Transforming Growth Factor- β 1/Smad3 Signaling and Redox Status in Experimentally Induced Nephrotoxicity: Impact of Carnosine

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Abstract Exposure to various organic compounds including drugs and environmental toxins causes cellular damage through generation of free radicals. Carnosine a dipeptide was used in this study to evaluate its effect against CCl₄-induced nephrotoxicity. Sixty male albino rats were involved in this study and were equally divided into four groups. CCl₄ (3 ml/kg body weight; biweekly for 4 weeks) was given to group II and III. Carnosine (10 mg/kg body weight; once daily for 4 weeks) was given to group III and VI. Transforming growth factor- β 1 (TGF- β 1) level by immunoassay and Smad3 mRNA level by real-time PCR were estimated in addition to cytochrome P450 2E1 (CYP2E1) activity, renal functions, redox status assessment and histopathological examination of the kidney. Carnosine significantly improved kidney function, renal redox status, decreased renal CYP2E1 activity, TGF- β 1 level and Smad3 gene expression when compared to CCl₄-intoxicated group. The protective effect of carnosine was confirmed by histopathological study. In conclusion: carnosine has the ability to protect against CCl₄-induced nephrotoxicity possibly by alleviating oxidative stress, normalizing kidney histopathological architecture in addition to the disruption of the inflammatory and fibrotic response induced by CCl₄.

Keywords Carnosine · Carbon tetrachloride · Cytochrome P450 2E1 · Transforming growth factor- β 1 · Smad 3

Introduction

Exposure to environmental pollutants, drug and ionizing radiation causes inflammation and oxidative stress. Carbon tetrachloride (CCl₄) is one of the chlorinated hydrocarbons with wide spread use in various industries so it is considered as an environmental toxin because of its leakage into air, water and soil. Hepatic and renal tubular necrosis is the typical manifestation of CCl₄-intoxication however; the pathogenesis of CCl₄-induced renal damage remains largely elusive [1]. CCl₄ acts through activation of phase I cytochrome P450 system especially cytochrome P450 2E1 (CYP2E1) with formation of reactive metabolites as trichloromethyl radicals (CCl₃) and peroxy trichloromethyl radicals (OOCCl₃). Free radicals cause oxidative damage of membrane lipids and proteins with subsequent impairment of kidney function [2].

Renal diseases occur as result of exposure to broad range of diverse injurious agents. Transforming growth factor-beta (TGF- β) is a cytokine controls the key cellular response to injurious agents and is the key mediator of tissue repair, inflammation, immune response and fibrosis [3]. TGF- β 1 acts through different signaling pathways, among them Smad pathway is the most important one. Smad proteins act as primary mediators of inflammatory and fibrotic response [4]. Carnosine (β -alanyl-L-histidine) is a dipeptide with anti-ischemic, anti-oxidant, membrane stabilizing, heavy metal chelating activities, and reduces matrix proteins synthesis such as fibronectin, collagen of podocytes and mesangial cells [5, 6].

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Materials and Methods

This research was carried out in accordance to the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) to minimize animal suffering and in accordance with the guidelines of the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt (Approval code: 3088/02/5).

Chemicals

Carnosine, CCl₄, other chemicals and solvents used unless otherwise described were purchased from Sigma (Sigma, St Louis, USA).

Induction of Nephrotoxicity by CCl₄

Sixty male albino rats (170–200 g) were obtained from Animal House of the National Research Center, Dokki, Cairo, Egypt. The animals were kept in ordinary cages at room temperature with 12 h dark/light cycles and allowed access ad libitum to commercial rodent chow and tap water (EL-Nasr Chemical Company, Cairo, Egypt) through the whole period of the experiment. All rats were acclimatized for 2 weeks before the experiment. Nephro-toxicity was induced by intra-peritoneal injection of CCl₄ (3 ml/kg body weight; 30 % in olive oil) biweekly for 4 weeks [7]. Rats were divided into four groups of 15 rats each. Group I: served as control received vehicle only (olive oil). Group II: (nephrotoxic group) rats were intoxicated with CCl₄ (3 ml/kg body weight; biweekly for 4 weeks). Group III: rats were intoxicated with CCl₄ as previously and co-treated with carnosine (10 mg/kg body weight; intra-peritoneal) once daily for 4 weeks [5]. Group IV: rats were treated with carnosine at the same previous dose.

Blood and Tissue Collection

At the end of the experiment, the overnight fasted rats were weighed, anesthetized by ether and sacrificed. Serum was separated after blood collection and then stored in aliquots at −80 °C until analysis; kidneys were removed, weighed then chilled on ice. Kidney of each rat was cut into small pieces. One piece was immersed in neutral buffered formalin 10 % and fixed for assessment of the morphological changes in the kidney by hematoxylin and eosin (H&E) stained sections. The second one was homogenized on ice with 50 mM phosphate buffer (pH 7.4) then centrifuged at 11,000×g for 20 min at 4 °C then the supernatant was frozen at −80 °C until analysis. Piece of 0.5 g was used for microsomal separation as previously described [8]. The

remaining piece was stored in liquid nitrogen at −80 °C for RNA extraction. Renal tissue protein content was determined by the method of Lowry et al. [9].

Biochemical Analysis

Assessment of Nephrotoxicity

Serum creatinine and urea levels were estimated by commercial kits (Diamond Diagnostic, Egypt).

Assessment of Oxidative Stress, Inflammation and Cytotoxicity in Renal Tissue

Oxidative damage to protein was quantified by measurement of protein carbonyl level as previously described [10]. Myeloperoxidase (MPO) and microsomal CYP2E1 activities were assayed as previously described [11, 12]. Total antioxidant capacity (TAC) level was assayed by commercial kit (Bio-diagnostic, Egypt).

Assessment of TGF-β1 Level

Renal TGF-β1 level was estimated by rat TGF-beta1 Platinum ELISA kit (Affymetrix eBioscience Inc., USA) according to the manufacturer's instructions.

Molecular Analysis

RNA Extraction, cDNA Synthesis and Real-Time PCR for Smad3 Gene

Total RNA was extracted from frozen kidney tissues after processing using Qiagen RNeasy Total RNA isolation kit (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer, followed by synthesis of the first strand using SuperScript[®] III First-Strand Synthesis System for RT-PCR kit (Life Technologies) according to the manufacturer's instructions. PCR reactions were performed using Power SYBR Green PCR Master Mix (Life Technologies) following the manufacturer's instructions. Smad3 mRNA transcripts were quantified, relative to the housekeeping gene, glyceraldehyde-3-phosphatedehydrogenase (GAPDH) which was used as an internal control. Sequence specific primers were designed by Primer3 software: (<http://bioinfo.ut.ee/primer3/>) as follows: rat Smad3 forward primer (5'-AGGGCTTTGAGGCTGTC-TACC-3') and reverse primer (5'-ACCCGATCCCTT-TACTCCA-3') (GenBank Accession No. NM_016769.4); rat GAPDH forward primer (5'-GGTGAAGTTCGGAGT-CAACGGA-3') and reverse primer (5'-GAGG-GATCTCGCTCCTGGAAGA-3') (GenBank Accession

No. NM_017008). The final results were automatically calculated from the cross-point values of the target and the reference gene by Rotor-Gene Q 6plex and its specific software (Qiagen, Valencia, CA, USA).

Statistical Analysis

Statistical analysis was conducted as mean and standard deviation using Statistical Package for Social Sciences (SPSS), version 16.0 for Windows (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for multiple comparisons to evaluate the statistical significance between experimental groups followed by post hoc test. *P* value <0.05 was considered significant.

Results

Body and Kidney Weighs

Rats intoxication with CCl₄ (group II) significantly decreased body and kidney weighs compared to control (group I). Meanwhile, carnosine co-treatment improved the effect of CCl₄ on body and kidney weighs (Table 1).

Nephrotoxicity Indices

Intoxication of rats with CCl₄ (group II) significantly increased serum urea and creatinine levels when compared to control (group I). Meanwhile, simultaneous administration of carnosine to CCl₄-intoxicated rats significantly improved renal functions to be normalised when compared to CCl₄-intoxicated rats (group II) (Table 1).

Cytotoxicity Study

Intoxication of rats with CCl₄ (group II) significantly increased CYP2E1 activity when compared to control (group I). Meanwhile, simultaneous administration of

carnosine to CCl₄-intoxicated rats significantly decreased CYP2E1 activity when compared with CCl₄ intoxicated rats (group II) (Table 2).

Assessment of Redox Status

Intoxication of rats with CCl₄ (group II) significantly decreased renal TAC level when compared to controls (group I). Meanwhile, simultaneous administration of carnosine to CCl₄-intoxicated rats significantly increased TAC level to be insignificant with control. Intoxication of rats with CCl₄ (group II) significantly increased renal tissue protein carbonyl level and MPO activity when compared to controls (group I). Meanwhile, significant decrease in renal tissue protein carbonyl and MPO activity were observed upon carnosine administration when compared with CCl₄-intoxicated rats (group II) (Table 2).

Assessment of Renal Smad3 mRNA and TGF-β1 Levels

Intoxication of rats with CCl₄ (group II) significantly increased renal Smad3 mRNA and renal TGF-β1 levels when compared to control (group I). Meanwhile, simultaneous administrations of carnosine to CCl₄-intoxicated rats (group III) significantly decreased TGF-β1 level and down-regulate *Smad3* gene expression (Table 2).

Results of Renal Histopathological Study

Kidney histopathological study of control rats (group I) and carnosine treated rats (group IV) showed normal architecture as regards to tubules, glomeruli, and tubulo-interstitial cells (Figs. 1, 4), respectively). However, Kidney sections of CCl₄-treated rats showed glomerular degenerations/segmentation, glomerular basement membrane thickening, mild dilatation of space of Bowman, tubule-interstitial inflammation, tubular dilatation and tubular cell necrosis with shedding of tubular epithelial cells into the tubular

Table 1 Effect of carnosine on body/kidney weighs and renal indices

Parameters/groups	Group I ^a (control group) (n = 15)	Group II ^b (CCl ₄ -intoxicated group) (n = 15)	Group III ^c (CCl ₄ -intoxicated + carnosine co-treated) (n = 15)	Group IV ^d (carnosine treated group) (n = 15)	<i>P</i> value
Body weight (g)	214.7 ± 1.26 ^{bc}	172.1 ± 12.3 ^{acd}	184.58 ± 7.05 ^{abd}	216.4 ± 2.05 ^{bc}	0.009*
Kidney weight (g)	2.74 ± 0.08 ^{bc}	4.14 ± 0.6 ^{acd}	3.03 ± 0.17 ^{abd}	2.68 ± 0.24 ^{bc}	0.0014*
Serum creatinine level (mg/dL)	0.56 ± 0.05 ^b	0.82 ± 0.04 ^{acd}	0.56 ± 0.04 ^b	0.54 ± 0.04 ^b	0.001*
Serum urea level (mg/dL)	49.4 ± 3.52 ^b	74.7 ± 3.03 ^{acd}	50.3 ± 3.56 ^b	47.8 ± 3 ^b	0.008*

Significant difference between groups at * *p* < 0.05 calculated by one-way ANOVA test followed by Tukey's post hoc test

^a Significance from group I; ^b significance from group II; ^c significance from group III; ^d significance from group IV

Table 2 Effect of carnosine on renal total antioxidant capacity (TAC) level, myeloperoxidase (MPO) activity, protein carbonyl level, cytochrome P450 2E1 (CYP2E1) activity, relative Smad3 mRNA level and transforming growth factor- β 1 (TGF- β 1) level

Parameters/groups	Group I ^a (control group) (n = 15)	Group II ^b (CCl ₄ intoxicated group) (n = 15)	Group III ^c (CCl ₄ intoxicated + carnosine co-treated) (n = 15)	Group IV ^d (carnosine treated group) (n = 15)	P value
Renal TAC (μ mol/g tissue)	5.26 $\pm$ 0.19 ^b	3.44 $\pm$ 0.22 ^{acd}	5.04 $\pm$ 0.36 ^b	5.26 $\pm$ 0.36 ^b	0.0012*
Renal MPO activity (μ mol/min/mg protein)	0.87 $\pm$ 0.1 ^{bc}	1.97 $\pm$ 0.94 ^{acd}	1.3 $\pm$ 0.15 ^{abd}	0.83 $\pm$ 0.1 ^{bc}	0.007*
Renal protein carbonyl level (nmol/mg protein)	6.85 $\pm$ 0.58 ^{bc}	16.59 $\pm$ 2.01 ^{acd}	8.2 $\pm$ 0.9 ^{abd}	6.69 $\pm$ 1.7 ^{bc}	0.001*
Renal CYP2E1 activity (pmol/min/mg protein)	326.6 $\pm$ 15.2 ^{bc}	890.1 $\pm$ 52.6 ^{acd}	600.5 $\pm$ 60.5 ^{abd}	316.4 $\pm$ 15.8 ^{bc}	0.0015*
Relative renal Smad3 mRNA level	0.32 $\pm$ 0.02 ^{bc}	1.89 $\pm$ 0.38 ^{acd}	0.89 $\pm$ 0.07 ^{abd}	0.33 $\pm$ 0.02 ^{bc}	0.008*
Renal TGF- β 1 level (Pg/mg protein)	49.55 $\pm$ 3.6 ^{bc}	96.6 $\pm$ 5.9 ^{acd}	61.4 $\pm$.54 ^{abd}	50.8 $\pm$ 2.9 ^{bc}	0.001*

Significant difference between groups at * $p < 0.05$ calculated by one-way ANOVA test followed by Tukey's post hoc test

^a Significance from group I; ^b significance from group II; ^c significance from group III; ^d significance from group IV

Fig. 1 Histopathological features of group I (control) kidney stained with H&E with normal glomerular and tubular architecture (magnification power $\times 400$)

lumen (Fig. 2). Meanwhile, Kidney section of CCl₄-intoxicated rats co-treated with carnosine revealed regeneration of renal cells, glomeruli and tubules nearly as seen in control rats except for mild tubulo-interstitial inflammatory infiltrate (Fig. 3).

Discussion

Kidney is the main route for elimination of unmodified drugs and metabolites. The mechanism underlying CCl₄-induced nephrotoxicity remains controversial. CCl₄-induced oxidative stress is an important element in initiation

Fig. 2 Histopathological features of group II (CCl₄-intoxicated rats) kidney stained with H&E with abnormal renal architecture in the form of tubular degeneration, tubular cell necrosis and epithelial shedding, tubulointerstitial inflammatory cellular infiltrates and in addition to glomerular necrosis and segmentation (magnification power $\times 400$)

and progression of organ damage through up-regulation of pro-inflammatory and chemotactic mediators like TGF- β 1 [3]. Therefore, it is valuable to identify new compounds which can antagonize the deleterious action of CCl₄-derived free radicals and protect kidney from damage. Carnosine an endogenously synthesized dipeptide with many beneficial effects was used in this study [5].

This study revealed impaired kidney functions in rats intoxicated with CCl₄ as a result of CCl₄-induced free radical generation which initiates lipid peroxidation of the cell membrane causing disturbance of membrane fluidity,

Fig. 3 Histopathological features of group III (CCl₄-intoxicated rats and co-treated with carnosine) kidney stained with H&E showing normal tubular and glomerular architecture except for some inflammatory cells infiltration (magnification power $\times 400$)

Fig. 4 Histopathological features of group IV (treated with carnosine only) kidney stained with H&E showing normal tubular and glomerular architecture (magnification power $\times 400$)

cation transport and Na^+/K^+ -ATPase activity with subsequent injury to the renal tubular cells [13]. These results were in concomitant with results obtained previously [13, 14]. Meanwhile, carnosine co-treatment improved kidney functions which may be endorsed to its membrane stabilizing and anti-oxidant effect which neutralize CCl₄ toxic effect and recover the injured kidney [5]. Aldini et al. [15] reported that carnosine improved dyslipidaemia, hypertension and renal injury in obese Zucker rat and proposed that some of its biological effects can be mediated by direct free radicals quenching mechanism.

Furthermore, this study showed significant increase in CYP2E1 activity in CCl₄-intoxicated rats which was significantly decreased upon carnosine co-treatment. These results suggested that the suppression of CYP2E1 activity

may be an important aspect of carnosine reno-protective effect. Yan et al. [16] suggested that carnosine has protective effects against acetaminophen-induced hepatotoxicity through improving redox, inflammatory status and decreasing cytochrome P450 2E1.

CCl₄ indirectly activates macrophages, neutrophils and inflammatory cells by producing tumor necrosis factor- α (TNF- α) and other cytotoxic cytokines with subsequent activation NF- κ B pathway.

Moreover, CCl₄-induced free radicals generation initiate DNA, proteins and lipid damage which signal activation of the transcription factor nuclear factor- κ B (NF- κ B). Upon activation, NF- κ B shifts to the nucleus and binds to promoters of genes involved in inflammatory and proliferative response as TGF- β 1 with more oxidative stress and vicious circle [3].

MPO is an enzyme secreted by white blood cells and catalyzes reaction of chloride ion with hydrogen peroxide (H_2O_2) generating hypochlorous acid (HOCl) which is a powerful chlorinated oxidant. MPO activity is a biochemical index for neutrophil infiltration and extent of inflammatory damage [17]. This study revealed that CCl₄-intoxication caused significant increase in MPO activity and protein carbonyl level however decreased renal TAC level which reflects the intensity of neutrophil infiltration, inflammation and protein oxidation. Meanwhile, carnosine co-treatment significantly decreased MPO activity and protein carbonyl level however increased renal TAC level. Baykara et al. [18] reported that carnosine improved redox status and MPO in alcohol-induced liver injury. Yilmaz-Ozden et al. [19] indicated that *Amaranthus lividus* L extract possesses protective and anti-oxidant effects against CCl₄-induced oxidative kidney damage through improved redox status and MPO activity. Moreover, Chen et al. [20] found that fraxetin significantly reduced protein carbonyl level compared with CCl₄ treated group.

Renal fibrosis is the excessive accumulation of extracellular matrix and is main underlying mechanism for chronic kidney disease progression. TGF- β 1 is one of the pro-fibrotic mediators and inflammatory cells chemo-attractant factor in chronic diseases which acts through different signaling pathways from which Smad3 pathway is the most important one [4]. This study revealed increased TGF- β 1 level and up-regulation of *Smad3* gene expression in CCl₄-intoxicated rats. These results may be due to excessive free radicals generation and activation of NF- κ B with more inflammation, oxidative stress and finally renal damage [21]. Meanwhile, carnosine co-treatment significantly decreased TGF- β 1 and down-regulate *Smad3* gene expression in the present study. Janssen et al. [22] reported that carnosine protects against the adverse effects of high glucose level on renal cells through decreased production of fibronectin and collagen type VI in podocyte and TGF-

β 1 level. Furthermore, Yao et al. [23] proved that curcumin with anti-oxidant effect, significantly attenuated the severity of CCl₄-induced liver inflammation and fibrosis through inhibition of TGF- β 1/Smad3 signaling pathway.

Results of renal histopathological study from CCl₄ intoxicated rats showed tubule-glomerular degeneration and necrosis in addition to tubulo-interstitial infiltration with inflammatory cells which were in harmony with results obtained previously [5, 24]. The improved histopathological findings with carnosine co-treatment gives an additional support that carnosine protected the kidney from damage induced by CCl₄ through quenching of the free radicals generated by CCl₄, reduced inflammation, improved kidney function, and induced healthy state of renal cells suggesting its role as renal protective agent. Yay et al. [25] reported that carnosine treatment prevented the renal morphological damage caused by diabetes in addition carnosine might be a useful therapeutic option to reduce oxidative stress and associated renal injury in diabetes mellitus.

Conclusion

Carnosine protects kidney against CCl₄-induced damage through the disruption of TGF- β /Smad3 signaling, improvement of inflammation, cytotoxicity and maintenance of oxidant/anti-oxidant balance. Therefore, carnosine may be beneficial as being a reno-protective agent against the toxic effect of CCl₄, other chemicals and drugs also it may be beneficial against progression of renal diseases.

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Compliance with Ethical Standards

Conflict of interest The author declares that there is no conflict of interest.

References

- Koc Y, Sokmen M, Unsal A, Cigerli S, Ozagari A, Basturk T, et al. Effects of human umbilical cord stem cells and granulocyte colony-stimulating factor (G-CSF) on carbon tetrachloride-induced nephrotoxicity. *Nephrourol Mon.* 2012;4(3):545–50. doi:10.5812/numonthly.2979.
- Khan RA, Khan MR, Sahreen S. Evaluation of *Launaea procumbens* use in renal disorders: a rat model. *J Ethnopharmacol.* 2010;128(2):452–61. doi:10.1016/j.jep.2010.01.026.
- Ma JQ, Ding J, Xiao ZH, Liu CM. Ursolic acid ameliorates carbon tetrachloride-induced oxidative DNA damage and inflammation in mouse kidney by inhibiting the STAT3 and NF- κ B activities. *Int Immunopharmacol.* 2014;21(2):389–95. doi:10.1016/j.intimp.2014.05.022.
- García-Sánchez O, López-Hernández FJ, López-Novoa JM. An integrative view on the role of TGF-beta in the progressive tubular deletion associated with chronic kidney disease. *Kidney Int.* 2010;77(11):950–5. doi:10.1038/ki.2010.88.
- Noori S, Mahboob T. Antioxidant effect of carnosine pretreatment on cisplatin-induced renal oxidative stress in rats. *Indian J Clin Biochem.* 2010;25(1):86–91. doi:10.1007/s12291-010-0018-x.
- Kiliš-Pstrušínska K. Carnosine, carnosinase and kidney diseases. *Postepy Hig Med Dosw.* 2012;66:215–21.
- Khan RA, Khan MR, Sahreen S, Bokhari J. Prevention of CCl₄-induced nephrotoxicity with *Sonchus asper* in rat. *Food Chem Toxicol.* 2010;48(8–9):2469–76. doi:10.1016/j.fct.2010.06.016.
- Guengerich FP, Churchill PF, Jung CY, Fleischer S. Target inactivation analysis applied to determination of molecular weights of rat liver proteins in the purified state and in microsomal membranes. *Biochim Biophys Acta.* 1987;915(2):246–53.
- Lowry OH, Rosenbrough NG, Farr AL, Randall RJ. Protein measurements with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265–75.
- Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 1990;186:464–78.
- Bradley PP, Priebat DA, Christensen RD, Rothstein GR. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol.* 1982;78(3):206–9.
- Koop DR. Hydroxylation of *p*-nitrophenol by rabbit ethanol-inducible cytochrome P-450 isozyme 3a. *Mol Pharmacol.* 1986;29(4):399–404.
- Metwally NS, Hamed MA, Ahmed SA. Association between efficiency of certain medicinal plants and severity of renal disorders in rats. *Int J Pharm Pharm Sci.* 2012;4(3):432–8.
- Hismiogullari AA, Hismiogullari SE, Karaca O, Sunay FB, Paksoy S, Can M, et al. The protective effect of curcumin administration on carbon tetrachloride (CCl₄)-induced nephrotoxicity in rats. *Pharmacol Rep.* 2015;67(3):410–6. doi:10.1016/j.pharep.2014.10.021 (Epub 2014 Nov 11).
- Aldini G, Orioli M, Rossoni G, Savi F, Braidotti P, Vistoli G, et al. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. *J Cell Mol Med.* 2011;15(6):1339–54. doi:10.1111/j.1582-4934.2010.01101.x.
- Yan SL, Wu ST, Yin MC, Chen HT, Chen HC. Protective effects from carnosine and histidine on acetaminophen-induced liver injury. *J Food Sci.* 2009;74(8):H259–65. doi:10.1111/j.1750-3841.2009.01330.x.
- Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta.* 2003;329:23–38.
- Baykara B, Micili SC, Tugyan K, Tekmen I, Bagriyanik H, Sonmez U, et al. The protective effects of carnosine in alcohol-induced hepatic injury in rats. *Toxicol Ind Health.* 2014;30(1):25–32. doi:10.1177/0748233712446722.
- Yilmaz-Ozden T, Can A, Karatug A, Pala-Kara Z, Okyar A, Bolkent S. Carbon tetrachloride-induced kidney damage and protective effect of *Amaranthus lividus* L. in rats. *Toxicol Ind Health.* 2014;0748233714555390. [Epub ahead of print].
- Chen X, Ying X, Zhang W, Chen Y, Shi C, Hou Y, et al. The hepatoprotective effect of fraxetin on carbon tetrachloride induced hepatic fibrosis by antioxidative activities in rats. *Int Immunopharmacol.* 2013;17(3):543–7. doi:10.1016/j.intimp.2013.08.006.
- Shimizu H, Bolati D, Adijiang A, Muteliefu G, Enomoto A, Nishijima F, et al. NF-kappaB plays an important role in indoxyl

- sulfate-induced cellular senescence, fibrotic gene expression, and inhibition of proliferation in proximal tubular cells. *Am J Physiol Cell Physiol.* 2011;301:C1201–12. doi:[10.1152/ajpcell.00471.2010](https://doi.org/10.1152/ajpcell.00471.2010).
22. Janssen B, Hohenadel D, Brinkkoetter P, Peters V, Rind N, Fischer C, et al. Carnosine as a protective factor in diabetic nephropathy: association with a leucine repeat of the carnosinase gene CNDP1. *Diabetes.* 2005;54(8):2320–7.
23. Yao QY, Xu BL, Wang JY, Liu HC, Zhang SC, Tu CT. Inhibition by curcumin of multiple sites of the transforming growth factor-beta1 signalling pathway ameliorates the progression of liver fibrosis induced by carbon tetrachloride in rats. *BMC Complement Altern Med.* 2012;12:156. doi:[10.1186/1472-6882-12-156](https://doi.org/10.1186/1472-6882-12-156).
24. Hamed MA, Ali SA, El-Rigal NS. Therapeutic potential of ginger against renal injury induced by carbon tetrachloride in rats. *Sci World J.* 2012;2012:840421. doi:[10.1100/2012/840421](https://doi.org/10.1100/2012/840421) (**Epub 2012 Apr 1**).
25. Yay A, Akkuş D, Yapıslar H, Balcioglu E, Sonmez MF, Ozdamar S. Antioxidant effect of carnosine treatment on renal oxidative stress in streptozotocin-induced diabetic rats. *Biotech Histochem.* 2014;89(8):552–7. doi:[10.3109/10520295.2014.913811](https://doi.org/10.3109/10520295.2014.913811).