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Renoprotection by α-mangostin is related to the attenuation in renal oxidative/nitrosative stress induced by cisplatin nephrotoxicity

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Abstract

Cisplatin (CDDP) is a chemotherapeutic agent that produces nephrotoxicity associated with oxidative/nitrosative stress. α-Mangostin (α-M) is a xanthone extracted from mangosteen with antioxidant and anti-inflammatory properties. The purpose of this study was to evaluate the renoprotective effect of α-M on the CDDP-induced nephrotoxicity. α-M was administered (12.5 mg/kg/day, i.g.) for 10 days (7 days before and 3 days after CDDP injection). On day 7, rats were treated with a single injection of CDDP (7.5 mg/Kg, i.p.); 3 days after the rats were killed. α-M attenuated renal dysfunction, structural damage, oxidative/nitrosative stress, decrease in catalase expression and increase in mRNA levels of tumour necrosis factor alpha and transforming growth factor beta. In conclusion the renoprotective effect of α-M on CDDP-induced nephrotoxicity was associated with the attenuation in oxidative/nitrosative stress and inflammatory and fibrotic markers and preservation of catalase activity.

Keywords: *Alpha-mangostin, cisplatin, antioxidant, anti-inflammatory, oxidative stress, catalase*

Introduction

Cisplatin (cis-diamminedichloroplatinum II, CDDP) is a potent anti-tumour agent widely used in the clinic [1]. CDDP is used for the treatment of testicular, head and neck, ovarian, cervical, non-small cell lung carcinoma and many other types of cancer [2]. However, its use is limited due to a number of adverse reactions in renal, neuronal and auditory systems. Nephrotoxicity is the most prevalent and serious toxicity of CDDP [3]. Despite its nephrotoxic effect, CDDP remains the drug of choice in several countries due to its low cost and effectiveness in comparison to new drugs. However, the mechanisms by which CDDP-induces nephrotoxicity are still not completely understood. Nevertheless, several authors have suggested that reactive oxygen (ROS) and nitrogen (RNS) species are implicated in the CDDP-induced nephrotoxicity *in vivo* and *in vitro* [4,5]. In addition it has been found that CDDP inhibits mitochondrial function and induces depletion of SH groups, DNA damage, apoptosis and inflammation [6,7].

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Some compounds with free radical scavenging and antioxidant activities have been used to attenuate the cytotoxic effect of CDDP [4,8].

On the other hand, there are a lot of plants that have biological activities with potential therapeutic applications. Mangosteen (*Garcinia mangostana* Linn) is a tropical tree from southeast of Asia that has been used in ayurvedic medicine against inflammation, diarrhoea, skin infections, etc. In fact, our laboratory has described the ROS scavenging and antioxidant capacity of the aqueous extract of mangosteen pericarp [9]. This pericarp contains a variety of secondary metabolites such as prenylated and oxygenated xanthones. Among the reported activities of xanthones isolated from mangosteen pericarp are the following: antioxidant, anti-tumoural, anti-inflammatory, anti-allergic, antibacterial, anti-fungal and anti-viral (reviewed in [10]). α-, β- and γ-mangostins are among the most studied xanthones of mangosteen. Several reports have shown the beneficial effect of α-mangostin (α-M) by their antioxidant and anti-inflammatory properties (reviewed in [10–13]). Studies in our laboratory found that α-M scavenges directly several ROS and prevents the neurotoxicity and ROS production induced by 3-nitropropionic acid in cultured neurons [14].

However there is no evidence of the effect of α-M on the CDDP-induced toxicity *in vivo*. So, the hypothesis of this study was that, due to its antioxidant and anti-inflammatory properties, α-M may attenuate the CDDP-induced nephrotoxicity in rats. The aim of this study was to evaluate the possible renoprotective effect of α-M on CDDP-induced nephrotoxicity and its potential association with the attenuation in the following alterations found in this experimental model: oxidative/nitrosative stress, decrease in antioxidant enzymes and increase in inflammation and fibrotic markers.

Materials and methods

Reagents

The dry powder of mangosteen pericarp used for the isolation of α-M was obtained from DNP International Inc. Co. (Whittier, CA) (Lot number NPMP37285). α-M was isolated as previously described [15]. The purity of α-M was established by chromatographic homogeneity using several chromatographic analyses (including high pressure liquid chromatography). These analyses revealed a single peak or spot with several mobile phases. In addition nuclear magnetic resonance (NMR) spectra were unable to show the presence of any other component. Finally, the melting point of our compound was similar to that reported in the literature [15]. CDDP (Cat. No. P4394) was from Sigma Chemical Co (St. Louis MO). Mouse monoclonal anti-4-hydroxy-2-nonenal (4-HNE) antibodies (Cat. No. 24325) were from Oxis International Inc. (Portland, OR). Mouse monoclonal anti-3-nitro-L-tyrosine (3-NT) antibodies (Cat. No. 189542) were from Cayman Chemical Co. (Ann Arbor, MI). Mayer's Hematoxylin (Lillie's Modification) (Cat. No. S3309) was from DAKO Corporation (Carpinteria, CA). The Amplex® Red hydrogen peroxide (H_2O_2)/peroxidase assay kit to measure urinary excretion of H_2O_2, TRIzol reagent, reverse transcriptase and primer sequences to amplify specific PCR fragments (Table I) were obtained from Invitrogen (Carlsbad, CA). All other chemicals used were of the highest quality available and were obtained from commercial sources.

Experimental design

Housing room was maintained under constant conditions of temperature (18–22°C) and lighting (12-h light/dark cycle). Thirty-four male Wistar rats weighing 270–320 g each and fed with a standard chow diet and water and were divided randomly in four groups. The first group was used as a control (C) and received a single intraperitoneal (i.p.) injection of vehicle (saline solution). The second group (α-M) was treated orally with 12.5 mg/Kg*day of α-M for 10 days (suspended in 0.5% carboxymethyl-cellulose). The third group (CDDP) was administered with a single i.p. injection of CDDP (7.5 mg/kg), which was previously dissolved in saline solution (1.5 mg/mL).

Table I. Oligonucleotide sequence to amplify specific PCR fragments.

Gen	GenBank number	Oligonucleotide sequence	Primer position	size, pb	Product region, pb
Catalase	NM_012520.1	5′- GGAGCAGGTGCTTTTGGATA-3′	315–334	243	315–557
		5′- GGCATCCCTGATGAAGAAAA-3′	538–557		
MnSOD	NM_017051.2	5′- CTGGACAAACCTGAGCCCTA-3′	386–405	132	386–517
		5′- GAACCTTGGACTCCCACAGA-3′	498–517		
TNFα	NM_012675.2	5′- AGTCCGGGCAGGTCTACTTT-3′	815–834	377	815–1191
		5'- GAGACAGCCTGATCCACTCC-3′	1171–1191		
TGFβ	NM_031131.1	5′- GCAGAGTTCAGGGTCTTTCG-3′	830–850	349	830–1178
		5′- CACCACTGGCATATGTGGAG-3′	1158–1178		
GAPDH	BC059110.1	5′- GGTGATGCTGGTGCTGAGTA-3′	332–352	369	332–700
		5′- GGATGCAGGGATGATGTTCT-3′	680–700		

MnSOD, manganese superoxide dismutase; TNFα, tumour necrosis factor alpha; TGFβ, transforming growth factor beta; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

The fourth group (CDDP + α-M) was administered with both compounds at the doses previously described; α-M was given for 10 days (7 days before and 3 days after CDDP injection). Along the study rats were placed in metabolic cages. Samples of blood and urine were collected at the beginning (day 0), middle (day 6) and end of the study (day 10).

Three days after CDDP-administration rats were killed by decapitation and blood was collected. A midline laparotomy was made; the right kidney was excised and frozen in liquid nitrogen and kept at −80°C until processing. The left kidney was placed in 10% formalin buffer for histopathological and immunohistochemical evaluation. All experiments involving animals were conducted in accordance with [16] and to the guidelines of Norma Official Mexicana Guide for the use and care of laboratory animals (NOM-062-ZOO-1999) and for the disposal of biological residues (NOM-087-ECOL-1995).

The α-M dose used in the present study was chosen in previous experiments performed in CDDP-injected rats treated with the following doses of this xanthone 12.5, 25, 50, 100 and 200 mg/Kg body weight for 10 days (7 days before and 3 days after CDDP-injection) and evaluating the following parameters: serum creatinine (SCr), blood urea nitrogen (BUN), creatinine clearance (CCr) and proteinuria. The doses of α-M of 12.5 and 50 mg/Kg induced the highest renoprotective effect against CDDP-inducted nephrotoxicity (Table II). Based on the above data, the dose of 12.5 mg/Kg was chosen to perform the present study. α-M by itself was unable to induce alterations in any of the parameters measured (Table II).

Studies to evaluate renal function

Serum and urine creatinine and BUN levels were measured with an autoanalyser (Technicon RA-1000, Bayer Tarrytown, NY). Sodium was measured by flame photometry (Flame Photometer 443, Instrumentation Laboratory, Lexington, MA) and urinary osmolality was measured with the advanced micro-Osmometer model 3300 (Instrument Inc., Lab products Division. Norwood, MA). CCr was calculated by the standard formula $CCr = U^{\star}V/P$, where U is the concentration in urine, V is urine flow rate and P is the concentration in plasma. CCr was corrected by 100 g of body weight. Urine protein and N-acetyl-β-glucosaminidase (NAG) were analysed as previously described [17]. Circulating glutathione peroxidase (GPx) activity was assayed in a coupled assay by the disappearance of NADPH at 340 nm [16]. This enzyme is a marker of tubular damage since it is synthesized and liberated to the circulation by the renal proximal tubules. Fractional excretion of sodium (FeNa) was calculated with the standard formula: (sodium clearance/CCr)*100.

Histological analysis

Kidneys stored in formaldehyde were dehydrated and embedded in paraffin [17]. Sections of 3 μm were stained with hematoxylin and eosin. The histological profile of 20 proximal tubules randomly selected per rat was recorded using a computerized image analyser KS-300 3.0 (Carl Zeiss, Jena, Germany). The percentage of tubular area with histopathological alterations like swelling, cytoplasmatic vacuolization, desquamation or necrosis was obtained. The percentage of damaged area of CDDP and CDDP + α-M groups was compared.

Markers of oxidative stress and activities of antioxidant enzymes

Urinary excretion of hydrogen peroxide (H_2O_2) was determined using a commercial kit. The following markers and antioxidant enzymes were measured in kidney tissue. Levels of malondialdehyde (MDA),

Table II. Dose-dependent protection of α-M on CDDP-induced nephrotoxicity evaluated by serum creatinine (SCr), blood urea nitrogen (BUN), creatinine clearance (CCr) and proteinuria.

	SCr, mg/dL	BUN, mg/dL	CCr, mL/min*100 g BW	Proteinuria, mg/24 h
Ct	0.41 ± 0.03	19.59 ± 0.52	0.51 ± 0.03	17.71 ± 0.90
CDDP	1.66 ± 0.29*	67.88 ± 9.80*	0.15 ± 0.05*	45.59 ± 8.90*
α-M (12.5)	0.50 ± 0.02	20.42 ± 0.85	0.40 ± 0.04	16.41 ± 1.17
CDDP + α-M (12.5)	0.56 ± 0.17	36.80 ± 8.23	0.41 ± 0.11	15.19 ± 2.66
α-M (25)	0.43 ± 0.01	19.65 ± 1.14	0.44 ± 0.02	15.91 ± 1.85
CDDP + α-M (25)	0.55 ± 0.08	36.42 ± 6.83	0.33 ± 0.09	18.79 ± 4.22
α-M (50)	0.34 ± 0.03	20.16 ± 0.94	0.49 ± 0.03	17.25 ± 2.28
CDDP + α-M (50)	1.06 ± 0.18*	59.39 ± 9.62*	0.14 ± 0.04*	31.58 ± 3.03
α-M (100)	0.25 ± 0.03	ND	0.54 ± 0.10	ND
CDDP + α-M (100)	2.05 ± 0.16*	98.73 ± 6.86*	0.03 ± 0.01*	31.84 ± 6.08
α-M (200)	0.43 ± 0.01	ND	0.62 ± 0.16	ND
CDDP + α-M (200)	1.56 ± 0.32*	88.28 ± 2.90*	0.16 ± 0.13*	41.28 ± 4.79*

Concentration of α-M appears between parentheses in mg/Kg. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin; BW, body weight; N.D. not determined; *$p < 0.05$ *vs* Ct Values are mean ± SEM of 3–6 rats/group.

a marker of lipid peroxidation, were determined according to the method of Gérard-Monnier et al. [18]. The content of protein carbonyl groups, an index of oxidized proteins, was measured by the reagent 2,4-dinitrophenylhydrazine [17]. The content of reduced glutathione (GSH) in renal tissue was determined with the fluorescence method of Fernandez-Checa and Kaplowitz [19]. The activity of catalase was measured by the disappearance of H_2O_2 at 240 nm [17]. GPx activity was measured as previously described [17]. Glutathione reductase (GR) activity was measured using oxidized glutathione as substrate and measuring the disappearance of NADPH at 340 nm [17]. Superoxide dismutase (SOD) was measured using the superoxide anion generator system xanthine/xhanthine oxidase and nitroblue tetrazolium as the indicator molecule [17].

Immunohistochemical studies

Immunohistochemical studies of 4-HNE, a marker of lipid peroxidation, and of 3-NT, a marker of nitrosative stress, were performed as previously described [20].

RNA isolation and semi-quantitative RT-PCR

Total RNA was extracted from each kidney using TRIzol reagent according to the manufacturer's instructions. Integrity of isolated total RNA was examined by 1% agarose gel electrophoresis and RNA concentration was determined by UV-light absorbance at 260 nm (Beckman DU640, Brea, CA). Reverse transcription (RT) was performed at 37°C for 60 min using at Moloney murine leukaemia virus reverse transcriptase.

The relative mRNA levels of catalase, MnSOD, tumour necrosis factor-α (TNFα), transforming growth factor β (TGFβ) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were assayed in total kidney by semi-quantitative RT-PCR. PCR cycles were performed in a DNA thermal cycler (Eppedndorf thermocycler, Model 5331). The control gene was amplified simultaneously in each reaction. The optimal number of cycles for each primer pair was assessed through kinetic amplification determination. PCR reaction was run for each primer pair and the reaction was stopped at different number of cycles to evaluate the linearity of the reaction. The number of cycles used for catalase, MnSOD, TNFα, TGFβ and GAPDH were: 30, 30, 33, 35 and 18, respectively. A PCR product was electrophoresed in a 2% agarose gel for analysis. Bands stained with ethidium bromide were visualized under UV light and the densitometric analysis was performed using the software ImageJ. All reactions were performed individually from each kidney's total RNA in duplicate. Genomic DNA contamination was checked by treating all RNA samples with RNAse-free DNAase I and by carrying samples through the PCR procedure without adding RT.

CDDP pharmacokinetics

This study was performed to evaluate if the administration of α-M produced change in the pharmacokinetics of CDDP by measuring platinum (Pt) concentration at different time intervals in rats treated or untreated with α-M (see above). Control rats and those treated with α-M were anaesthetized with the mix of isofurane/oxygen 3%. The caudal artery was catheterized with PE-50 polyethylene tubing and the rats were placed in individual cages to facilitate sample collection. Once awake, the rats were administered with a single i.p. injection of CDDP (7.5 mg/Kg), blood samples were taken at 0, 5, 10, 15, 30, 60, 90, 120 and 240 min. Based on the fact that CDDP highly join to plasma proteins, the pharmacokinetic study was performed by only 4 h in CDDP and CDDP + α-M groups. The samples obtained were centrifuged at 11 180 × g at 4°C and immediately after the plasma was ultrafiltered at 11 180 × g and 4°C for 30 min using Millipore filter (30 000 Da). The ultrafiltered was frozen to quantify the content of Pt. The following pharmacokinetic parameters were calculated by the software GraphPad Prism 4 (San Diego, CA): area under the curve (AUC), maximum concentration of Pt (C_{max}) and maximum time of concentration of Pt in plasma (T_{max}). Kidneys were obtained at the end of the study (4 h) to measure Pt concentration.

Pt quantification

Pt concentrations were analysed by autoanalyser atomic absorption spectroscopy with graphite oven (sample: 20 µl; wavelength: 265.9 nm; slit: 0.2 nm; apparatus: Varian SpectrAA 220A model with graphite oven GTA-110). Pt standard solution for atomic absorption (High-purity 1 000 µg/mL in 5% de HCl) was used to construct the calibration curve, which was linear for the concentration range 25–400 ppb of Pt. At Pt concentration > 400 ppb, samples were appropriately diluted with deionization water. The coefficient of variation for the assay was less than 4%.

Statistical analysis

Results are presented as means ± SEM. The data were analysed by ANOVA and Student Newman Keuls correction for multiple comparisons. GraphPad Prism 4 software (San Diego, CA) was used to perform statistical analysis. P-value < 0.05 was considered significant.

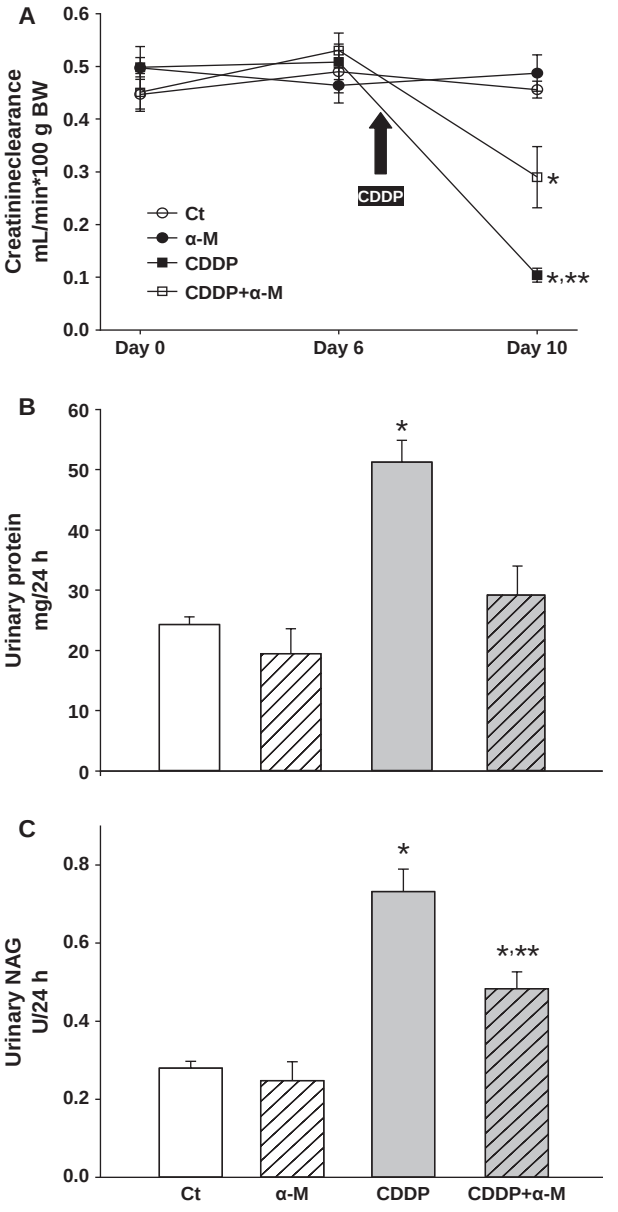


Figure 1. Markers of nephrotoxicity in the four groups of rats studied. (A) creatinine clearance, (B) total protein and (C) urinary NAG excretion. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. $^{\star}p<0.01$ vs Ct; $^{\star\star}p<0.01$ vs CDDP. Values are mean ± SEM of 6–8 rats/group. The arrow indicates that CDDP was injected on day 7.

Results

First of all, the effect of α-M on CDDP-induced renal dysfunction was evaluated by the following markers: SCr, CCr, BUN levels, circulating GPx activity, proteinuria, urinary excretion of NAG, polyuria, FeNa and osmolality (Figure 1 and Tables III and IV). α-M prevents the increase in SCr and BUN levels and the decrease in circulating GPx induced by CDDP (Table III). It was clear that the beneficial effect of α-M on CDDP-induced nephrotoxicity.

CCr at 0, 6 and 10 days is shown in Figure 1A. At the beginning, the rats of all groups had similar renal function that was not modified by α-M (day 6). Rats receiving CDDP show a significant reduction of CCr at the end of the study (day 10) which was attenuated by α-M. In addition, α-M attenuates the CDDP-induced proteinuria (Figure 1B), increased urinary excretion of NAG (Figure 1C), polyuria, increased FeNa and decreased urinary osmolality (Table IV).

At the end of the study one of the kidneys was fixed to determine the presence of structural alteration. Microscopic examination of kidney tissue with hematoxylin-eosin revealed that CDDP-treatment produces necrosis, vacuolization and hyaline casts. Representative photomicrographs (Figure 2A) and the percentage of the area affected in CDDP and CDDP + α-M groups (Figure 2B) are presented. CDDP-injected rats presented structural renal injury with 30% of damaged area which decreased significantly to 11% in the CDDP + α-M group. Taken together, the above data suggest that α-M had the capacity to protect the renal damage induced by CDDP at functional and structural levels.

Our next question was to know how α-M protects the kidney of the CDDP-induced nephrotoxicity. α-M has antioxidant properties and CDDP induces cellular damage through oxidative stress. Our next battery of experiments was designed to evaluate markers of oxidative/nitrosative stress. The following markers were evaluated: urinary excretion of H_2O_2 and MDA levels, protein carbonyl content, GSH levels and abundance of 4-HNE and 3-NT in kidney tissue. It was found that α-M attenuates the increased

Table III. Renal function parameters in CT, α-M, CDDP and CDDP + α-M groups. These parameters were unaltered by the sole administration of α-M whereas CDDP injection induced a significant increase in BUN and serum creatinine and a decrease in GPx. These CDDP-induced changes were attenuated by α-M in the CDDP + α-M group.

	SCr, mg/dL	BUN, mg/dL	Serum GPx, U/mL
Ct	0.51 ± 0.01	23.9 ± 1.5	1.54 ± 0.09
α-M	0.47 ± 0.01	19.2 ± 1.2	1.61 ± 0.19
CDDP	1.88 ± 0.15*	67.8 ± 6.4*	0.85 ± 0.06*
CDDP + α-M	0.96 ± 0.05*,**	40.2 ± 7.2*,**	1.17 ± 0.09*,**

Ct, control; α-M, alpha-mangostin; CDDP, cisplatin; SCr, serum creatinine; BUN, blood urea nitrogen; Serum GPx, glutathione peroxidase. $^{\star}p<0.05$ vs Ct; $^{\star\star}p<0.05$ vs CDDP. Values are mean ± SEM of 6–8 rats/group.

Table IV. Effect of α-M and CDDP on polyuria, osmolality and fractional excretion of sodium (FeNa). These parameters were unaltered by the sole administration of α-M, whereas CDDP injection induced a significant increase in urinary volume and FeNa and a decrease in urinary osmolality. These CDDP-induced changes were prevented by α-M in the CDDP + α-M group.

	Urinary volume (mL/24 h)	Osmolality (mOsm)	FeNa (%)
Ct	12.65 ± 1.12	1936 ± 76	0.89 ± 0.06
α-M	10.00 ± 1.96	1734 ± 143	0.61 ± 0.08
CDDP	16.77 ± 0.76*	787 ± 60*	1.73 ± 0.25*
CDDP + α-M	11.52 ± 1.07**	1398 ± 237**	1.06 ± 0.18**

Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. *$p < 0.05$ vs Ct; **$p < 0.01$ vs CDDP. Values are mean ± SEM of 6–8 rats/group.

urinary of excretion of H_2O_2 (Figure 3D) and the enhanced MDA levels (Figure 3A) and protein carbonyl content (Figure 3B) and the GSH depletion (Figure 3C) in kidney tissue. In addition, it was found that α-M attenuates the CDDP-induced increase in the renal abundance of 4-HNE (Figure 4A and B).

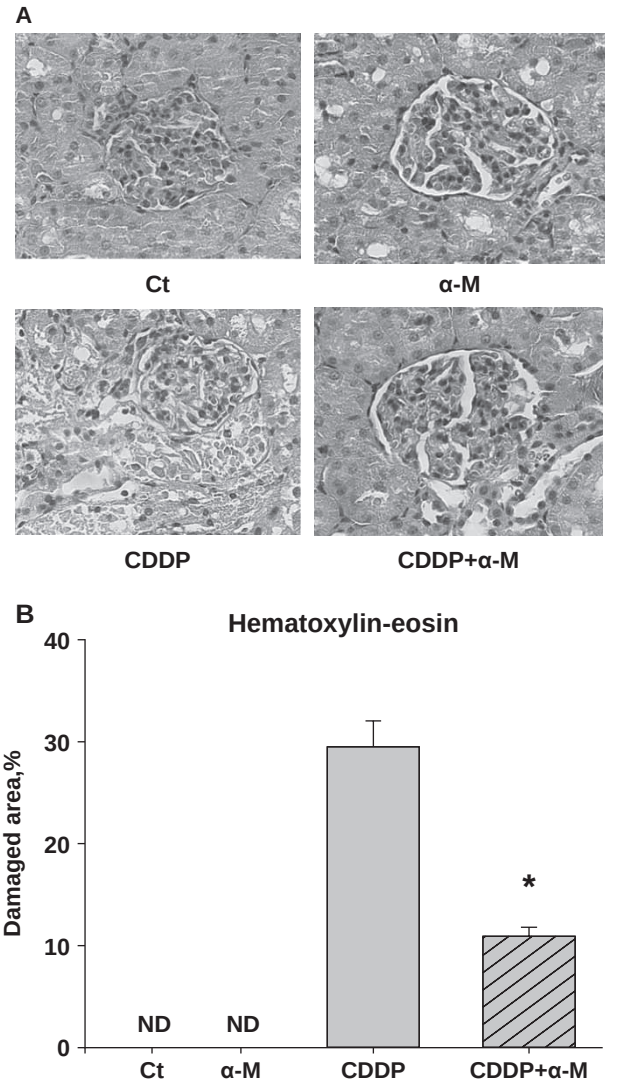


Figure 2. Histological analysis by hematoxylin-eosin staining in the four groups of rats studied. (A) Representative images. (B) Percentage area affected in CDDP and CDDP + α-M groups. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. ND, Not determined. *$p < 0.03$ vs CDDP. Values are mean ± SEM of 6–8 rats/group.

Taking into account that α-M not only scavenges ROS, but also RNS; the immunohistochemistry for 3-NT, a marker of nitrosative stress, was performed (Figure 4C and D). It was found that α-M also attenuated the CDDP-induced increase in this parameter. All theses results together suggest that the renoprotective effect induced by α-M is associated with the decrease in oxidative/nitrosative stress induced by CDDP.

Having known that α-M attenuates oxidative and nitrosative stress during CDDP-induced nephropathy, it was explored if an antioxidant enzyme may be involved. No significant differences were found in the activity of SOD, GPx and GR (data not shown) as well as in the mRNA levels of MnSOD (data not shown). In contrast it was found that CDDP decreases both mRNA levels (Figure 5A and B) and activity of catalase (Figure 5C) and α-M treatment attenuates the decrease in both parameters. It is interesting to note that the α-M-induced improvement in catalase expression, an H_2O_2 metabolizing enzyme, was correlated with the attenuation in urinary excretion of H_2O_2 in CDDP + α-M group.

α-M also has anti-inflammatory properties and it is know that inflammation is associated with the CDDP-induced renal damage; therefore it was decided to quantify the mRNA levels of TNFα, a marker of inflammation. It was found that α-M completely prevented the increase in TNFα mRNA levels (Figure 6A), suggesting that α-M also confers renoprotection by this pathway. In addition, the mRNA levels of TGFβ, a marker of fibrosis, were assessed. It was found that α-M attenuates the CDDP-induced increase in this parameter (Figure 6B).

To ensure that all these findings of renoprotection provided by α-M are due to α-M itself and not by interfering with the pharmacokinetics of CDDP, this parameter was evaluated by measuring Pt levels in plasma at the time intervals previously described. Figure 7 shows that α-M did not alter the CDDP pharmacokinetic; both curves as well as the pharmacokinetic parameters AUC, C_{max} and T_{max} are similar in the α-M and CDDP + α-M groups.

In addition, α-M was unable to alter renal Pt concentration 4 h after CDDP-injection: 6.2 ± 2.0 μg Pt/g tissue in the CDDP group vs 6.7 ± 1.0 μg Pt/g tissue in the α-M + CDDP group.

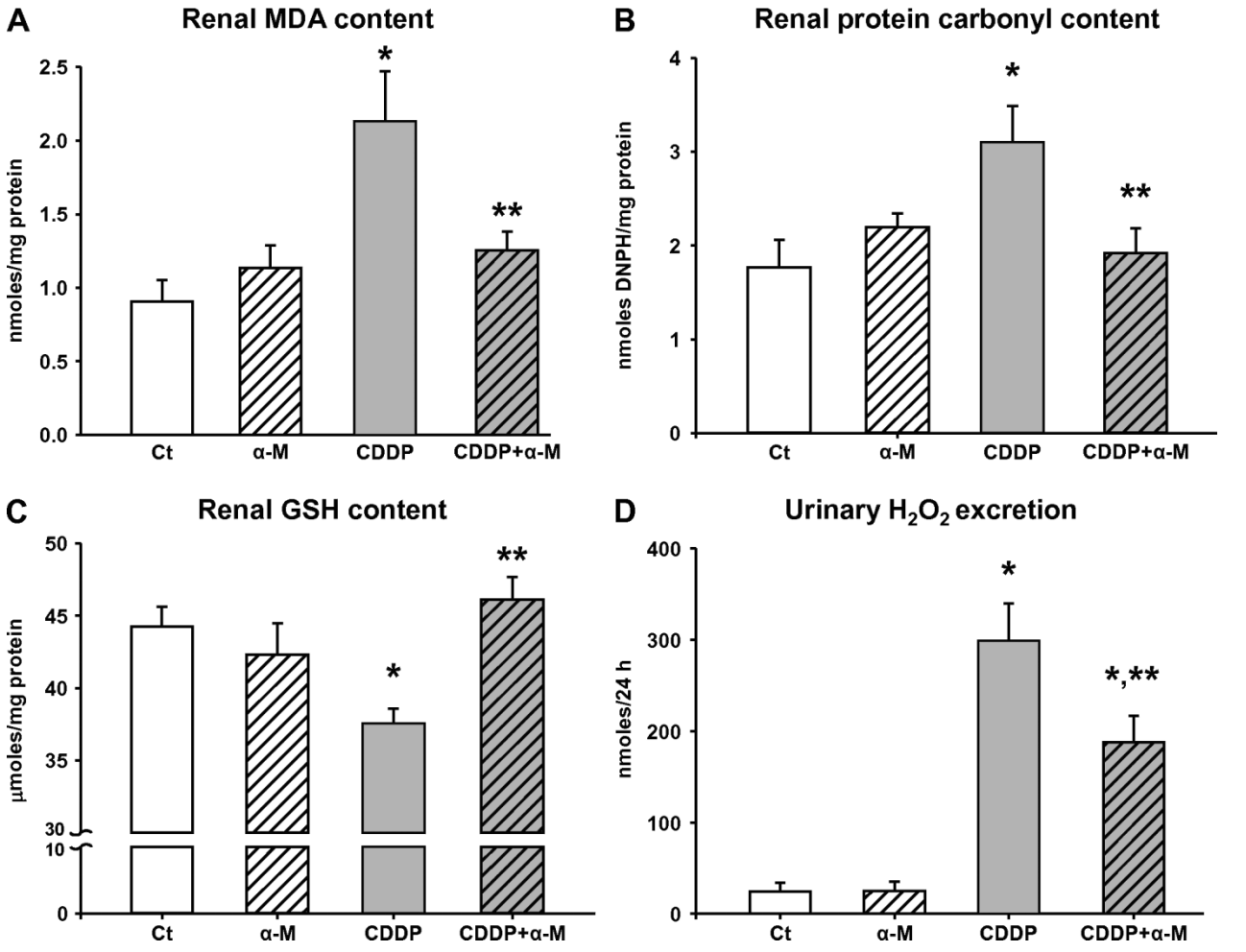


Figure 3. Oxidative stress markers in the four groups of rats studied. (A) Malondialdehyde, MDA, (B) Protein carbonyl content, (C) Glutathione (GSH) content and (D) urinary hydrogen peroxide (H_2O_2) excretion. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. $^{*}p < 0.05$ vs Ct; $^{**}p < 0.05$ vs CDDP. Values are mean ± SEM of 6–8 rats/group.

Discussion

CDDP is one of the most potent chemotherapeutic agents used around the world for the treatment of many kinds of tumours; unfortunately its adverse effects have limited its use. CDDP causes dose-related renal dysfunction through preferential accumulation in cells of the S3 segment of the renal proximal tubule [1]. Oxidative/nitrosative stress and diminished antioxidant system are involved in the CDDP-induced renal tubular injury and cell death (reviewed in [4,20–22]). For the above reason several compounds, including synthetic or natural antioxidant agents and free radical scavengers, have been used to attenuate CDDP-induced nephrotoxicity (reviewed in [4,20,22]).

In this context, α-M, a natural product with antioxidant properties, was used in this work with the purpose to analyse its potential renoprotective effect on CDDP-induced nephrotoxicity. α-M is a compound extracted from pericarp of mangosteen which has antioxidant and anti-inflammatory properties, among others (reviewed in [10]). Mahabusarakam et al. [23] found that α-M can inhibit the oxidation of low density lipoproteins and changes in its structure can modify the antioxidant activity of this xanthone. In addition, *in vitro* studies performed by Jung et al. [24] and Pedraza-Chaverri et al. [14] have shown that α-M possesses potent peroxynitrite anion scavenging activity. Moreover, *in vivo* studies performed by Devi Sampath and Vijayaraghavan [11] have shown that protective effect of α-M against isoproterenol-induced myocardial infarction in the rat was associated with the prevention of cardiac lipid peroxidation and protection of intracellular antioxidant defense system. For all that reasons, it was decided to investigate the potential protective effect of α-M against CDDP-induced nephrotoxicity.

This is the first report where the renoprotective effect of α-M on CDDP-induced nephrotoxicity is demonstrated. It was observed that a single injection of CDDP causes significant kidney damage evaluated by histological analysis and by the injury markers CCr, BUN levels, circulating GPx activity, proteinuria, polyuria, fractional excretion of sodium and urinary NAG excretion. Here, we show that administration of

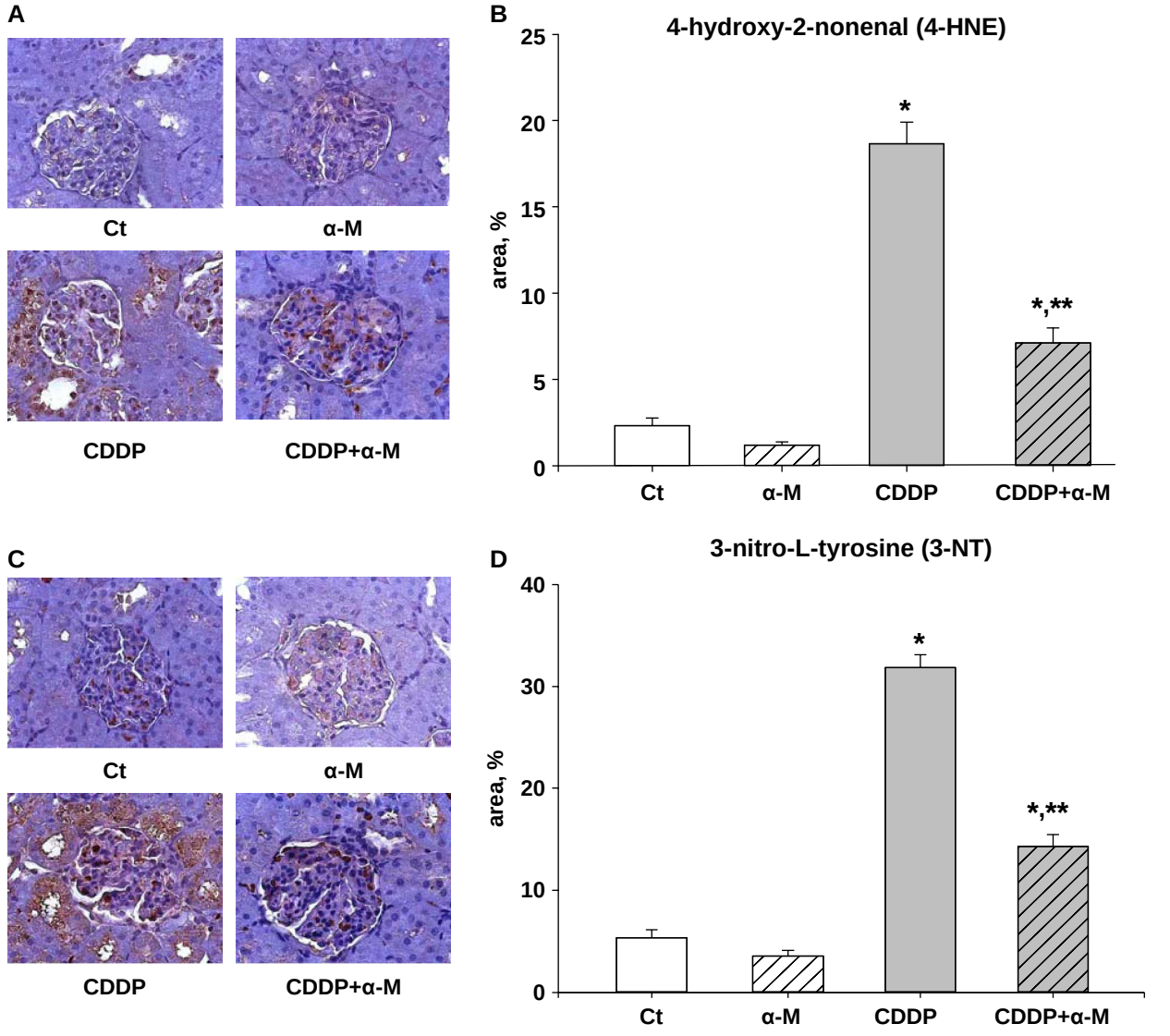


Figure 4. Renal immunostaining of 4-HNE and 3-NT in the four groups studied. (A) Representative microphotographs of 4-HNE. (B) Percentage area of 4-HNE abundance. (C) Representative microphotographs of 3-NT. (D) Percentage area of 3-NT abundance. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. $^{\star}p < 0.001$ vs Ct; $^{\star\star}p < 0.01$ vs CDDP. Values are mean ± SEM of 6–8 rats/group.

α-M before CDDP injection was able to attenuate renal tubular injury at both structural and functional levels. These renoprotective effects of α-M are not secondary to alteration in CDDP pharmacokinetics in CDDP + α-M treated rats. This xanthone was unable to modify renal concentration of Pt and the pharmacokinetic parameters measured (AUC, C_{max} and T_{max}) in CDDP-treated rats.

In this study the participation of oxidative stress was evaluated. GSH is one of the most important intracellular antioxidant compounds. The depletion of GSH induces cellular oxidative stress. A number of studies have disclosed that the metabolism of xenobiotics often produces GSH depletion [25]. In this work we found that CDDP decreases GSH content and α-M treatment was able to totally prevent this diminution. Consistent with the above finding and with previous reports of the literature, it was found that CDDP increases both ROS and RNS [20–22] and that the protective effect of α-M was associated with the improvement of CDDP-induced oxidative and nitrosative stress which were evaluated by measuring urinary (H_2O_2 excretion) and renal (MDA content, protein carbonyl content and the abundance of 3-NT and 4-HNE) markers (Figures 3 and 4). These data suggest that α-M protects the kidney from the CDDP-induced damage through the decrease in oxidative/nitrosative stress.

Our next point was to know which of the antioxidant enzymes may be involved in the α-M protection. In this study several antioxidant enzymes were analysed; it was only found that α-M improved catalase activity during the CDDP-induced nephrotoxicity which is closely associated with the decrease

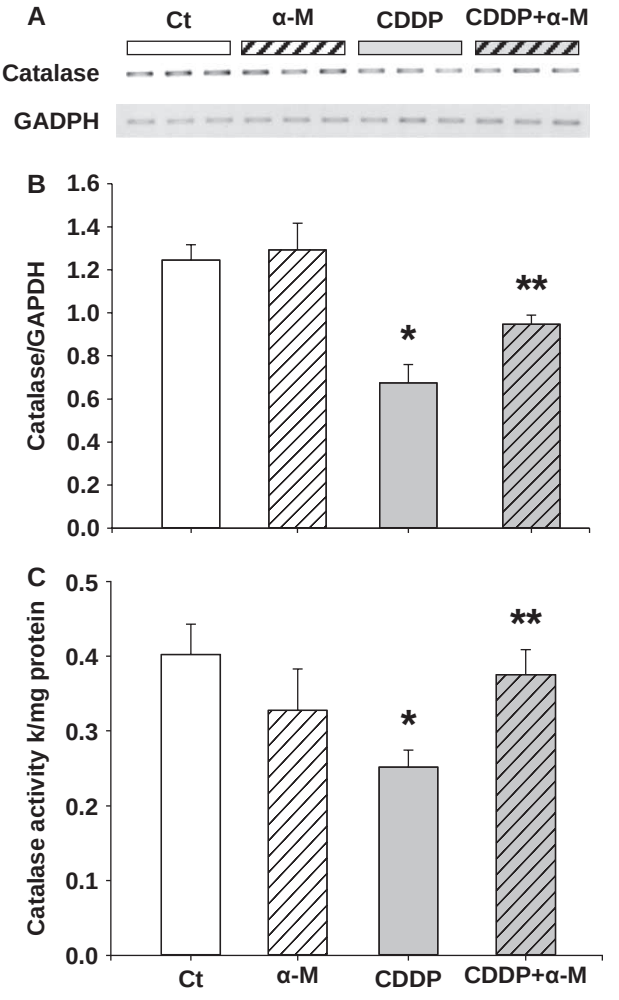


Figure 5. mRNA levels and enzymatic activity of catalase in the kidneys of the four groups studied. (A) Representative gels showing mRNA expression of catalase and GAPDH, (B) mRNA quantification of catalase, (C) catalase activity. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. *$p < 0.05$ vs Ct; **$p < 0.05$ vs CDDP. Values are mean ± SEM of 5–8 rats/group.

in urinary excretion of H_2O_2 observed in the CDDP + α-M group. The above results suggest that the preservation of catalase activity is involved in the renoprotective effect of α-M against CDDP. In contrast with other authors [8,26], we did not find changes in GPx and SOD activities in CDDP-treated animals. These differences may be due to the strain of animals used, dose of CDDP, route of administration and time of exposition to the drug. Consistent with the above data, it was found that MnSOD mRNA levels were unchanged in all groups, whereas CDDP-treatment decreased catalase mRNA levels which were totally prevented by α-M.

It was found that in addition to the attenuation of oxidative/nitrosative stress and the preservation of catalase activity, the anti-inflammatory properties of α-M are involved in its renoprotective effect on CDDP-induced nephrotoxicity. In the past years, there is growing evidence that the inflammation is another pathway implicated in the CDDP-induced

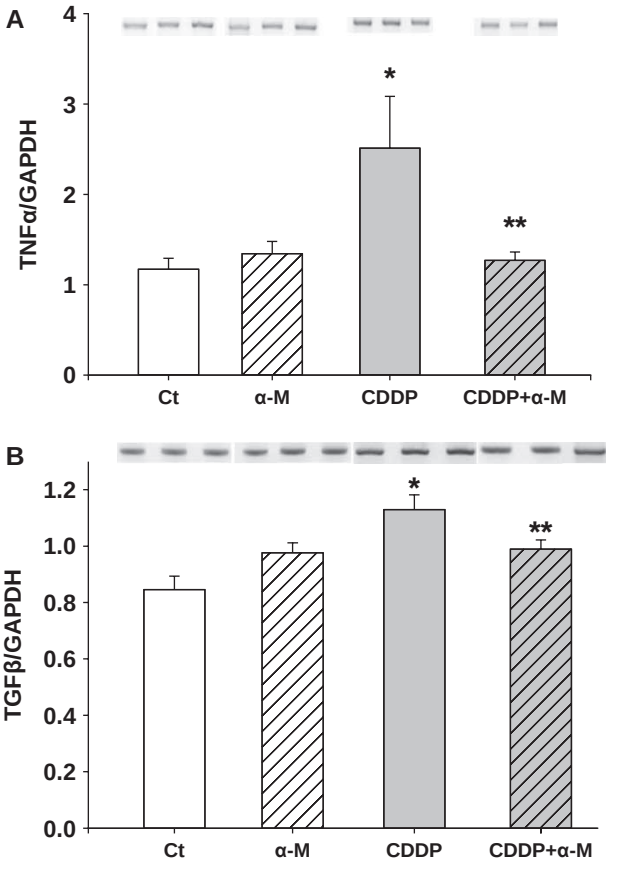
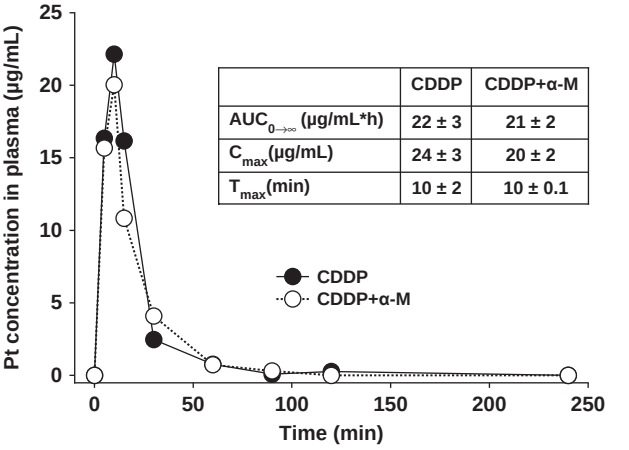


Figure 6. Intrarenal gene expression of (A) TNFα and (B) TGFβ in the four groups studied. Ct, control; α-M, alpha-mangostin; CDDP, cisplatin. *$p < 0.05$ vs Ct; **$p < 0.05$ vs CDDP. Values are mean ± SEM of five rats/group.

nephrotoxicity; this drug promotes the recruitment of inflammatory cells, as well as some cytokines as TNFα [27]. We previously reported that CDDP promotes infiltration of monocytes and macrophage



	CDDP	CDDP+α-M
$AUC_{0\rightarrow\infty}$ (µg/mL*h)	22 ± 3	21 ± 2
C_{max}(µg/mL)	24 ± 3	20 ± 2
T_{max}(min)	10 ± 2	10 ± 0.1

Figure 7. Effect of α-M on CDDP pharmacokinetics. Pt concentration was measured in plasma at different interval times after CDDP injection in CDDP and CDDP + α-M groups. AUC = area under the curve, C_{max} = maximum concentration of Pt and T_{max} = maximum time of Pt in plasma. Values are mean ± SEM of five rats/group.

and the inhibition of inducible nitric oxide synthase (this enzyme is up-regulated by external factors and cytokines) was capable of preventing that effect [20]. Taking into account the above data, the pro-inflammatory molecule TNFα was measured; it was clear how α-M treatment totally prevented the increase of TNFα mRNA levels expression during CDDP-induced nephrotoxicity. In this same context, macrophages infiltration promotes growth factors as TGFβ that induces extracellular matrix such as collagen and fibronectin [28]. It was found that α-M prevents the increase in this molecule in rats with CDDP-induced nephrotoxicity.

In summary, according to the data obtained in this work, the possible mechanisms by which α-M produces the renoprotective effects are: (1) attenuation of oxidative/nitrosative stress and prevention of the decrease in catalase activity and (2) attenuation the inflammatory and fibrotic pathways.

In conclusion, it was demonstrated that the renoprotective effect of α-M in CDDP-induced nephrotoxicity seems to be multifactorial; it is related to the attenuation of free radical-induced damage (oxidative/nitrosative stress), inflammatory responses, fibrotic pathways and in the preservation of the catalase activity. α-M can be an alternative therapeutic strategy in CDDP-treated patients, which may improve the life style and avoid drug discontinuity. However, further experiments in humans are needed to test this hypothesis.

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