Ameliorative Effect of Sesamin in Cisplatin-Induced Nephrotoxicity in Rats by Suppressing Inflammation, Oxidative/Nitrosative Stress, and Cellular Damage

BADRELDIN H. ALI[1*], SUHAIL AL SALAM[2], YOUSUF AL SULEIMANI[1], MOHAMED AL ZA'ABI M, MOHAMMED ASHIQUE[1], PRIYADARSINI MANOJ[1], MANJUSHA SUDHADEVI[2], MOHAMMED AL TOUBI[3], ABDERRAHIM NEMMAR[4]

1. Department of Pharmacology and Clinical Pharmacy, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman.
2. Department of Pathology, College of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.
3. Department of Biochemistry and Clinical Pharmacy, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman.
4. Department of Physiology, College of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates

Running title: Sesamin and cisplatin nephrotoxicity

Corresponding author: B. H. Ali, Department of Pharmacology, College of Medicine, Sultan Qaboos University, P O Box 35 Al Khod, Postal code 123, Oman

Summary

Nephrotoxicity of cisplatin (CP) involves renal oxidative stress and inflammation, and sesamin (a major liganin in many plants) has strong antioxidant and antiinflammatory actions. Therefore, we investigated here the possible mitigative action of sesamin on CP nephrotoxicity in rats. Sesamin was given orally (5 mg/kg/day, 10 days), and on the 7^{th} day, some of the treated rats were injected intraperitoneally with either saline or CP (5mg/kg). On the 11^{th} day, rats were sacrificed, and blood and urine samples and kidneys were collected for biochemical estimation of several traditional and novel indices of renal damage in plasma and urine, several oxidative and nitrosative indices in kidneys, and assessment of histopathological renal damage. CP significantly and adversely altered all the physiological, biochemical and histopathological indices of renal function measured. Kidneys of CP-treated rats had a moderate degree of necrosis. This was markedly lessened when CP was given simultaneously with sesamin. Sesamin treatment did not significantly alter the renal CP concentration. The results suggested that sesamin had ameliorated CP nephrotoxicity in rats by reversing the CP – induced oxidative stress and inflammation. Pending further pharmacological and toxicological studies sesamin may be considered a potentially useful nephroprotective agent.

Key words: Cisplatin • nephrotoxicity • sesame oil • sesamin • rats

Introduction

Cisplatin (CP) is a major and potent platinum-based antineoplastic agent that is used in the treatment of a wide range of cancers, including otherwise resistant solid tumors. It is currently among the most widely used agents in the chemotherapy of lymphomas, stomach, esophageal, pancreatic, bladder, head and neck, lung, ovarian, and testicular cancers (Dasari and Tchounwou. 2016, Ojima *et al*. 2018). Nephrotoxicity is known to be the dose–limiting side effect of CP-based chemotherapy in cancer patients that leads to acute kidney injury (AKI), followed by chronic renal problems (Latcha *et al*. 2016, Skinner. 2018). CP – induced AKI is a complex process, and has often poor prognosis (Sharp and Siskind. 2017). Wider usage of CP is hampered by nephrotoxicity, as about 25-40% of the cancer patients exhibit a progressive decline in renal function after one dose of CP, and more than 70% of children given CP experience renal dysfunction (Hoek *et al*. 2016, Karasawa and Steyger. 2015).

Currently, there are no completely effective approaches available to prevent CP nephrotoxicity during chemotherapy (Crona *et al.* 2017). Although the pathogenesis of CP nephrotoxicity is not entirely clear, it is known that CP activates multiple signaling pathways in renal tubular cells, leading to inflammation, oxidative stress, tubular cell injury, and death (Karasawa and Steyger. 2015, Zhu *et al*. 2015).

Various strategies, including the use of drugs and phytochemical supplements, have been attempted to either ameliorate or prevent CP nephrotoxicity (Heidari-Soreshjani. 2017, Crona. 2017). These include naturally-occurring and synthetic antioxidants, modulators of nitric oxide, diuretics, cytoprotective and antiapoptotic agents (Ali and Al Moundhri.

2006, Pabla and Dong. 2008, Nematbakhsh *et al*. 2017). Data from animal and limited human studies suggest that use of these approaches may improve oncological outcomes and mitigate toxicity (Prasad. 2004).

Sesame seeds and oil have been widely used in human diet as a healthy food for thousands of years (Namiki 2007, Guo *et al*. 2016). Sesame oil has also been utilized in traditional medicine in, for example, India and the Middle East (Namiki 2007). Sesamin is the most abundant lignan in sesame oil, usually about 0.4% (Fukuda *et al*. 1998, Namiki 2007). It has antiinflammatory (Fan *et al*. 2017a), antioxidant (Wan *et al*. 2015, and antiapoptotic (Fan *et al*. 2017b) actions. As CP nephrotoxicity involves inflammation, oxidative stress and apoptosis (Pabla and Dong *et al*. 2008, Sharp and Siskind. 2017), we thought it worthwhile testing whether sesamin, at a safe dose that have been successfully used before (Tomimori *et al*. 2017), could ameliorate CP-induced nephrotoxicity.

Materials and methods

Ethics statement

Ethical approval for conducting the work was obtained from Sultan Qaboos University (SQU) Animal Ethics Committee (SQU/AEC/2017-15). All procedures involving animals and their care were carried out in accordance with international laws and policies (EEC Council directives 2010/63/EU, 22 September, 2010 and NIH Guide for the Care and Use of Laboratory Animals, NIH Publications, 8th edition, 2011).

Chemicals

Sesamin, carboxymethyl cellulose and platinum standard solution were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA), CP from Mylan S.A.S. (Saint-Priest,

France), and tumor necrosis factor alpha (TNF-α), cystatin C, interleukin-1β (IL-1β), transforming growth factor (TGF-β1) and neutrophil gelatinase-associated lipocalin (NGAL) ELISA kits from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Renalase ELISA kit was purchased from Cusabio Biotech Co., Ltd. (Wuhan, Hubei Province, P. R. China), and superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), total antioxidant capacity (TAC), and malondialdehyde (MDA) assay kits from BioVision, Inc. (Milpitas, CA, USA). Total nitrite oxide assay kit, T-cell immunoglobulin and mucin domain 1(TIM-1) or kidney injury molecule -1 (KIM-1), and liver-type fatty acid–binding protein (L-FABP) ELISA kits were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). *N*-acetyl-β-D-glucosaminidase (NAG) was obtained from Diazyme Laboratories, Inc. (Poway, California, USA), and myeloperoxidase (MPO) assay kit from Abcam, Inc. (Cambridge, UK). Nuclear factor erythroid 2–related factor 2 (Nrf 2) ELISA kit was obtained from Cloud Clone Corp. (Katy, TX, USA). Urea, creatinine, uric acid, calcium, phosphorus and albumin were measured using an automated biochemical analyzer, Mindray BS-120 chemistry analyzer, from Shenzhen Mindray Bio-Medical Electronics Co. (Shenzhen, P. R. China).

Animals and treatments

Male Wistar rats, eight-weeks old and weighing about 200 g were obtained from Sultan Qaboos University Small Animal House. The rats (n = 36) were given free access to water, and a standard nutritionally-adequate laboratory chow diet (Oman Flour mills, Muscat, Oman). They were kept at an ambient temperature of $22 \pm 2°C$, humidity of 60 % and maintained at 12 h/12 h light: dark cycle (light from 6:00 to 18:00).

Two experiments were conducted as follows:

A. After five days of acclimatization, the rats were randomly assigned to four groups and treated as follows:

1. The **first** (Control) received oral saline (0. 4 ml/ kg) only for 10 consecutive days.
2. The **second** group was treated as in group one, but also injected intraperitoneally (i.p.) with CP at a dose of 5 mg/kg, once on the 7th day of treatment
3. The **third** group was treated as in group one, but also given sesamin (5 mg/kg/day) orally for 10 days
4. The **fourth** group was treated with sesamin (5 ml/kg/day) orally for 10 days, and CP on the 7th day of treatment, as in the second group.

B. A similar experiment was carried out in which sesamin was replaced with sesame oil (5 ml/kg/day) orally for 10 days, and CP was injected i.p. on the 7th day.

The doses of sesamin, sesame oil, and CP were selected encompassing doses mentioned in previously published studies, viz Soliman *et al*. 2014, Tomimori *et al*. 2017, and Ali *et al*. 2011 respectively.

One day before the rats were killed, urine of each rat was collected over a 24-hr period, and its volume measured. Immediately after the end of the treatment period, rats were anesthetized with a combination of ketamine (60 mg/ kg) and xylazine (5 mg/kg) given i.p. Blood was then collected from the inferior vena cava in heparinized tubes and centrifuged at 900 *g* for 15 min, at 5°C to separate plasma. The plasma harvested was stored frozen at −80°C pending biochemical analyses within ten days. The rats were then sacrificed by an overdose of anesthesia. The kidneys were removed from the rats, washed with ice-cold saline, blotted with a piece of filter paper and weighed. A small piece from

the left kidney was fixed in 10% buffered formalin. The cortex of the right kidney was excised from the medulla. Part was stored immediately deep frozen at −80°C for measuring platinum concentration and part was rapidly homogenized in ice-cold saline to produce 10% (w/v) tissue homogenate for other biochemical measurements.

Biochemical analysis

Plasma urea, creatinine, uric acid, calcium (Ca), phosphorus (P) and urine albumin and creatinine were measured by an autoanalyzer, as described before (Al Suleimani et al. 2017). Plasma TNF-α, cystatin-C, IL-1β, renalase and NGAL were measured using ELISA kits.

Kidney SOD, CAT, GR, TAC, nitrite, nitrate and total nitric oxide (NO) as well as urinary albumin, creatinine and urine albumin-to-creatinine ratio UACR), kidney injury molecule-1 (KIM-1), fatty acids -binding proteins (FABP), and N-acetyl-β-D-glucosaminidase (NAG) activity were measured as described before using spectrophotometry and ELISA kits (Ali *et al*. 2013, Ali *et al*. 2018).

Measurement of plasma platinum concentration

The concentration of CP (as platinum) in plasma was measured by a standard method of inductively coupled plasma atomic emission spectrometry using an emission wavelength of 265.945 nm, at the Central Analytical and Applied Research Unit, College of Science, SQU, Oman. Platinum atomic absorption spectrophotometer standard solution was used to construct the standard curve.

Histopathological analysis

Kidneys were excised, washed with ice-cold saline, blotted with filter paper and weighed. Each kidney was cassetted and fixed directly in 10% neutral formalin for 24 h, which was followed by dehydration in increasing concentrations of ethanol, clearing with xylene and embedding in paraffin. Four-μm sections were prepared from paraffin blocks and stained with hematoxylin and eosin (H &E). The stained sections were evaluated blindly using light microscopy. The extent of necrosis was measured using Image J software (NIH, USA).

Statistical analysis

Data were given as mean ± SEM, and were analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test (GraphPad Prism version 5.03, San Diego, CA, USA); *P* less than 0.05 was considered statistically significant.

Results

Physiological findings

CP treatment caused a significant reduction in the body-weight of the rats and a significant rise in their kidney weight, relative to the body-weight, and in their urinary output 24 hr after the end of treatment ($P < 0.05$). These changes were significantly mitigated when sesamin was given concomitantly with CP. Treatment with sesamin alone did not cause significant changes in the above parameters (Table 1).

Plasma biochemical indices of renal damage

As shown in Table 2, CP treatment significantly increased creatinine, urea and uric acid, and P concentrations, and decreased that of Ca in plasma, when compared with the control values. CP also increased the activity of the enzyme NGAL. Sesamin was without

any significant effect on these analytes. Sesamin given together with CP significantly mitigated the actions of CP mentioned above.

Urinary biochemical indices of renal function

These results are shown in Table 3. CP treatment significantly increased the urinary concentrations of KIM and L-FABP, NAG activity, and the urine albumin-to-creatinine ratio ($P < 0.05$). CP treatment significantly decreased creatinine clearance ($P < 0.05$).

Inflammatory biomarkers in plasma

The results are shown in Fig 1. CP treatment significantly increased the concentrations of TNF-α, cystatin C, IL-1β, TGF-β1, and MPO activity, and significantly decreased that of renalase ($P < 0.001$). These actions were significantly mitigated when sesamin was given with CP. Treatment with sesamin alone did not significantly affect any of these analytes.

Plasma platinum concentrations

The concentration of platinum in the plasma (in ppm) from cisplatin-treated rats was 0.063 ± 0.009, and 0.056 ± 0.003 in the plasma from rats treated with cisplatin and sesamin ($P > 0.1$). The insignificant reduction in the latter group amounted to about 11%.

Oxidative and nitrosative stress indices in renal homogenates

The results of the oxidative stress are depicted in Fig 2. CP treatment significantly decreased the activities of SOD, CAT and GR, and TAC concentration ($P < 0.0001$, except for the result of CAT, which was at the level of $P < 0.05$). Treatment with sesamin alone significantly increased renal SOD activity ($P < 0.0001$), and slightly and insignificantly increased the levels of the other analytes. CP treatment significantly

increased the Nrf_2 and MDA concentrations ($P < 0.0001$), an action that was significantly reversed by concomitant treatment with sesamin ($P < 0.0001$).

The results on the nitrosative stress are shown in Fig 3. CP significantly increased the renal nitrate concentration and the nitrate / nitrite ratio ($P < 0.05$). Concomitant sesamin treatment to CP – treated rats significantly decreased the nitrate / nitrite ratio (($P < 0.05$) to control level.

Renal Histopathology

The results of renal histopathology examinations are presented in fig. 4 and table 4. The control group (saline IP) showed normal kidney architecture and histology (score 0) {fig. 4 A & B}. The cisplatin (IP) treated group, showed acute tubular necrosis in 52 ± 3.6% of examined tissue areas (score 3), showing tubular distention with necrotic material involving loss of brush border, tubular dilatation, tubular cells necrosis, tubular nuclear pyknosis, tubular nuclear enlargement with hyperchromasia, tubular cells flattening, macrophages within the lumen, epithelial cells within the lumen and intra-luminal eosinophilic material {fig. 4 (C & D)}. The sesamin-treated group showed normal kidney architecture and histology (score 0) {fig. 4 (E & F)}. The (cisplatin + sesamin)-treated group showed dramatic improvement in the histological appearance when compared with the cisplatin-treated group. There was no morphological evidence of acute tubular necrosis in the examined areas (score 0) {fig. 4 (G & H)}.

Sesame oil

Sesame oil, at the dose used, did not significantly alter any of the parameters studied in the sesamin experiment described above (data not shown).

Discussion

In this work, we have found that sesamin (5 mg/kg) was highly and significantly effective in mitigating the physiological, biochemical and histopathological damage induced by CP, without causing any overt adverse effects.

Sesame oil, at the dose used, did not significantly alter any of the parameters studied in the sesamin experiment described above. Raw sesame oil contains 0.5–1.1 % sesamin (Mahendra Kumar and Singh, 2015). The low amount of sesamin the oil may account for its inability to mitigate the indices of renal damage caused CP.

CP is considered the cornerstone of therapy for many types of solid tumors (Crona *et al*. 2017). Despite many studies, the mechanisms underlying the side effects induced by CP are not fully elucidated, but have been suggested to be multi-factorial in nature (Miller *et al*. 2010, Wilmes *et al*. 2015). These mechanisms include the generation of reactive oxygen and nitrogen species, inflammation and apoptosis. The former interferes with the antioxidant defense system and causes oxidative damage in tissues, and reaction with thiols in protein and glutathione, leading to cell dysfunction. Inflammation could be instigated by damage to the renal epithelial cells, and may augment renal injury and dysfunction *in vivo* (Miller *et al*. 2010).

In this work, injection of rats with CP (5 mg/kg) caused a significant decrease in total body weight as well as an increase in kidney/body weight ratio when compared with the control group. The decrease in body weight seen in the CP-treated rats may be due to the gastrointestinal toxicity of the drug (Shahid *et al*. 2018) or possibly to the injured renal tubules, and the consequent inability of the tubular cells to reabsorb water, increased

urine volume voided leading to dehydration and loss of body weight (Ali *et al*. 2008). This renal tubular damage resulted in acute nephrotoxicity reflected in the significant changes in the physiological, biochemical and other indices, as previously reported (Ali *et al*. 2014, Ali *et al*. 2018). These results were supported by light microscopic examination of renal tissues that showed clear pathological changes. Renal structure and function were significantly improved by treatment with sesamin (5 mg/kg).

Among the mechanisms involved in sesamin amelioration of CP-induced nephrotoxicity, are the significant reduction in oxidative and nitrosative stress, apoptosis and inflammation. Oxidative stress is known to have a critical and causal role in CP-induced nephrotoxicity and that it mediates a rise in lipid peroxidation, which is a reliable oxidative stress marker, and nitric oxide increases inflammation and oxidative processes (Meng *et al*. 2017). Sesamin at the relatively low dose used here was effective in significantly abrogating these actions, confirming its strong antioxidant actions. CP is taken up in renal tubular cells in high concentrations, leading to its accumulation and tubular cell injury and death, culminating in acute renal failure (Miller *et al*. 2010).

We have found that CP treatment significantly increased the renal content of (Nrf2), an essential homeostasis master regulator of tolerance to redox stress, and this action was mitigated by sesamin administration. The increase in Nrf2 has recently been implicated in the cellular defense against CP nephrotoxicity (Shelton et al, 2013).

It has recently been reported that sesamin (at a single dose of 25–100 mg/kg) given orally one hour before intraperitoneal injection of lipopolysaccharide (LPS) was effective in protecting mice from LPS-induced acute kidney injury (AKI) by reducing renal oxidative stress, inflammation, and apoptosis (Rousta *et al*. 2018).

The pathogenesis of CP nephrotoxicity, involves inflammation as a major factor, and CP is known to activate the NF-κB pathway, that facilitate the increase in several inflammatory cytokines such as TNF- α and the IL-1β. Both cytokines have been increased in our experiment by CP, an action that was significantly antagonized by sesamin treatment. Cystatin, in addition to being a reliable renal biomarker in both acute kidney injury and chronic kidney disease (Ali et al, 2018), is also considered a biomarker for inflammation (Deyà-Martínez *et al*. 2016).

We used in this work the relatively novel biomarkers TGF-β1, which is used as biomarker in cardiovascular diseases (Mukherji et al. 2017), and lately as a biomarker in surgery-induced renal fibrosis in rat (Ma *et al*. 2018). Here, we found that its concentration in plasma is significantly increased in CP-induced nephrotoxicity, and that sesamin significantly ameliorated that action.

Probably for the first time, we used the activity of the relatively novel enzyme renalase as a biomarker of renal health in rats with CP nephrotoxicity. Renalase was used before as a biomarker for cardiovascular disease (Schlaich *et al*. 2018). Here we have found that CP treatment induces a significant inhibition of the enzyme, whereas sesamin alone causes the opposite action. (Fig. 1). When sesamin was given to CP-treated rats, the enzyme activity was significantly raised to about 80% of the control value. Our results indicate that plasma renalase activity is negatively correlated with renal health in rats, and is different from results in humans with chronic kidney disease, where a significant increase has been observed. This reason(s) for the difference is not certain, but may be due to species difference (Quelhas-Santos and Pestana, 2014, Baek *et al*. 2017).

Previous studies have shown that some agents can reduce CP-induced nephrotoxicity by decreasing the accumulation of platinum renal levels (Kimoto et al. 2013), and some agents mitigate CP nephrotoxicity without significantly affecting these levels (Ali *et al.* 2013).

It was of interest to note that the ameliorative action of sesamin on CP nephrotoxicity was not related to any possible reduction of CP accumulation in the plasma, as we found only a slight (about 11%) and insignificant reduction in the concentration of platinum in plasma of sesamin-treated rats. It is established that a significant rise in CP concnetrations in the kidneys and plasma occurs in cases of nephrotoxicity (Darwish *et al*. 2017).

Further experiments are warranted to determine the specific molecular pathways by which the nephroprotective action of sesamin is carried out. Several molecular pathways have previously been suggested to explain the salutary action of sesamin in various experimentally-induced conditions. For example, sesamin has been reported to protect against cardiac remodeling in rodents induced by transverse aortic constriction via the Sirt3/ROS pathway (Fan *et al.* 2017), several dietary restriction-related signaling pathways, including processes requiring SIRT1, TOR, and AMPK in *Caenorhabditis elegans*, and the inhibition of the TLR4 expression and NF-κB activation in LPS-induced acute lung injury in mice (Qiang *et al*. 2016). NF-κB activation, toll-like receptor 4 (TLR4), cyclooxygenase-2 (COX2), tumor necrosis factor α (TNF-α), interleukin-6, DNA fragmentation), and Nrf2 have been suggested to be the mechanisms involved in LPS-induced AKI in mice (Rousta *et al*. 2018).

Conclusion

The present work has presented experimental evidence that sesamin is useful in mitigating adenine – induced CKD in rats, through attenuation of several inflammatory, oxidative and nitrosative stress parameters. No overt untoward actions have been found from sesamin treatment. Further studies into the specific molecular mechanism (s) of the beneficial action of sesamin are warranted. Pending further pharmacological and toxicological studies, clinical testing of sesamin as a dietary supplement in patients with AKI and other renal diseases may also be warranted.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments:

The author(s) received no specific funding for this work. However, two of the co-authors (MA and PM) were financially supported by research grants from Sultan Qaboos University. We thank Professor G. Blunden for reading the manuscript the researchers at Central Analytical and Applied Research Unit, College of Science, SQU for their help with measuring platinum in this work.

References

AL SULEIMANI YM, AL MAHRUQI AS, AL ZA'ABI M, SHALABY A, ASHIQUE M, NEMMAR A, ALI BH: Effect of diesel exhaust particles on renal vascular responses in rats with chronic kidney disease. Environ Toxicol **32**: 541-549, 2017.

ALI BH, ABDELRAHMAN AM, AL-SALAM S, SUDHADEVI M, ALMAHRUQI AS, AL-HUSSENI IS, BEEGAM S, DHANASEKARAN S, NEMMAR A, AL-MOUNDHRI

M: The effect of sildenafil on cisplatin nephrotoxicity in rats. Basic Clin Pharmacol Toxicol **109**: 300-308, 2011.

ALI BH, AL MOUNDHRI MS: Agents ameliorating or augmenting the nephrotoxicity of CP and other platinum compounds: a review of some recent research. Food Chem Toxicol **44**: 1173-1183, 2006.

ALI BH, RAMKUMAR A, MADANAGOPAL TT, WALY MI, TAGELDIN M, AL-ABRI S, FAHIM M, YASIN J, NEMMAR A. Motor and behavioral changes in mice with cisplatin-induced acute renal failure. Physiol Res.;63: 35-45, 2014.

ALI BH, AL-MOUNDHRI M, ELDIN MT, NEMMAR A, AL-SIYABI S, ANNAMALAI K: Amelioration of cisplatin-induced nephrotoxicity in rats by tetramethylpyrazine, a major constituent of the Chinese herb *Ligusticum wallichi*. Exp Biol Med (Maywood) 233: 891-896, 2008.

ALI BH, AL-SALAM S, AL HUSSEINI IS, AL-LAWATI I, WALY M, YASIN J, FAHIM M, NEMMAR A: Abrogation of cisplatin-induced nephrotoxicity by emodin in rats. Fundam Clin Pharmacol **27**: 192–200, 2013.

ALI BH, AL-SALAM S, AL SULEIMANI Y, AL KALBANI J, AL BAHLANI S, ASHIQUE M, MANOJ P, AL DHAHLI B, AL ABRI N, NASER HT, YASIN J, NEMMAR A, AL ZA'ABI M, HARTMANN C, SCHUPP N: Curcumin ameliorates kidney function and oxidative stress in experimental chronic kidney disease. Basic Clin Pharmacol Toxicol **122**: 65-73, 2018

ALI BH, CAHLIKOVÁ L, OPLETAL L, KARACA T, MANOJ P, RAMKUMAR A, AL SULEIMANI YM, AL ZA'ABI M, NEMMAR A, CHOCHOLOUSOVA-

HAVLIKOVA L, LOCAREK M, SIATKA T, BLUNDEN G: Effect of aqueous extract and anthocyanins of calyces of *Hibiscus sabdariffa* (Malvaceae) in rats with adenine-induced chronic kidney disease. J Pharm Pharmacol **69**: 1219-1229, 2017.

BAEK SH, CHA RH, KANG SW, PARK CW, CHA DR, KIM SG, YOON SA, KIM S, HAN SY, PARK JH, CHANG JH, LIM CS, KIM YS, NA KY: Circulating renalase predicts all-cause mortality and renal outcomes in patients with advanced chronic kidney disease. Korean J Intern Med, 2017. doi: 10.3904/kjim.2017.058.

CRONA DJ, FASO A, NISHIJIMA TF, MCGRAW KA, GALSKY MD, MILOWSKY MI: A systematic review of strategies to prevent cisplatin -induced nephrotoxicity. Oncologist **22**: 609-619, 2017.

DARWISH MA, ABO-YOUSSEF AM, KHALAF MM, ABO-SAIF AA, SALEH IG, ABDELGHANY TM: Vitamin E mitigates cisplatin-induced nephrotoxicity due to reversal of oxidative /nitrosative stress, suppression of inflammation and reduction of total renal platinum accumulation. J Biochem Mol Toxicol **31**: 1-9, 2017.

DASARI S, TCHOUNWOU PB: Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol **740**: 364-378, 2014.

DEYÀ-MARTÍNEZ À, FORTUNY C, SOLER-PALACÍN P, NETH O, SÁNCHEZ E, MARTÍN-NALDA A, FALCÓN-NEYRA L, VILA A, VALLS A, NOGUERA-JULIAN A: Cystatin C: A Marker for inflammation and renal function among HIV-infected children and adolescents. Pediatr Infect Dis J **35**(2): 196-200, 2016.

FAN D, YANG Z, LIU FY, JIN YG, ZHANG N, NI J, YUAN Y, LIAO HH, WU QQ, XU M, DENG W, TANG QZ: Sesamin protects against cardiac remodeling via Sirt3/ROS Pathway. Cell Physiol Biochem **44**: 2212-2227, 2017a.

FAN D, YANG Z, YUAN Y, WU QQ, XU M, JIN YG, TANG QZ: Sesamin prevents apoptosis and inflammation after experimental myocardial infarction by JNK and NF-κB pathways. Food Funct **8**: 2875-2885, 2017b.

FUKUDA N, ZHANG L, KODAMA M, SAKONO M, IDE T, YAMAMOTO K, SUGANO M: Effect of dietary sesamin on metabolic fate of an exogenous linolelaidic acid in perfused rat liver. J Nutr Sci Vitaminol (Tokyo) **45**: 437-48, 1999.

HEIDARI-SORESHJANI S, ASADI-SAMANI M, YANG Q, SAEEDI-BOROUJENI A: Phytotherapy of nephrotoxicity-induced by cancer drugs: an updated review. J Nephropathol **6**: 254-263, 2017.

GUO H, LIU Y, WANG L, ZHANG G, SU S, ZHANG R, ZHANG J, LI A, SHANG C, BI B, LI Z: Alleviation of doxorubicin-induced hepatorenal toxicities with sesamin via the suppression of oxidative stress. Hum Exp Toxicol **35**: 1183-93, 2016.

HOEK J, BLOEMENDAL KM, VAN DER VELDEN LA, VAN DIESSEN JN, VAN WERKHOVEN E, KLOP WM, TESSELAAR M: Nephrotoxicity as a dose-limiting factor in a high-dose CP-based chemoradiotherapy regimen for head and neck carcinomas. Cancers (Basel) **8** (2). pii: E21, 2016.

KARASAWA T, STEYGER PS: An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. Toxicol Lett **237**: 219-227, 2015.

KIMOTO Y, NISHINOHARA M, SUGIYAMA A, HARUNA A, TAKEUCHI T: Protective effect of lactoferrin on Cisplatin-induced nephrotoxicity in rats. J Vet Med Sci **75**(2): 159 -164, 2013.

LATCHA S, JAIMES EA, PATIL S, GLEZERMAN IG, MEHTA S, FLOMBAUM CD: Long-term renal outcomes after CP treatment. Clin J Am Soc Nephrol **11**: 1173-1179, 2016.

MA L, LI H, ZHANG S, XIONG X, CHEN K, JIANG P, JIANG K, DENG G: Emodin ameliorates renal fibrosis in rats via TGF-β1/Smad signaling pathway and function study of Smurf 2. Int Urol Nephrol **50**: 373-382, 2018.

MAHENDRA KUMAR C, SINGH SA. Bioactive lignans from sesame (*Sesamum indicum* L.): evaluation of their antioxidant and antibacterial effects for food applications. J Food Sci Technol. **52**: 234-241, 2015.

MENG H, FU G, SHEN J, SHEN K, XU Z, WANG Y, JIN B, PAN H: Ameliorative effect of daidzein on cisplatin-induced nephrotoxicity in mice via modulation of inflammation, oxidative stress, and cell death. Oxid Med Cell Longev **2017**: 3140680, 2017.

MILLER RP, TADAGAVADI RK, RAMESH G, REEVES WB: Mechanisms of cisplatin nephrotoxicity. Toxins (Basel) **2**: 2490-2518, 2010.

MUKHERJI A, ANSARI U, BORGGREFE M, AKIN I, BEHNES M: Clinically relevant biomarkers in acute heart failure: An update. Curr Pharm Biotechnol **18**: 482-490, 2017.

NAKATANI Y, YAGUCHI Y, KOMURA T, NAKADAI M, TERAO K, KAGE-NAKADAI E, NISHIKAWA Y: Sesamin extends lifespan through pathways related to dietary restriction in *Caenorhabditis elegans*. Eur J Nutr **57**: 1137-1146, 2018.

NAMIKI M: Nutraceutical functions of sesame: a review. Crit Rev Food Sci Nutr **47**: 651-73, 2007.

NEMATBAKHSH M, PEZESHKI Z, ESHRAGHI JAZI F, MAZAHERI B, MOEINI M., SAFARI T, AZARKISH F, MOSLEMI F, MALEKI M, REZAEI A, SABERI, S. H, DEHGHANI A, MALEK M, MANSOURI A, GHASEMI M, ZEINALI F, ZAMANI Z, NAVIDI M, JILANCHI S, SHIRDAVANI S, ASHRAFI F: CP-induced nephrotoxicity; protective supplements and gender differences. Asian Pac J Cancer Prev **18**: 295-314, 2017.

OJIMA T, NAKAMURA M, NAKAMORI M, KATSUDA M, HAYATA K, MARUOKA S, SHIMOKAWA T, YAMAUE H: Phase I/II trial of chemotherapy with docetaxel, cisplatin, and S-1 for unresectable advanced squamous cell carcinoma of the esophagus. Oncology **95**: 116-120, 2018.

PABLA N, DONG Z: CP nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int **73**: 994-1007, 2008.

PRASAD KN: Multiple dietary antioxidants enhance the efficacy of standard and experimental cancer therapies and decrease their toxicity. Integr Cancer Ther **3**: 310-322, 2004.

QIANG L, YUAN J, SHOUYIN J, YULIN L, LIBING J, JIAN-AN W: Sesamin attenuates lipopolysaccharide-induced acute lung injury by inhibition of TLR4 signaling pathways. Inflammation **39**: 467-472, 2016.

QUELHAS-SANTOS J, PESTANA M: Plasma renalase in chronic kidney disease: differences and similarities between humans and rats. Curr Hypertens Rev **10**: 166-170, 2014.

ROUSTA AM, MIRAHMADI SM, SHAHMOHAMMADI A, NOURABADI D, KHAJEVAND-KHAZAEI MR, BALUCHNEJADMOJARAD T, ROGHANI M:. Protective effect of sesamin in lipopolysaccharide-induced mouse model of acute kidney injury via attenuation of oxidative stress, inflammation, and apoptosis. Immunopharmacol Immunotoxicol **29**: 1-7, 2018.

SCHLAICH MP, LAMBERT GW, EIKELIS N: Renalase - a potential biomarker for risk of atrial fibrillation? Kardiol Pol **76**(8): 1201-1202, 2018.

SHAHID F, FAROOQUI Z, KHAN F. Cisplatin-induced gastrointestinal toxicity: An update on possible mechanisms and on available gastroprotective strategies. Eur J Pharmacol **827**: 49-57, 2018.

SHARP CN, SISKIND LJ: Developing better mouse models to study cisplatin-induced kidney injury. Am J Physiol Renal Physiol **313**: F835-F841, 2017.

SHELTON LM, PARK BK, COPPLE IM. Role of Nrf2 in protection against acute kidney injury. Kidney Int **84**: 1090 –1095, 2013.

SKINNER R: Late renal toxicity of treatment for childhood malignancy: risk factors, long-term outcomes, and surveillance. Pediatr Nephrol **33**: 215-225, 2018.

SOLIMAN MM, ATTIA HF, EL-ELLA GA: Genetic and histopathological alterations induced by cypermethrin in rat kidney and liver: Protection by sesame oil. Int J Immunopathol Pharmacol **28**: 508-520, 2015.

TOMIMORI N, ROGI T, SHIBATA H: Absorption, distribution, metabolism, and excretion of [(14) C] sesamin in rats. Mol Nutr Food Res **61**: 2017. doi: 10.1002/mnfr. 201600844

WAN Y, LI H, FU G, CHEN X, CHEN F, XIE M: The relationship of antioxidant components and antioxidant activity of sesame seed oil. J Sci Food Agric **95**: 2571-2578, 2015.

WILMES A, BIELOW C, RANNINGER C, BELLWON P, ASCHAUER L, LIMONCIEL A, CHASSAIGNE H, KRISTL T, AICHE S, HUBER CG, GUILLOU C, HEWITT P, LEONARD MO, DEKANT W, BOIS F, JENNINGS P: Mechanism of cisplatin proximal tubule toxicity revealed by integrating transcriptomics, proteomics, metabolomics and biokinetics. Toxicol *In Vitro* **30**: 117-27, 2015.

ZHU S, PABLA N, TANG C, HE L, DONG Z: DNA damage response in cisplatin-induced nephrotoxicity. Arch Toxicol 89: 2197-2205, 2015.

Figure legends

Figure 1. The plasma concentration of tumor necrosis factor (TNF-α), interleukin (IL-1β), transforming growth factor (TGF-β1), cystatin C, renalase, and the renal activity of myeloperoxidase (MPO) in control rats, and rats treated with cisplatin or sesamin (separately or in combination). Each column and vertical bar represents mean ± SEM (n = 6).

Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.

Figure 2. The renal concentration or activity of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), total antioxidant capacity (TAC), malondialdehyde (MDA), and nuclear factor erythroid 2-related factor 2 (Nrf2) in control rats, and rats treated with cisplatin or sesamin (separately or in combination). Each column and vertical bar represents mean ± SEM (n = 6).

Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.

Figure 3. The renal concentration of total nitric oxide (NO), nitrite and nitrate, and the nitrate/nitrite ratio in control rats, and rats treated with cisplatin or sesamin (separately or in combination). Each column and vertical bar represents mean ± SEM (n = 6).

Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.

Figure 4. Representative micrographs of kidney sections from control rats, and rats treated with cisplatin or sesamin (separately or in combination), stained with hematoxylin and eosin (H & E). The control (A and B) and sesamin-treated group (E & F) show normal kidney architecture and histology. The cisplatin-treated group (C & D) shows acute tubular necrosis in 52 ± 3.62% of examined tissue areas (thin arrows) with tubular distention with necrotic material. The (cisplatin + sesamin)-treated group (G & H) shows dramatic improvement in the histologic appearance with absence of acute tubular necrosis and complete recovery of injured tubules.

Table 1. Effect of treatment with sesamin (SM) on some physiological parameters in rats with cisplatin (CP)-induced acute kidney injury (AKI).

Parameters/ Treatment	Control	CP (5 mg/kg)	SM (5 mg/kg)	CP + SM
Change in body weight (%)	16.77 ± 1.29	8.44 ± 1.76[a]	20.07 ± 1.10	14.94 ± 0.99[b]
Relative kidney weight (%)	0.58 ± 0.01	0.69 ± 0.03[a]	0.57 ± 0.01	0.62 ± 0.01[b]
Urine flow (µl/min)	6.25 ± 0.36	9.95 ± 0.66[a]	5.67 ± 0.42	8.68 ± 0.61[a]
Food intake (g)	21.33 ± 1.20	17.20 ± 2.14[a]	21.63 ± 1.13	19.30 ± 0.48
Feces output (g)	8.17 ± 0.91	5.97 ± 0.76[a]	9.70 ± 0.80	6.82 ± 0.40

The values in the tables are mean ± SEM (n = 6).

Sesamin (5 mg/kg/day) was given to the rats by oral gavage for 10 days, and on the 7th day, AKI was induced by injecting a single dose of CP (5 mg/kg), intraperitoneally. On the 10th day of treatment, the rats were placed in metabolic cages to collect urine. The rats were sacrificed on the 11th day.

Different superscripts indicate significance as follows ($P < 0.05$ was considered significant):
[a] denotes significance of Control group vs different groups,
[b] denotes significance of CP group vs. (CP + SM)-treated group.

Table 2. Effect of treatment with sesamin (SM) on some indices of renal damage in plasma of rats with cisplatin (CP)-induced acute kidney injury (AKI).

Parameters/ Treatment	Control	CP (5 mg/kg)	SM (5 mg/kg)	CP + SM
NGAL (ng/ml)	27.36 ± 2.27	138.86 ± 9.05[a]	30.76 ± 2.81	95.06 ± 7.26[a,b]
Creatinine (µmol/l)	18.35 ± 1.08	121.37 ± 7.72[a]	14.88 0.54	56.72 ± 2.48[a,b]
Urea (mmol/l)	5.70 ± 0.37	13.42 ± 0.81[a]	4.76 ± 0.24	9.88 ± 0.57[a,b]
Uric acid (µmol/l)	46.67 ± 3.85	89.97 ± 5.75[a]	38.95 ± 2.22	67.28 ± 5.56[a,b]
Phosphorus (mmol/l)	0.55 ± 0.05	1.27 ± 0.12[a]	0.54 ± 0.05	0.89 ± 0.05[a,b]
Calcium (mmol/l)	1.15 ± 0.06	0.69 ± 0.05[a]	1.03 ± 0.09	0.92 ± 0.05[a,b]

The values in the tables are mean ± SEM (n = 6).

Sesamin (5 mg/kg/day) was given to the rats by oral gavage for 10 days, and on the 7th day, AKI was induced by injecting a single dose of cisplatin (5 mg/kg), intraperitoneally.

NGAL: Neutrophil gelatinase-associated lipocalin.

Different superscripts indicate significance as follows ($P < 0.05$ was considered significant):
[a] denotes significance of Control group vs different groups,
[b] denotes significance of CP group vs. (CP + SM)-treated group.

Table 3. Effect of treatment with sesamin (SM) on some urinary indices in rats with cisplatin (CP)-induced acute kidney injury (AKI).

Parameters/ Treatment	Control	CP (5 mg/kg)	SM (5 mg/kg)	CP + SM
KIM-1 (pg/ml)	221.64 ± 13.08	532.40 ± 6.86[a]	202.09 ± 20.61	399.69 ± 21.05[a,b]
L-FABP (ng/ml)	0.82 ± 0.07	6.83 ± 0.38[a]	0.90 ± 0.10	5.35 ± 0.32[a,b]
NAG activity (IU/l)	3.16 ± 0.27	14.72 ± 0.84[a]	1.12 ± 0.19[a]	5.26 ± 0.34[a,b]
Creatinine clearance (mL/min)	3.10 ± 0.22	0.54 ± 0.04[a]	3.24 ± 0.20	1.28 ± 0.10[a,b]
UACR (mg/mmol)	423.89 ± 10.55	789.83 ± 27.36[a]	405.33 ± 13.13	571.16 ± 28.84[a,b]

Values in the table are means ± SEM (n = 6).

Sesamin (5 mg/kg/day) was given to the rats by oral gavage for 10 days, and on the 7th day, AKI was induced by injecting a single dose of cisplatin (5 mg/kg), intraperitoneally. On the 10th day of treatment, the rats were placed in metabolic cages to collect urine.

KIM-1: Kidney injury molecule-1; L-FABP: Liver-type fatty acid–binding protein; NAG: *N*-acetyl-β-D-glucosaminidase (NAG); UACR: Urine albumin-to-creatinine ratio.

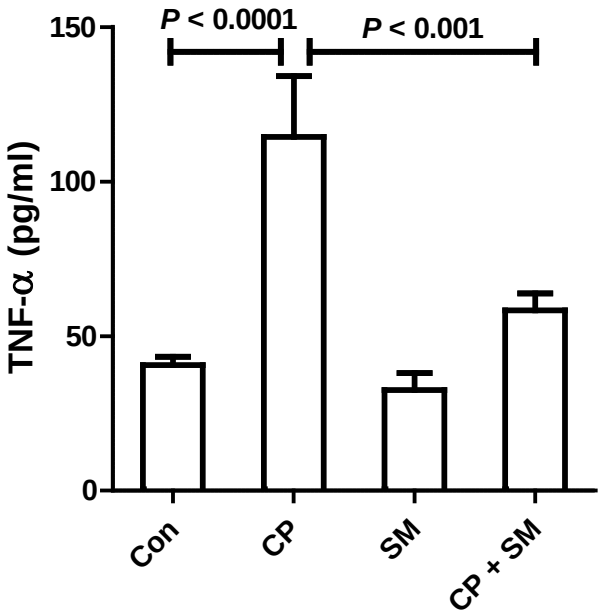
Different superscripts indicate significance as follows ($P < 0.05$ was considered significant):
[a] denotes significance of Control group vs different groups,
[b] denotes significance of CP group vs. (CP + SM)-treated group.

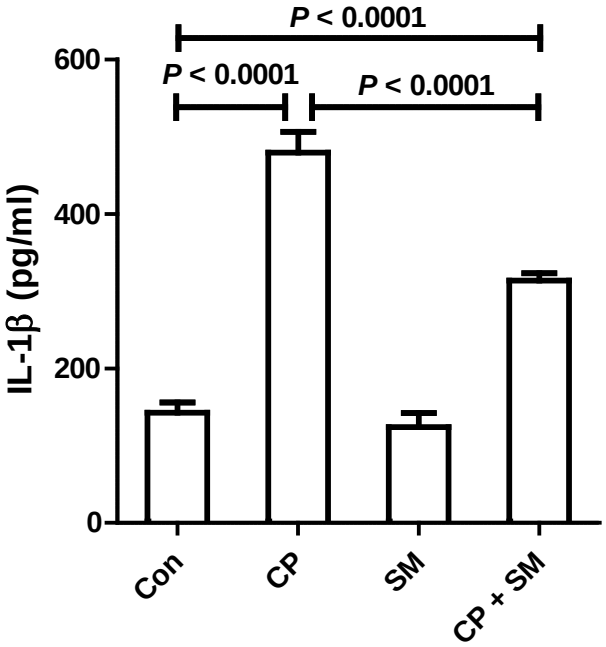
Table 4. Effect of treatment with sesamin (SM) on necrosis in the kidneys of rats with cisplatin (CP)-induced acute kidney disease (AKD).

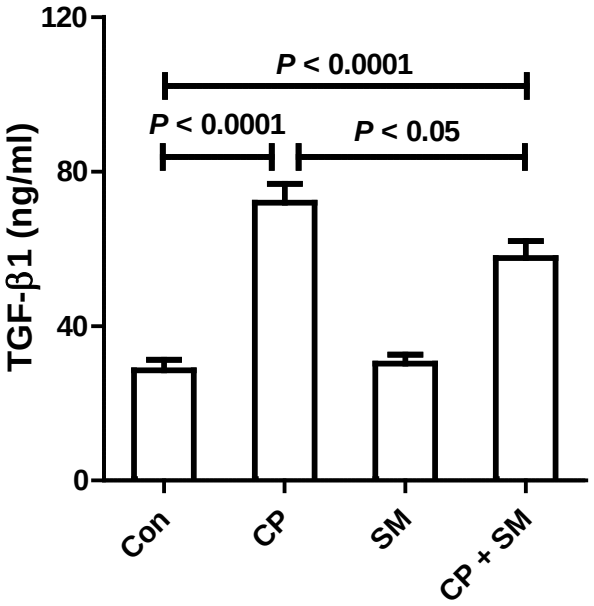
Parameters/ Treatments	**Control**	**CP (5 mg/kg)**	**SM (5 mg/kg)**	**CP + SM**
Percentage of necrosis	0.0 ± 0.0	52.0 ± 3.6[a]	0.0 ± 0.0	0.0 ± 0.0[b]
Score of necrosis	0.0 ± 0.0	3.0 ± 0.0[a]	0.0 ± 0.0	0.0 ± 0.0[b]

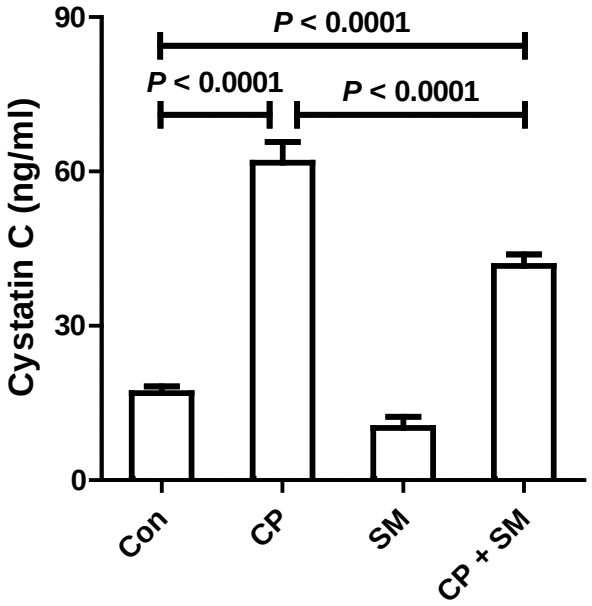
The values in the tables are mean ± SEM (n = 6)

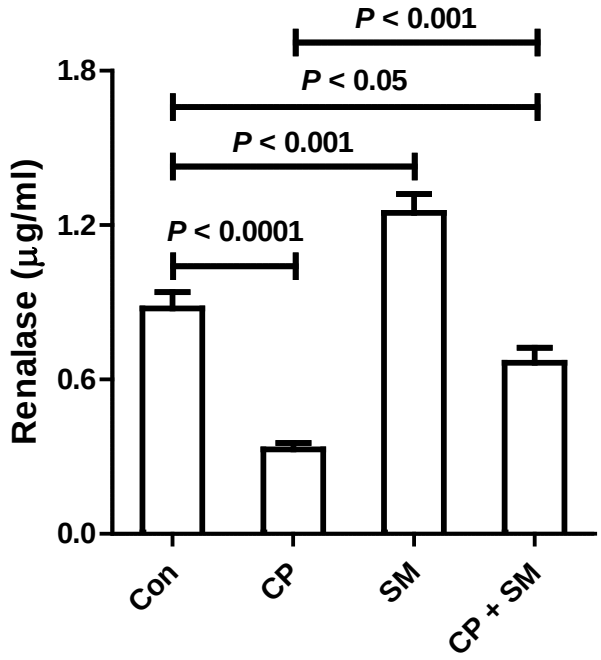
Different superscripts indicate significance as follows, where $P < 0.05$:
[a] denotes significance of control group vs different groups,
[b] denotes significance of CP group vs. (CP + SM)-treated group.

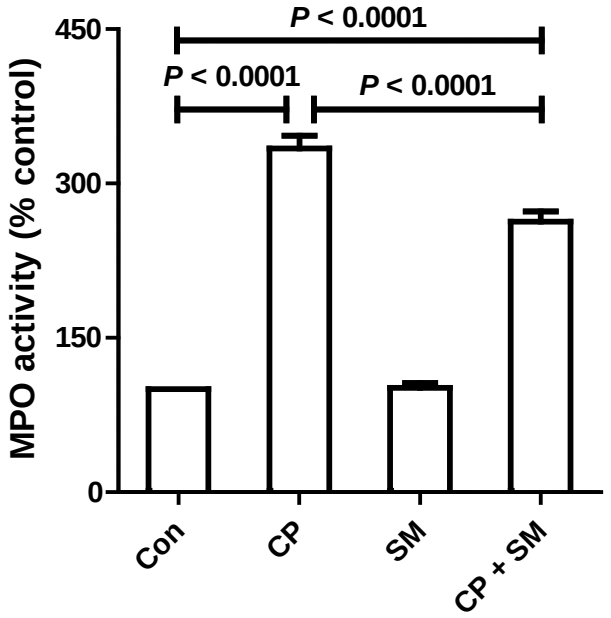

P < 0.0001
P < 0.001
TNF-α (pg/ml)
150
100
50
0
Con
CP
SM
CP + SM


P < 0.0001
P < 0.0001
P < 0.0001
IL-1β (pg/ml)
600
400
200
0
Con
CP
SM
CP + SM


P < 0.0001
P < 0.0001
P < 0.05
TGF-β1 (ng/ml)
120
80
40
0
Con
CP
SM
CP + SM


Cystatin C (ng/ml)
90
60
30
0
P < 0.0001
P < 0.0001
P < 0.0001
Con
CP
SM
CP + SM


Renalase (μg/ml)
1.8
1.2
0.6
0.0
P < 0.001
P < 0.05
P < 0.001
P < 0.0001
Con
CP
SM
CP + SM


MPO activity (% control)
450
300
150
0
P < 0.0001
P < 0.0001
P < 0.0001
Con
CP
SM
CP + SM

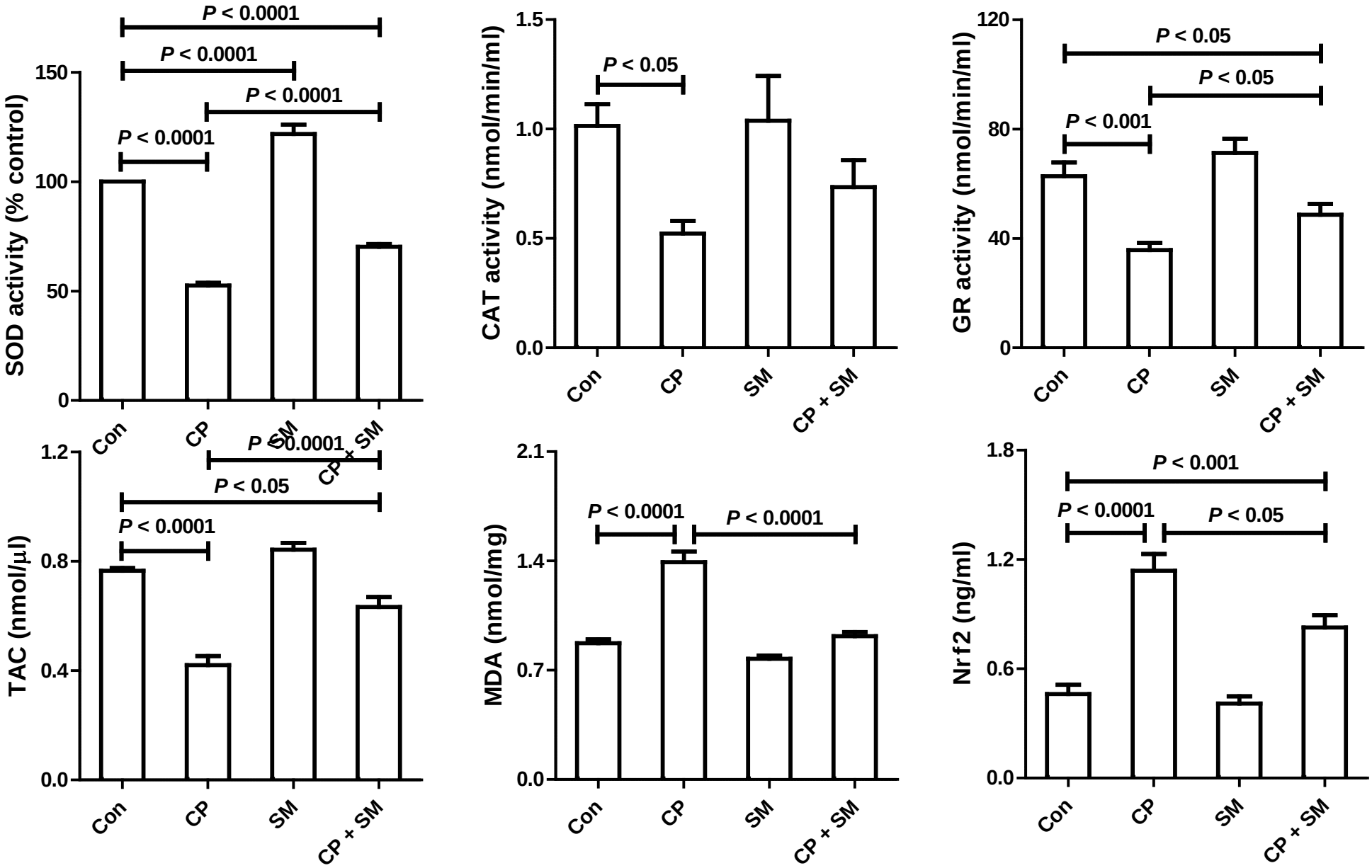
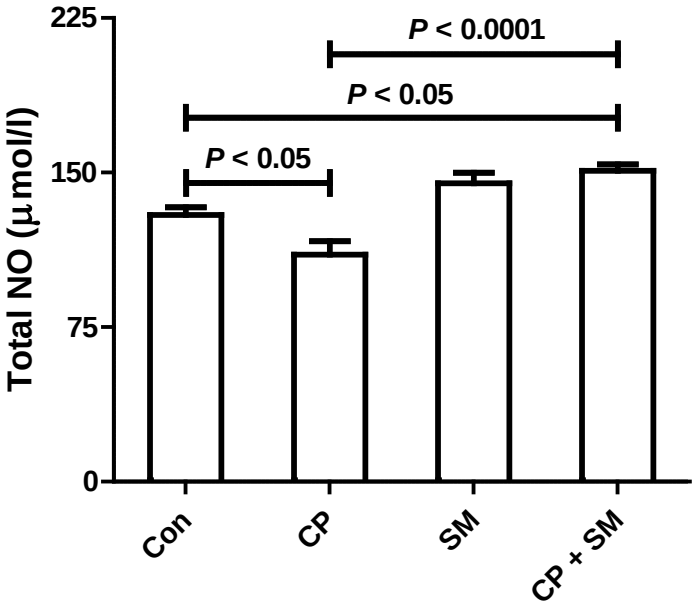
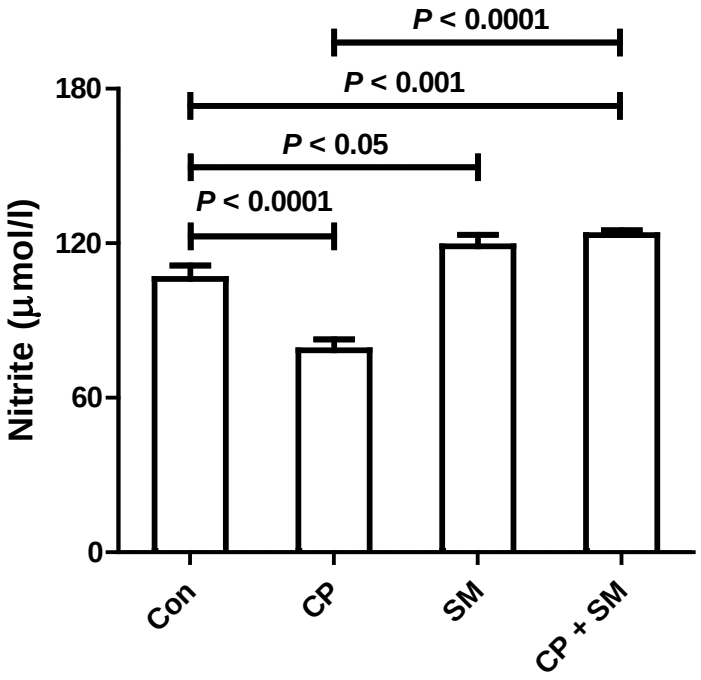
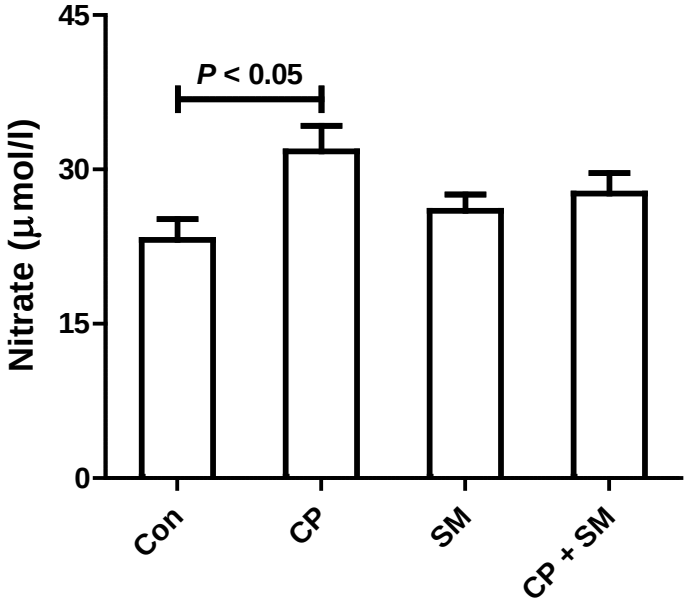

SOD activity (% control)
P < 0.0001
P < 0.0001
P < 0.0001
P < 0.0001
150
100
50
0
Con
CP
SM
CP + SM
CAT activity (nmol/min/ml)
P < 0.05
1.5
1.0
0.5
0.0
GR activity (nmol/min/ml)
P < 0.05
P < 0.05
P < 0.001
120
80
40
TAC (nmol/µl)
P < 0.0001
P < 0.05
P < 0.0001
1.2
0.8
0.4
MDA (nmol/mg)
P < 0.0001
P < 0.0001
2.1
1.4
0.7
Nrf2 (ng/ml)
P < 0.001
P < 0.0001
P < 0.05
1.8
1.2
0.6

Fig. 2.


Total NO (µmol/l)
225
150
75
0
P < 0.0001
P < 0.05
P < 0.05
Con
CP
SM
CP + SM


Nitrite (µmol/l)
180
120
60
0
P < 0.0001
P < 0.001
P < 0.05
P < 0.0001
Con
CP
SM
CP + SM


Nitrate (µmol/l)
45
30
15
0
P < 0.05
Con
CP
SM
CP + SM

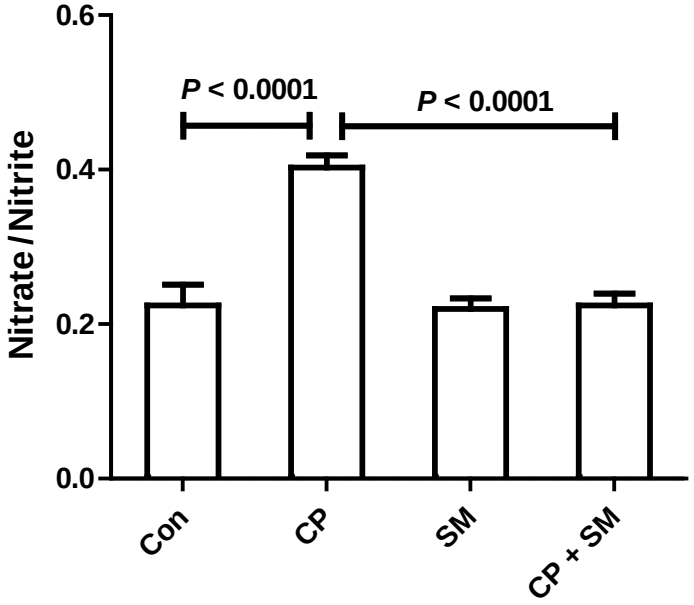
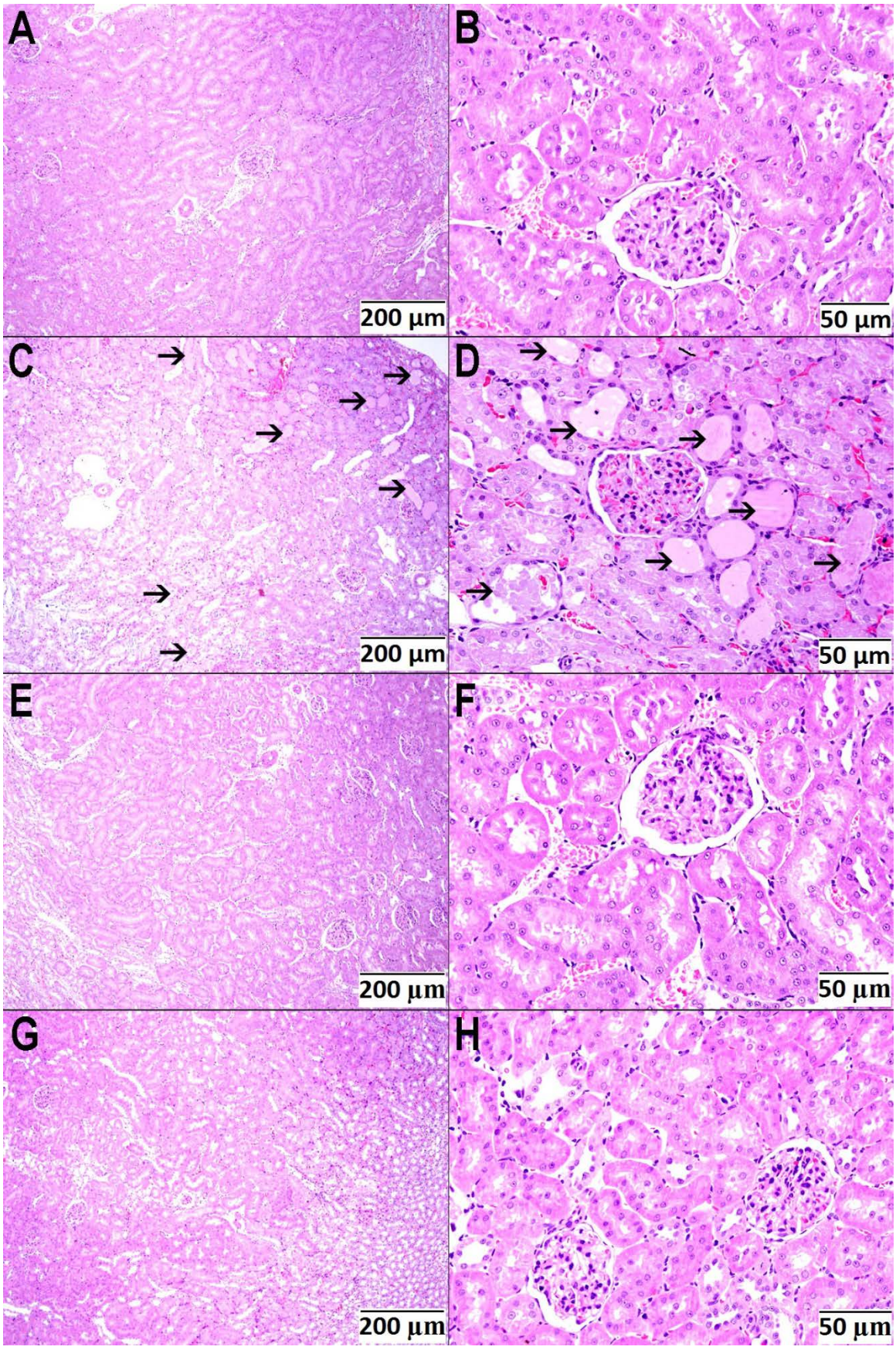

Nitrate/Nitrite
0.6
0.4
0.2
0.0
P < 0.0001
P < 0.0001
Con
CP
SM
CP + SM

Fig. 3


A
200 µm
B
50 µm
C
200 µm
D
50 µm
E
200 µm
F
50 µm
G
200 µm
H
50 µm