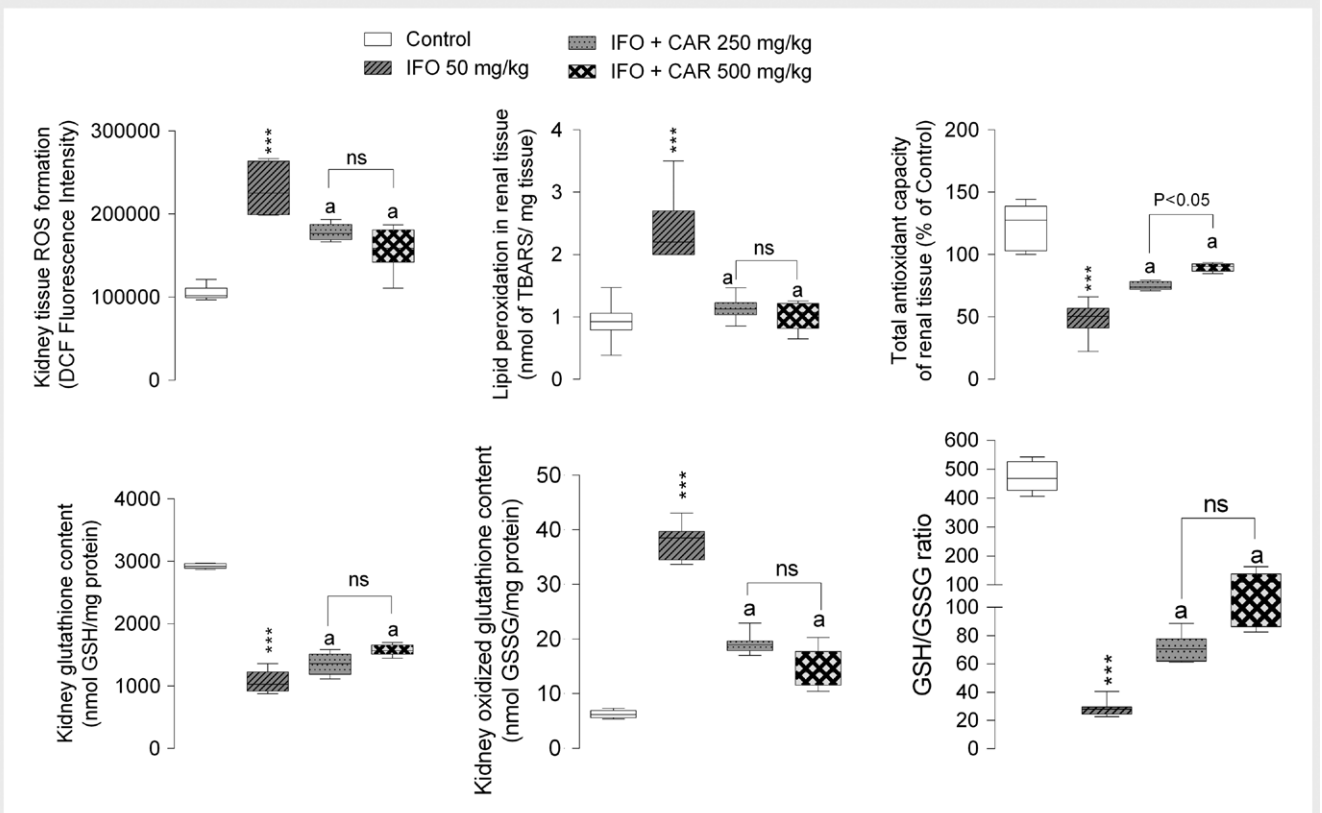
Serum marker	Control	IFO 50 mg/kg	IFO + CAR 250 mg/kg	IFO + CAR 500 mg/kg
Ca^{2+}(mg/dl)	5.07 ± 0.07	4.81 ± 0.14^{ns}	5.13 ± 0.23	4.82 ± 0.21
K^{+}(mmol/l)	5.72 ± 1.3	3.53 ± 0.9 *	4.98 ± 0.3[a]	5 ± 0.6[a]
Na^{+}(mmol/l)	78.6 ± 4	69.5 ± 5	77 ± 4	78 ± 5
Glucose (mg/dl)	109 ± 10	113 ± 2^{ns}	111 ± 8	110 ± 7
Uric acid (mg/dl)	1.7 ± 0.2	0.50 ± 0.4 *	0.96 ± 0.6[a]	0.92 ± 0.1[a]
Phosphate (mg/dl)	3.02 ± 0.41	2.33 ± 0.22 *	3.06 ± 0.41[a]	2.95 ± 0.39[a]
Total protein (mg/dl)	7.4 ± 0.5	6.6 ± 0.5	6.7 ± 0.3	6.8 ± 0.2
BUN (mg/dl)	44 ± 6	71 ± 7 *	54 ± 7[a]	56 ± 4[a]
Creatinine (mg/dl)	0.48 ± 0.1	0.74 ± 0.09 *	0.58 ± 0.07[a]	0.57 ± 0.06[a]

Data are given as mean ± SD (n = 8). IFO: Ifosfamide; CAR: Carnosine. * Indicates significantly different as compared with the control group (P < 0.01). [a]Indicates significantly different as compared with IFO 50 mg/kg group (P < 0.05). ns: not significant as compared with the control group.

▶ **Table 2** Urine markers of kidney injury in ifosfamide (IFO)-treated rats.

Urine marker	Control	IFO 50 mg/kg	IFO + CAR 250 mg/kg	IFO + CAR 500 mg/kg
ALP (U/l)	2043 ± 213	4414 ± 855 *	3167 ± 432[a]	2247 ± 345[a]
Glucose (mg/dl)	77 ± 7	106 ± 10 *	79 ± 3[a]	81 ± 7[a]
γ-GT	1995 ± 423	3998 ± 563 *	2744 ± 398[a]	1746 ± 392[a]
Protein (mg/dl)	0.51 ± 0.04	1.08 ± 0.04 *	0.7 ± 0.09[a]	0.71 ± 0.04[a]

Data are given as mean ± SD (n = 8). IFO: Ifosfamide; CAR: Carnosine. * Indicates significantly different as compared with the control group (P < 0.01). [a]Indicates significantly different as compared with IFO 50 mg/kg group (P < 0.05).



▶ **Fig. 1** Kidney tissue markers of oxidative stress in ifosfamide (IFO)-treated animals and the effect of carnosine supplementation. CAR: Carnosine.

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Statistical analysis

Data are given as the Mean ± SD. Data comparison was performed by the one-way analysis of variance (ANOVA) with Tukey's multiple comparisons as the *post hoc* test. $P<0.05$ was considered a statistically significant difference.

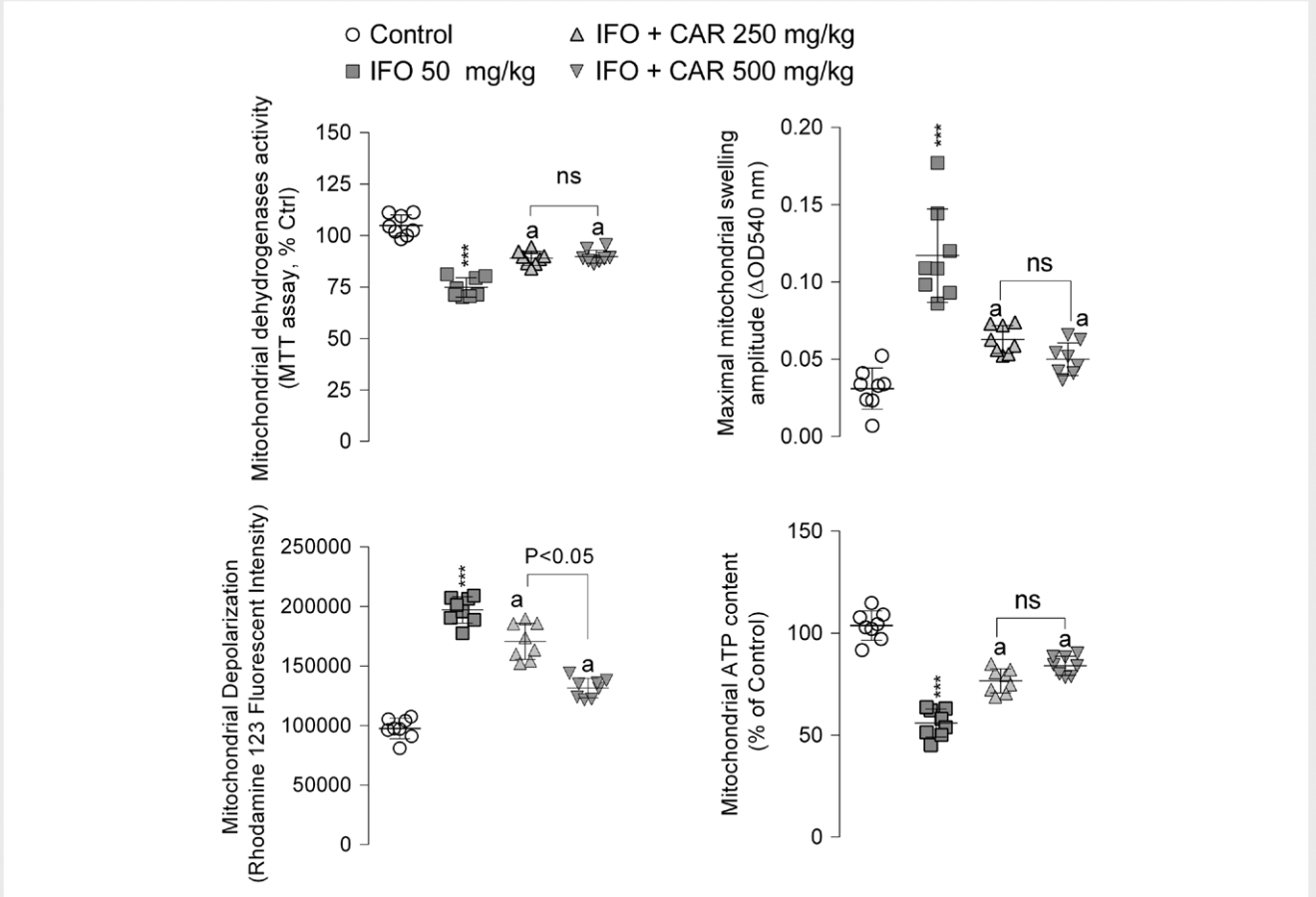
Results

A significant increase in serum biomarkers of renal injury was evident in IFO (50 mg/kg)-treated rats (▶ **Table 1**). Urine markers of renal injury including urine glucose, ALP, γ-GT, and protein level were also elevated in IFO-treated animals (▶ **Table 2**). It was found that CAR treatment (250 and 500 mg/kg) significantly decreased IFO-induced elevation in serum Cr and BUN (▶ **Table 1**). A significant decrease in urine biomarkers of renal injury was also evident after CAR (250 and 500 mg/kg) therapy (▶ **Table 1** and ▶ **2**).

Signs of disturbances in serum electrolytes including hypophosphatemia and hypokalemia were detected in IFO-treated rats (▶ **Table 1**). No significant changes in serum protein, Na^+, glucose, and Ca^{2+} were detected when animals were treated with IFO (▶ **Table 1**). It was found that CAR (250 and 500 mg/kg) significantly mitigated IFO-induced disturbances in serum electrolytes and ions (▶ **Table 1**).

Biomarkers of oxidative stress including ROS formation, increased GSSG levels, and lipid peroxidation were significantly increased in the kidney tissue of IFO-treated animals (▶ **Fig. 1**). On the other hand, tissue antioxidant capacity and GSH stores were depleted when animals received IFO (50 mg/kg, i.p) (▶ **Fig. 1**). It was found that CAR supplementation (250 and 500 mg/kg, i.p) alleviated IFO-induced oxidative stress in the kidney tissue (▶ **Fig. 1**).

Markers of kidney mitochondrial function were evaluated in the IFO-treated animals (▶ **Fig. 2**). A significant decrease in mitochondrial dehydrogenases activity, as well as dissipation of mitochondrial membrane potential, were evident in kidney mitochondria isolated from IFO-treated rats (▶ **Fig. 2**). Mitochondrial ATP level was also significantly lower in the IFO-treated group (▶ **Fig. 2**). On the other hand, significant mitochondrial permeabilization, and swelling were detected in kidney mitochondria when animals received IFO (50 mg/kg, i.p) (▶ **Fig. 2**). Biomarkers of oxidative stress including significant lipid peroxidation, increased GSSG levels, and depletion of GSH reservoirs were also evident in kidney mitochondria isolated from IFO-treated animals (▶ **Fig. 3**). It was found that CAR supplementation (250 and 500 mg/kg, i.p for five consecutive days) preserved kidney mitochondrial function in IFO-treated rats (▶ **Fig. 2** and ▶ **3**).



▶ **Fig. 2** Kidney tissue mitochondrial indices of functionality in ifosfamide (IFO)-treated rats. CAR: Carnosine.

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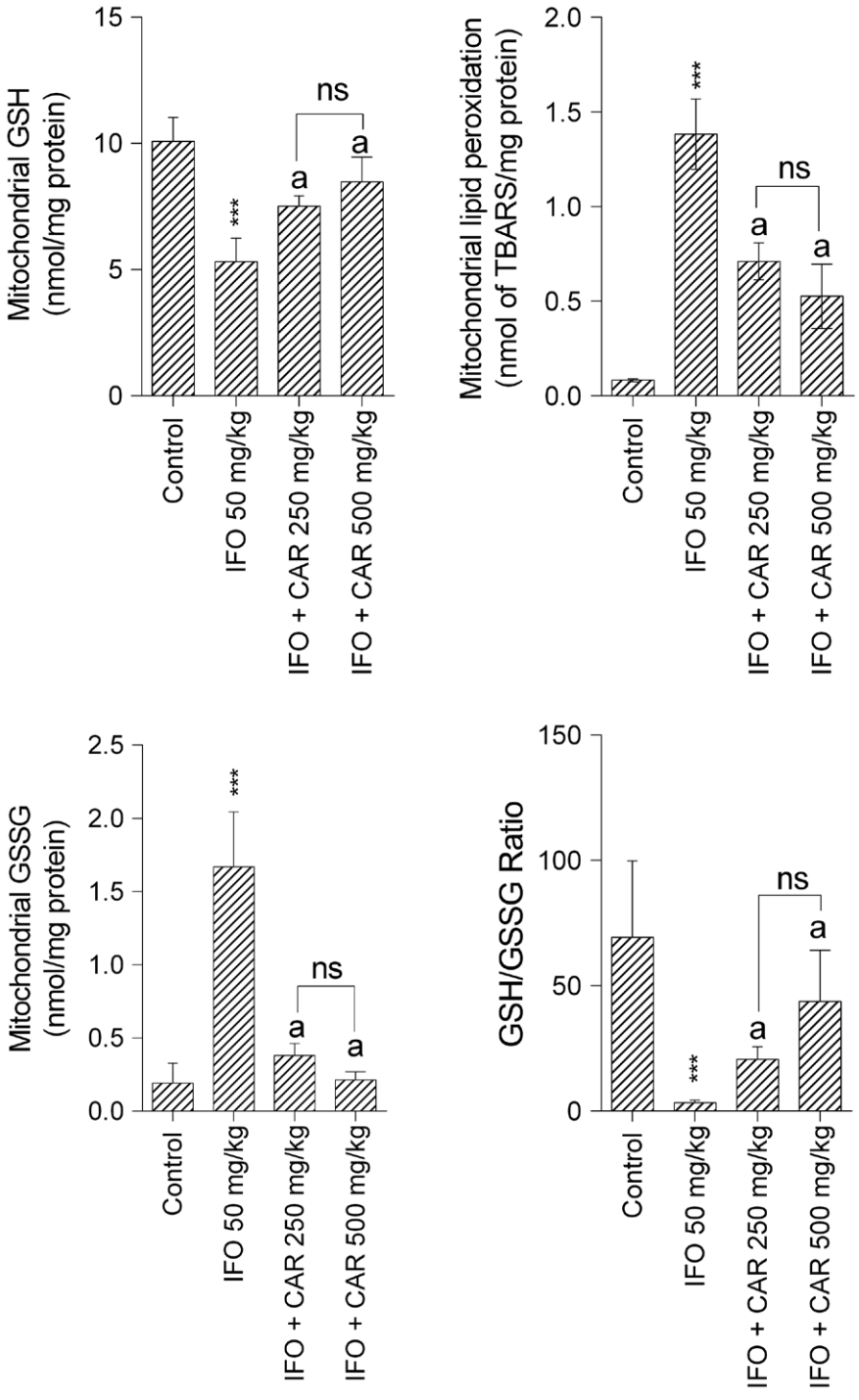

▶ **Fig. 3** Biomarkers of oxidative stress in the kidney mitochondria isolated from ifosfamide (IFO)-treated animals. CAR: Carnosine.

Severe interstitial inflammation, tissue necrosis, dilated Bowman capsule, vascular congestion, and hemorrhage were detected in real tissue of IFO-treated animals (▶ **Fig. 4** and ▶ **Table 3**). Carnosine administration (250 and 500 mg/kg) significantly mitigated renal tissue histopathological changes in IFO-treated rats (▶ **Fig. 4** and ▶ **Table 3**).

Discussion

Renal injury and serum electrolytes abnormalities are clinical complications linked to a range of xenobiotics including many pharmaceuticals [9]. IFO is an anticancer agent which its clinical use is connected with several adverse drug reactions including renal injury [24]. Several cases of electrolytes imbalance and renal injury have

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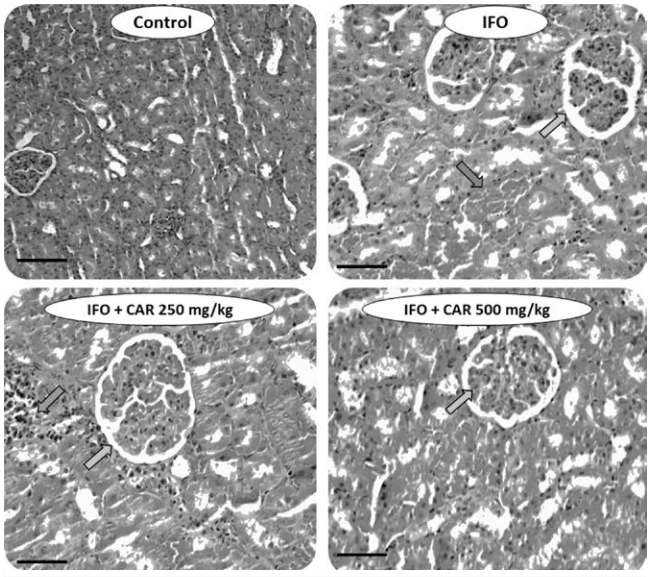

▶**Fig. 4** Renal tissue histopathological alterations in ifosfamide (IFO)-treated rats. IFO treatment caused severe interstitial inflammation (Blue arrow), dilated Bowman capsule (Green arrow), vascular congestion, and hemorrhage (Red arrow) in the rat kidney (▶**Table 3**). Carnosine (CAR) supplementation mitigated renal tissue histopathological changes induced by IFO (▶**Table 3**). H&E staining; Magnification: ×400; Scale bars: 50 µm.

▶**Table 3** Kidney tissue histopathological alterations in ifosfamide (IFO)-treated animals.

Treatments	Tubular necrosis and degenerative dilation	Interstitial inflammation	Dilated Bowman, capsule	Vascular congestion and haemorrhage
Control	-	-	-	-
IFO	+ +	+ +	+ + +	+ + +
IFO + Carnosine 250 mg/kg	+ +	+	+ +	+
IFO + Carnosine 500 mg/kg	+	-	+	-

+: Mild; + +: Moderate; and + + +: Severe histopathological changes.

been documented in association with IFO therapy [25, 26]. Therefore, finding ancillary therapeutic options against IFO-induced renal injury has clinical value.

In the current study, we found that mitochondrial indices of functionality were impaired in the kidney tissue of IFO-treated animals (▶**Fig. 2**). Some other investigations also mentioned the effect of IFO or its suspected cytotoxic metabolites on cellular mitochondria [12]. It has been found that acrolein and chloroacetaldehyde significantly decreased mitochondrial respiration and depolarized kidney mitochondria [12]. These data mention that cellular mitochondria are critical targets for IFO-induced nephrotoxicity. Several investigations also mentioned that the reactive aldehyde metabolite of IFO is responsible for its adverse effects on the kidney [27, 28]. Reactive aldehydes target different cellular components including proteins, lipid, DNA, and organelles such as mitochondria. These events could lead to severe oxidative stress, mitochondrial impairment, and cell death. On the other hand, cellular mitochondria are the major source of ROS [29]. On the other hand, oxidative stress impairs mitochondrial function [29]. Hence, oxidative stress and mitochondrial impairment are two mechanistically interconnected events. IFO-induced mitochondrial impairment could enhance oxidative stress in the kidney tissue and *vice versa*. A wide range of other mechanisms including inflammatory response and endoplasmic reticulum (ER) stress could be involved in chemotherapy drugs (including IFO)-induced nephrotoxicity. The importance of such mechanisms could be the subject of future investigations in this field.

CAR is a well-known carbonyl trap [30]. This peptide can scavenge reactive species including highly reactive and cytotoxic aldehydes [30]. Hence, a crucial part of the protective properties of CAR against IFO-induced oxidative stress, mitochondrial dysfunction, and renal injury might be mediated through the scavenging of reactive aldehyde metabolites of IFO in the kidney tissue. This might also serve as a great superiority of CAR against IFO-induced renal injury in comparison with other protective agents and antioxidants.

The chemical reabsorption process in the kidney is highly dependent on cellular energy (ATP) status. The Na^+/K^+ ATPase pump in renal proximal tubule consumes ATP to produce a Na^+ chemical gradient which is further used for the absorption of many chemicals including amino acids, glucose, phosphate, and calcium [31]. Hence, IFO-induced mitochondrial dysfunction could lead to cellular ATP exhaustion and energy crisis (▶**Fig. 2**). These events might finally impair Na^+/K^+ ATPase activity and result in serum electrolytes imbalance. All these data might mention the importance of targeting cellular mitochondria to protect the kidney against IFO. Several studies mentioned the positive effects of carnosine on mitochondrial function and cellular energy status [14, 32, 33]. CAR could also prevent mitochondria-mediated cell death [32]. These unique properties make this peptide as a potential therapeutic option against IFO-induced renal injury.

Collectively, the data obtained from the current study mention the fundamental role of oxidative stress and mitochondrial impairment in the pathogenesis of IFO-induced renal injury. On the other hand, CAR as a protective agent effectively preserved renal mitochondrial function and counteracted oxidative stress in IFO-treated animals. Further investigations of the renoprotective properties of CAR in tumor-bearing animals, as well as the potential interaction of this peptide with chemotherapy regimens, could be the subject of future investigations.

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Conflict of Interest

Authors declare that they have no conflicts of interest.

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