Coixol attenuated cisplatin induced hepatotoxicity and nephrotoxicity, and might not interfere with anti-cancer effects of cisplatin

Sheng-Lei YAN[1,†], Mei-Chin MONG[2,†], Zhi-Hong WANG[2,†], Mei-Chin YIN[3,4,*]

[1]Department of Internal Medicine, Chang Bing Show-Chwan Memorial Hospital, Changhua County, Taiwan

[2]Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan

[3]Office of Research and Development, Asia University, Taichung, Taiwan

[4]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan

Correspondence: mcyin@hotmail.com.tw

[†]These authors contributed equally to this study.

ORCIDS:

Sheng-Lei Yan: https://orcid.org/0000-0002-6715-1650

Mei-Chin Mong: https://orcid.org/0000-0001-7051-2464

Zhi-Hong Wang: https://orcid.org/0000-0002-2931-6688

Mei-Chin Yin: https://orcid.org/0000-0001-6851-2684

Acknowledgement: This study was partially supported by a grant from Asia University, Taichung, Taiwan (ASIA-107-CMUH-05).

Conflicts of Interest: All authors declare that no Conflict of Interest.

Consent to Publish: The final content of this paper was read and approved by all authors.

Informed consent: This study had no human subject.

Abstract

Background/aim: Effects of coixol (Coi) against cisplatin (CP) induced hepatotoxicity and nephrotoxicity in mice were investigated. The cytotoxicity of Coi alone, and combined with CP against HepG2 and A549, liver and lung cancer cells, was also examined.

Materials and methods: There were five groups of mice: normal groups, 0.25% Coi groups, CP groups, 0.125% Coi+CP groups, and 0.25% Coi+CP groups. Coi was supplied in diet for 2 weeks. Histological and biochemical analyses were processed.

Results: Histological results indicated that CP caused necrosis, inflammatory infiltration and vacuolization in liver and kidney. Coi pre-treatments alleviated these damage and improved hepatic and renal cells integrity. CP exposure increased reactive oxygen species and nitric oxide production, decreased glutathione level, and reduced hepatic and renal activity of glutathione peroxidase and glutathione reductase. Coi pre-treatments reversed these changes. CP exposure increased hepatic and renal levels or activities of interleukin-1beta, tumor necrosis factor-alpha, prostaglandin E_2, cytochrome P450 2E1 and cyclooxygenase-2. Coi pre-treatments lowered these inflammatory mediators. CP enhanced mRNA expression of nuclear factor kappa B, p38 mitogen-activated protein kinase, nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3, and decreased mRNA expression of nuclear factor E2-related factor 2 in liver and kidney. Coi pre-treatments reversed these alterations. Cell lines study indicated that Coi alone did not affect the growth of HepG2 and A549 cancer cells, and the co-use of Coi and CP resulted in similar cytotoxic effects as CP alone.

Conclusion: These data suggest that coixol is a potent hepatic and renal protective agent against cisplatin.

Key words: Coixol, cisplatin, hepatoxicity, nephrotoxicity, protection

1. Introduction

Cisplatin (CP) is a platinum-based anti-cancer agent, and widely used to treat lung, breast, bladder and cervix malignancies [1]. Although CP displays markedly therapeutic efficacy for several cancers, the clinical application of this agent leads to many adverse effects including hepatoxicity and nephrotoxicity [2,3]. These adverse effects cause other complications, restrict anti-cancer therapy and deteriorate health status for cancer patients. Thus, the exploration for safe compound with organ protective capability against CP is necessary.

It has been documented that oxidation and inflammation are major contributors toward the development of CP induced hepatoxicity and nephrotoxicity [4,5]. The accumulated CP in liver cells and renal parenchymal cells promoted the expression of upstream regulators such as nuclear factor kappa B (NF-κB) p65, p38 mitogen-activated protein kinase (MAPK), nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inflammasome, cyclooxygenase (COX)-2, and suppressed the expression of nuclear factor E2-related factor 2 (Nrf2) [6-8]. These regulators consequently accelerated downstream oxidative and inflammatory responses in liver and kidney, and facilitated the production of oxidants and inflammatory cytokines including reactive oxygen species (ROS), nitric oxide (NO), interleukin (IL)-1beta, tumor necrosis factor (TNF)-alpha and prostaglandin E $(PGE)_2$ [9-11]. As a result, these oxidative and inflammatory factors impaired integrity and/or biofunctions of hepatic and renal cells, and further evoked other pathological complications.

So far, the protective effects from plant derived natural products for organs against CP caused toxicity have been attractive [12]. Coixol (6-methoxy-2(3H)-benzoxazolone, Coi), a polyphenol, is naturally presented in seed of adlay (*Coix lachryma-jobi* L.) [13]. Adlay seed is often consumed as a cereal crop in

many Asian countries [14], or used as an herb for the treatments of chronic inflammatory diseases including rheumatism and neuralgia [15]. Coi has attracted more attention recently due to its biological activities such as anti-oxidative, anti-diabetic and anti-inflammatory potent [16]. Chen et al. [17] reported that Coi possessed anti-mutagenic activity. Amen et al. [18] found that Coi inhibited melanogenesis in B16-F10 melanoma cells. Sharma et al. [19] indicated that Coi exerted anti-diabetic actions via stimulating insulin secretion on isolated mice islets. The study of Hameed et al. [20] revealed that Coi through regulating cyclic AMP signaling pathway increased insulin response to glucose in rats. Hu et al. [21] reported that Coi could limit the activation of MAPK, NF-κB and NLRP3 inflammasome in lipopolysaccharide treated macrophage cells. Those previous studies suggest that Coi might possess multiple nutritional and medical benefits.

In our current study, the hepatic and renal protection from Coi against CP induced oxidative and inflammatory injury was evaluated. The effects of this agent upon histological alteration, and messenger RNA (mRNA) expression of associated genes were analyzed. Furthermore, the cytotoxicity of Coi alone and combined with CP against HepG2 and A549, liver and lung cancer cells, was also examined to ensure the application of Coi did not interfere with the anti-cancer therapy of CP. These results could support the future application of Coi to alleviate the adverse effects of CP.

2. Materials and methods

2.1. Materials

Coi (97.5%) was purchased from Chem-Impex Int. Inc. (Wood Dale, IL, USA). CP (98.5%) was obtained from Sigma Chem. Co. (St. Louis, MO, USA). Male ICR mice, six-week (wk) old, were obtained from National Laboratory Animal Center (Taipei City,

Taiwan), and maintained under the conditions of 22 °C, 60% humidity and 12 hours (h) light/dark cycle. After one wk acclimation, mice were used for experiments. This animal study was approved by the animal care and use committee of Asia University (Taichung, Taiwan), and the permission number was 106-asia-03.

2.2. Experimental design

There were five groups of mice: normal groups, 0.25% Coi groups, CP groups, 0.125% Coi+CP groups, and 0.25% Coi+CP groups. Our preliminary experiments revealed that 0.125% Coi alone led to similar histological and biochemical results as normal groups. Thus, 0.125% Coi groups were not included in this present study. Each group had eight mice. Coi at 0.125 or 0.25 g was mixed with 99.875 or 99.75 g standard power diet. All mice were free to access feed and water. Initial and final body weight (BW), feed intake and water intake were recorded. After a two-wk supplementation, CP at 25 mg/kg was given via one intraperitoneal injection to mice in CP and Coi+CP groups. At 48 h CP post-treatment, mice were kept in metabolic cages and urine was collected for 24 h. Then, mice after fasted overnight were inhaled by carbon dioxide for scarification. Blood, liver and kidney were collected. Plasma was separated from whole blood by centrifugation. Liver and kidney were perfused with 1 mL phosphate buffer saline (PBS, pH 7.2) to remove blood residue. Hepatic or renal tissue at 100 mg was homogenized. Protein concentration of tissue homogenate was measured by assay reagents (Bio-Rad Laboratories Inc., Hercules, CA, USA). Tissue homogenate was diluted to 1 mg protein/mL by PBS for further analyses.

2.3. Assays for hepatic and renal functions

Plasma activity or level of alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN) and creatinine (Cr), and urinary Cr were determined by assay kits obtained from Wako Chem. Co. (Tokyo, Japan). Glomerular filtration rate

(GFR) was calculated according to this equation: (urine volume x urinary Cr) ÷ plasma Cr.

2.4. Histological examination

Partial fresh hepatic or renal tissue from each mouse was treated with 4% paraformaldehyde for fixation. After dehydrated with increased ethanol concentrations and cleaned with xylene, tissue samples were paraffin embedded. Subsequently, paraffin section with 5 μm thickness was cut. Each section was processed by hematoxylin and eosin (H&E) staining. Four random fields per mouse liver or kidney were evaluated by an experienced and blinded pathologist. Liver injury was evaluated by four features: hepatic lobules destruction, inflammatory cells infiltration, necrotic hepatocyte and hemorrhage. Kidney injury was evaluated by four features: tubular epithelial swelling, brush border loss, necrotic tubules and vacuolar degeneration. The criteria of Baranova et al. [22] were modified for grading histological injury score (IS), which were graded 0-4 to represent the degree of severity for each feature, 0 = none, 1 = < 25% of damage, 2 = 25 to 50% of damage, 3 = 50 to 75% of damage, 4 = 75 to 100% of damage. IS was in the range of 0-16.

2.5. Measurements for oxidative and inflammatory mediators

ROS level of hepatic and renal tissues was measured by using 2',7'-dichlorofluorescein diacetate (DCFH-DA). Tissue homogenate at 100 μL was mixed with DCFH-DA at 1 mg/mL. After a 30-minute (min) incubation at 37 °C, fluorescence value was recorded. Result was shown as relative fluorescence unit (RFU)/mg protein. NO level was quantified by the method of Moshage et al. [23]. In brief, tissue homogenate at 100 μL was mixed with flavin adenine dinucleotide, nicotinamide adenine dinucleotide phosphate (NADPH) and nitrate reductase. After a 60-min incubation in the dark, this mixture was centrifuged at 6,000 xg for 15 min. Supernatant was collected and

reacted with Griess reagent. The optical density (OD) value at 540 nm was recorded. A sodium nitrite prepared standard curve was used for data qualification. Glutathione (GSH), IL-1beta, TNF-alpha and PGE_2 levels, and glutathione reductase (GR) and glutathione peroxidase (GPX) activities in tissue homogenate were assessed by commercial kits obtained from Wako Chem. Co. (Tokyo, Japan).

2.6. Measurement of cytochrome P450 2E1 (CYP2E1) and COX-2 activities

CYP2E1 activity was measured by monitoring the formation rate of p-nitrocatechol from p-nitrophenol (PNP). Tissue homogenate at 150 μL was mixed with PNP at 0.2 mM and potassium-phosphate buffer at 100 mM. NADPH at 1 mM was added to initiate reaction. After a 60-min incubation at 37 °C for 1 h, 30 μL 20% trichloroacetic acid was added to terminate the reaction. After centrifugation, supernatant was collected to react with sodium hydroxide. The OD value at 546 nm was read. COX-2 activity was analyzed by an assay kit purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA).

2.7. Quantification of mRNA expression

Real-time polymerase chain reaction (RT-PCR) was processed to determine mRNA expression of NF-κB, p38, NLRP3 and Nrf2. Total RNA was extracted from hepatic or renal tissue by TRIzol® reagents obtained from Thermo Fisher Scientific (Waltham, MA, USA), and total RNA concentration was quantified by measuring the OD at 260 nm. Subsequently, 2 μg RNA was used to synthesize complementary DNA (cDNA) by a synthesis kit (Legene Biosciences, San Diego, CA, USA), and the synthesized cDNA was further applied for RT-PCR process. AB7500 RT-PCR system (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex TaqTM II was used to run RT-PCR. PCR primers for target genes are shown in Table 1. The mRNA expression of each target gene was normalized by a house keeping gene, glyceraldehyde

3-phosphate dehydrogenase.

2.8. Cancer cell line study

HepG2 and A549 cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), and routinely cultured at 37 °C in 5% CO_2 in RPMI 1640 medium (pH 7.4), which contained fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (100 units/mL). The culture medium was refreshed every three days. Cell number was adjusted by PBS (pH 7.2) to 10^5/mL for experiments. HepG2 and A549 cells were treated with Coi at 0, 2, 4, 8, 16 or 32 μM at 37 °C for 48 h to examine the impact of Coi upon cancer cell survival. Furthermore, HepG2 and A549 cells (10^5/mL) were treated with CP (5 μM), Coi (32 μM) or CP (5 μM) plus Coi (32 μM) at 37 °C for 48 h to examine the influence of Coi upon the cytotoxic effects of CP. Normal groups contained no CP or Coi. Viability was assayed at 0 and 48 h.

2.9. Assay for cell viability

Cell viability was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were reacted with MTT at 0.25 mg/mL at 37 °C for 3 h. The formed formazan product was quantified via monitoring the OD variation at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability was shown as a percent of normal groups.

2.10. Statistical analysis

In animal study, each group had 8 mice (n = 8). In cell line study, 5 replications were processed (n = 5). Data are expressed as mean ± standard deviation (SD). Statistical analyses were handled using SPSS Statistics 24.0 (IBM, Boston, MA, USA). The comparisons between groups were processed by using one-way analysis of variance (ANOVA) followed by post hoc Tukey test. *P* value lower than 0.05 was considered as statistically significant.

3. Results

3.1. Animal study

3.1.1. Coi improved hepatic and renal functions

As shown in Table 2, Coi and CP treatments did not affect feed and water intake (p >0.05). CP exposure decreased final BW and led to lower BW gain (p <0.05). Coi pre-treatments at 0.25% increased BW gain (p <0.05). CP exposure increased liver and kidney weights (p <0.05). Coi pre-treatments at 0.25% decreased liver weight, and at two doses reduced kidney weight (p <0.05). CP exposure increased ALT and AST activities, BUN and Cr levels, and decreased GFR (Table 3, p <0.05). Coi pre-treatments at two doses reduced ALT and AST activities, lowered BUN and Cr levels, and increased GFR (p <0.05), in which Coi pre-treatments at 0.25% showed greater effects than 0.125% (p <0.05). Histological image (Figure 1A) and IS values (Figure 1B) revealed that CP caused severe injury in liver and kidney including necrosis, inflammatory infiltration, degeneration, vacuolization or boundary disturbance. Coi pre-treatments at two doses abated the above injury features, improved cells integrity and decreased IS values in both organs, in which Coi pre-treatments at 0.25% exhibited greater improvement than 0.125% in both organs (p <0.05).

3.1.2. Coi mitigated hepatic and renal oxidative and inflammatory stress

CP exposure increased ROS and NO production, decreased GSH level, and reduced GPX and GR activities in liver and kidney (Table 4, p <0.05). Coi pre-treatments reversed these changes (p <0.05). Coi pre-treatments at 0.25% showed greater effects than 0.125% in decreasing ROS and NO levels, increasing GSH level and GR activity in both organs (p <0.05). CP exposure increased IL-1beta, TNF-alpha and PGE_2 levels in liver and kidney (Table 5, p <0.05). Coi pre-treatments at two doses decreased

these inflammatory mediators (p <0.05), in which Coi pre-treatments at 0.25% were greater than at 0.125% in both organs (p <0.05). CP enhanced CYP2E1 and COX-2 activities in liver and kidney (Figure 2, p <0.05). Coi pre-treatments at two doses declined the activity of CYP2E1 and COX-2 in both organs (p <0.05). Coi pre-treatments at 0.25% exhibited greater effects than 0.125% in lowering CYP2E1 activity in both organs, and decreasing COX-2 activity in liver (p <0.05). CP increased mRNA expression of NF-κB, p38, NLRP3, and decreased Nrf2 mRNA expression in liver and kidney (Figure 3, p <0.05). Coi pre-treatments at two doses reversed these changes (p <0.05). Coi pre-treatments at 0.25% showed greater effects than 0.125% in down-regulating mRNA expression of NF-κB in kidney, p38 and NLRP3 in both organs (p <0.05). Coi pre-treatments at 0.25% also displayed greater effects than 0.125% in up-regulating renal Nrf2 mRNA expression (p <0.05).

3.2. Cell line study

Coi at 2-32 μM did not exhibit inhibitory effects against HepG2 and A549 cells when compared with normal groups (Figure 4, p >0.05). As shown in Figure 5, compared with cell viability at time 0, CP exposure at 5 μM for 48 h decreased HepG2 and A549 cells viability at 52 and 43%, respectively (p <0.05). The co-treatments of Coi at 32 μM and CP at 5 μM for 48 h resulted in similar viability as CP groups alone (5 μM) in both HepG2 and A549 cells (p >0.05).

4. Discussion

It has been documented that the medical application of CP activated upstream oxidative, inflammatory and apoptotic pathways, stimulated massive production of oxidants and inflammatory mediators, and induced many adverse effects in organs, even led to acute organ failure [24-27]. These negative impacts also resulted in complicated

physiological burden and limited the therapeutic efficiency of CP for cancer patients. Many studies have reported that phytochemicals such as taxifolin and gliclazide could improve CP induced organs damage, and benefit the anti-cancer therapy of CP [28,29]. Our histological and biochemical results agreed that CP enhanced oxidative and inflammatory responses in liver and kidney, which contributed to the observed multiple hepatic and renal injury such as necrosis and abnormal biofunctions. However, we found that the pre-treatments of Coi, a phytochemical, at 0.125 and 0.25% for two weeks markedly protected mice liver and kidney to ameliorate subsequent CP evoked oxidative and inflammatory stress. Our histological pictures and IS values supported that Coi pre-treatments abated hepatic and renal necrosis, degeneration and inflammatory infiltration caused by CP. Since histological injury has been effectively alleviated, the improved hepatic and renal biofunctions including less release of ALT, AST, BUN and Cr, greater GFR and body weight gain in Coi-treated mice could be partially explained. Moreover, our data indicated that Coi could mediate hepatic and renal mRNA expression of associated crucial genes. It is highly possible that Coi could exert its actions at the molecular level. In addition, the data from our cell lines study revealed that Coi did not affect the cytotoxic effects of CP to HepG2 and A549 cells. These novel findings suggest that the co-use of CP and Coi might display anti-cancer effects and reduce the incidence or severity of CP induced toxicity.

It is reported that increased activity of CYP2E1 and COX-2 played important roles in pathological progression of CP associated tissue damage, apoptosis and organ failure because higher activity of CYP2E1 and COX-2 facilitated the generation of ROS and PGE_2, two crucial factors responsible for the progression of CP associated oxidative and inflammatory toxicity [30,31]. In our present work, CP increased both CYP2E1 and COX-2 activities in liver and kidney, which subsequently contributed to the greater

oxidative and inflammatory stress in these two organs. However, Coi pre-treatments at two doses substantially reduced the activity of CYP2E1 and COX-2. Thus, the lower hepatic and renal oxidative and inflammatory responses in Coi-treated mice could be partially ascribed to the suppression of Coi upon CYP2E1 and COX-2 activities.

The up-regulation of NF-κB and p38MAPK pathways accelerated both oxidative and inflammatory reactions, which led to the over-production of oxidants and inflammatory cytokines such as ROS and TNF-alpha [32,33]. CP could facilitate the maturation and release of IL-1beta and IL-18, and mediate cancer cell pyroptosis via activating NLRP3 inflammasome [34,35]. Consequently, the increased circulating levels of ROS, IL-1beta and IL-18 from those activated signaling pathways promoted the progression of hepatotoxicity and nephrotoxicity [36,37]. In our present study, CP exposure increased hepatic and renal mRNA expression of NF-κB, p38MAPK and NLRP3. Thus, it seems reasonable to observe the massive release of ROS, NO, IL-1beta and TNF-alpha in liver and kidney. These oxidants and inflammatory cytokines certainly contributed to the development of observed hepatotoxicity and nephrotoxicity. Nrf2 is a master transcriptional regulator responsible for basal and inducible expression of genes encoded for xenobiotic transporters and anti-oxidative proteins [38]. The suppression of Nrf2 due to CP further diminished the expression of down-stream antioxidant genes and phase II detoxifying enzymes, which subsequently weakened the anti-oxidative defensive capability of organs or systems [39]. We found that pre-treatments of Coi effectively mediated mRNA expression of NF-κB, p38MAPK, NLRP3 and Nrf2, which in turn mitigated oxidative and inflammatory stress, and promoted anti-oxidative protection. The observed higher GSH level, greater GPX and GR activities, as well as lower ROS, NO, IL-1beta and TNF-alpha levels also agreed that Coi lessened CP induced oxidative and inflammatory stress in

liver and kidney. These results also implied that Coi was an inhibitor against NF-κB, p38MAPK and NLRP3, and an enhancer for Nrf2.

HepG2 and A549 cells are liver and lung cancer cells. Both cell lines have been widely used as cancer cell model for pathological and pharmacological investigations [40,41]. In our present work, these two cancer cell lines were used to evaluate whether Coi interfered with the anti-cancer effects of CP. Our data revealed that Coi alone did not affect the growth of these cancer cells. Furthermore, we found that the co-use of Coi and CP resulted in similar cytotoxic effects as CP alone to these two cancer cells. These findings suggest that Coi could protect liver and kidney against CP induced toxicity, and might not interfere with the anti-cancer activity of CP. Further *in vivo* studies, especially cancer animal models, are necessary to verify the effects and appropriate doses of co-use of Coi and CP. It is reported that the combined use of CP and oleanolic acid co-loaded calcium carbonate nanoparticles enhanced liver cancer cell apoptosis and reduced hepatoxicity [42]. Thus, the co-use of CP and other agent(s) with organ's protective activities could be considered to perform effective therapy for cancers and mitigate CP associated adverse effects.

Coi is a polyphenol and naturally occurred in adlay seed. Kuo et al. [16] reported that adlay was a potential cancer chemopreventive agent. Our present study further indicated that Coi, a component compound of adlay, could provide multiple blocking properties against cisplatin. It is highly possible that Coi contributes to the chemopreventive action of adlay. In addition, our data revealed that Coi alone at 0.25% exhibited similar histological and biochemical results as normal groups. Thus, the application of Coi at this dose and lower doses like 0125% should be safe, and might be helpful for cancer patients.

5. Conclusions

Coixol pre-treatments markedly protected mice liver and kidney against cisplatin evoked multiple injury. Coixol alleviated histological oxidative and inflammatory injury, regulated mRNA expression of associated crucial genes and mediated activity of several enzymes. Consequently, hepatic and renal biofunctions were maintained. Furthermore, coixol did not affect cisplatin induced apoptosis in HepG2 and A549 cancer cells. These findings suggest that coixol is a potent hepatic and renal protective agent against cisplatin.

Acknowledgement

This study was partially supported by a grant from Asia University, Taichung, Taiwan (ASIA-107-CMUH-05).

Conflicts of Interest

All authors declare that no Conflict of Interest.

Consent to Publish

The final content of this paper was read and approved by all authors.

Informed Consent

This study had no human subject.

References

1. Marullo R, Werner E, Degtyareva N, Moore B, Altavilla G et al. Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. PLoS One 2013; 8: e81162. https://doi.org/10.1371/journal.pone.0081162
2. Arany I, Safirstein RL. Cisplatin nephrotoxicity. Seminars in Nephrology 2003; 23 (5): 460-464. https://doi.org/10.1016/s0270-9295(03)00089-5
3. Qi L, Luo Q, Zhang Y, Jia F, Zhao Y et al. Advances in toxicological research of the anticancer drug cisplatin. Chemical Research in Toxicology 2019; 32 (8): 1469-1486. https://doi.org/10.1021/acs.chemrestox.9b00204
4. Chirino YI, Pedraza-Chaverri J. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. Experimental and Toxicologic Pathology 2009; 61 (3): 223-242. https://doi.org/10.1016/j.etp.2008.09.003
5. Bilgic Y, Akbulut S, Aksungur Z, Erdemli ME, Ozhan O et al. Protective effect of dexpanthenol against cisplatin-induced hepatotoxicity. Experimental and Therapeutic Medicine 2018; 16 (5): 4049-4057. http://doi.org/10.3892/etm.2018.6683
6. Neamatallah T, El-Shitany NA, Abbas AT, Ali SS, Eid BG. Honey protects against cisplatin-induced hepatic and renal toxicity through inhibition of NF-kappaB-mediated COX-2 expression and the oxidative stress dependent BAX/Bcl-2/caspase-3 apoptotic pathway. Food & Function 2018; 9 (7): 3743-3754. https://doi.org/10.1039/c8fo00653a
7. Qu X, Gao H, Tao L, Zhang Y, Zhai J et al. Autophagy inhibition-enhanced assembly of the NLRP3 inflammasome is associated with cisplatin-induced acute injury to the liver and kidneys in rats. Journal of Biochemical and Molecular

Toxicology 2018; 33: e22208. https://doi.org/10.1002/jbt.22228

8. Verma VK, Malik S, Mutneja E, Sahu AK, Rupashi K et al. Mechanism involved in fortification by berberine in CDDP-induced nephrotoxicity. Current Molecular Pharmacology 2020; 13 (4): 342-352. https://doi.org/10.2174/1874467213666200220142202

9. Cetin R, Devrim E, Kiliçoğlu B, Avci A, Candir O et al. Cisplatin impairs antioxidant system and causes oxidation in rat kidney tissues: possible protective roles of natural antioxidant foods. Journal of Applied Toxicology 2006; 26 (1): 42-46. https://doi.org/10.1002/jat.1103

10. Kuai CP, Ju LJ, Hu PP, Huang F. Corydalis saxicola alkaloids attenuate cisplatin-induced neuropathic pain by reducing loss of IENF and blocking TRPV1 activation. American Journal of Chinese Medicine 2020; 48 (2): 407-428. https://doi.org/10.1142/S0192415X20500214

11. Un H, Ugan RA, Kose D, Bayir Y, Cadirci E et al. A novel effect of aprepitant: protection for cisplatin-induced nephrotoxicity and hepatotoxicity. European Journal of Pharmacology 2020; 880: 173168. https://doi.org/10.1016/j.ejphar.2020.173168

12. Iskander A, Yan LJ. Cisplatin-induced kidney toxicity: potential roles of major NAD^+-dependent enzymes and plant-derived natural products. Biomolecules 2022; 12: 1078. http://doi.org/10.3390/biom12081078

13. He C, Li Z, Liu H, Zhang H, Wang L et al. Chemical compositions and antioxidant activity of adlay seed (Coixlachryma-jobi L.) oil extracted from four main producing areas in China. Journal of Food Science 2020; 85 (1): 123-131. https://doi.org/10.1111/1750-3841.14982

14. Chang SW, Hwang BK. Relationship of host genotype to Bipolaris leaf blight

severities and yield components of adlay. Plant Diseases 2002; 86 (7): 774-779. https://doi.org/10.1094/PDIS.2002.86.7.774

15. Kuo CC, Chen HH, Chiang W. Adlay (yi yi; "soft-shelled job's tears"; the seeds of Coix lachryma-jobi L. var. ma-yuen Stapf) is a potential cancer chemopreventive agent toward multistage carcinogenesis processes. Journal of Traditional and Complementary Medicine 2012; 2: 267-275. https://doi.org/10.1016/s2225-4110(16)30112-2

16. Yin H, Wang S, Nie S, Xie M. Coix polysaccharides: Gut microbiota regulation and immunomodulatory. Bioactive Carbohydrates and Dietary Fibre 2018; 16: 53-61. https://doi.org/10.1016/j.bcdf.2018.04.002

17. Chen HH, Chiang W, Chang JY, Chien YL, Lee CK et al. Antimutagenic constituents of adlay (Coix lachryma-jobi L. var. ma-yuen Stapf) with potential cancer chemopreventive activity. Journal of Agricultural and Food Chemistry 2011; 59 (12): 6444-6452. https://doi.org/10.1021/jf200539r

18. Amen Y, Arung ET, Afifi MS, Halim AF, Ashour A et al. Melanogenesis inhibitors from Coix lacryma-jobi seeds in B16-F10 melanoma cells. Natural Product Research 2017; 31 (23): 2712-2718. https://doi.org/10.1080/14786419.2017.1292270

19. Sharma KR, Adhikari A, Hafizur RM, Hameed A, Raza SA et al. Potent insulin secretagogue from Scoparia dulcis Linn of Nepalese origin. Phytotherapy Research 2015; 29 (10): 1672-1675. https://doi.org/10.1002/ptr.5412

20. Hameed A, Hafizur RM, Khan MI, Jawed A, Wang H et al. Coixol amplifies glucose-stimulated insulin secretion via cAMP mediated signaling pathway. European Journal of Pharmacology 2019; 858: 172514. https://doi.org/10.1016/j.ejphar.2019.172514

21. Hu Y, Zhou Q, Liu T, Liu Z. Coixol suppresses NF-kappaB, MAPK pathways and NLRP3 inflammasome activation in lipopolysaccharide-induced RAW 264.7 cells. Molecules 2020; 25: 894. https://doi.org/10.3390/molecules25040894

22. Baranova IN, Souza AC, Bocharov AV, Vishnyakova TG, Hu X et al. Human SR-BI and SR-BII potentiate lipopolysaccharide-induced inflammation and acute liver and kidney injury in mice. Journal of Immunology 2016; 196 (7): 3135-3147. https://doi.org/10.4049/jimmunol.1501709

23. Moshage H, Kok B, Huizenga JR, Jansen P. Nitrite and nitrate determinations in plasma: A critical evaluation. Clinical Chemistry 1995; 41: 892-896. https://doi.org/10.1093/clinchem/41.6.892

24. Oun R, Moussa YE, Wheate NJ. The side effects of platinum-based chemotherapy drugs: a review for chemists. Dalton Transactions 2018; 47 (19): 6645-6653. https://doi.org/10.1039/c8dt00838h

25. Mohamadi Yarijani Z, Godini A, Madani SH, Najafi H. Reduction of cisplatin-induced renal and hepatic side effects in rat through antioxidative and anti-inflammatory properties of Malva sylvestris L. extract. Biomedicine & Pharmacotherapy 2018; 106: 1767-1774. https://doi.org/10.1016/j.biopha.2018.07.115

26. Pratibha R, Sameer R, Rataboli PV, Bhiwgade DA, Dhume CY. Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. European Journal of Pharmacology 2006; 532 (3): 290-293. https://doi.org/10.1016/j.ejphar.2006.01.007

27. Zager RA, Johnson AC, Lund S, Hanson S. Acute renal failure: determinants and characteristics of the injury-induced hyperinflammatory response. American Journal of Physiology-Renal Physiology 2006; 291 (3): 546-556.

https://doi.org/10.1152/ajprenal.00072.2006

28. Kurt N, Türkeri ÖN, Suleyman B, Bakan N. The effect of taxifolin on high-dose-cisplatin-induced oxidative liver injury in rats. Advances in Clinical and Experimental Medicine 2021; 30 (10): 1025-1030. https://doi.org/10.17219/acem/138318

29. Taghizadeh F, Hosseinimehr SJ, Zargari M, Karimpour Malekshah A, Mirzaei M et al. Alleviation of cisplatin-induced hepatotoxicity by gliclazide: Involvement of oxidative stress and caspase-3 activity. Pharmacology Research & Perspectives 2021; 9: e00788. https://doi.org/10.1002/prp2.788

30. Quintanilha JCF, de Sousa VM, Visacri MB, Amaral LS, Santos RMM et al. Involvement of cytochrome P450 in cisplatin treatment: implications for toxicity. Cancer Chemotherapy and Pharmacology 2017; 80: 223-233. https://doi.org/10.1007/s00280-017-3358-x

31. Fernández-Martínez AB, Benito Martínez S, Lucio Cazaña FJ. Intracellular prostaglandin E2 mediates cisplatin-induced proximal tubular cell death. Biochimica et Biophysica Acta 2016; 1863 (2): 293-302. https://doi.org/10.1016/j.bbamcr.2015.11.035

32. Zhao K, Wen LB. DMF attenuates cisplatin-induced kidney injury via activating Nrf2 signaling pathway and inhibiting NF-kB signaling pathway. European Review for Medical and Pharmacological Sciences 2018; 22 (24): 8924-8931. https://doi.org/10.26355/eurrev_201812_16662

33. Sherif IO, Al-Mutabagani LA, Alnakhli AM, Sobh MA, Mohammed HE. Renoprotective effects of angiotensin receptor blocker and stem cells in acute kidney injury: Involvement of inflammatory and apoptotic markers. Experimental

Biology and Medicine (Maywood). 2015; 240 (12): 1572-1579. https://doi.org/10.1177/1535370215577582

34. Tavakoli Dargani Z, Singla DK. Embryonic stem cell-derived exosomes inhibit doxorubicin-induced TLR4-NLRP3-mediated cell death-pyroptosis. American Journal of Physiology-Heart and Circulatory Physiology 2019; 317 (2): H460-H471. https://doi.org/10.1152/ajpheart.00056.2019

35. Eisa NH, El-Sherbiny M, Abo El-Magd NF. Betulin alleviates cisplatin-induced hepatic injury in rats: Targeting apoptosis and Nek7-independent NLRP3 inflammasome pathways. International Immunopharmacology 2021; 99: 107925. https://doi.org/10.1016/j.intimp.2021.107925

36. Wei S, Ma W, Zhang B, Li W. NLRP3 Inflammasome: a promising therapeutic target for drug-induced toxicity. Frontiers in Cell and Developmental Biology 2021; 9: 634607. https://doi.org/10.3389/fcell.2021.634607

37. Mahmoud AM, Hussein OE, Abd El-Twab SM, Hozayen WG. Ferulic acid protects against methotrexate nephrotoxicity via activation of Nrf2/ARE/HO-1 signaling and PPARgamma, and suppression of NF-kappaB/NLRP3 inflammasome axis. Food & Function 2019; 10 (8): 4593-4607. https://doi.org/10.1039/c9fo00114j

38. Shelton LM, Park BK, Copple IM. Role of Nrf2 in protection against acute kidney injury. Kidney International 2013; 84 (6): 1090-1095. https://doi.org/10.1038/ki.2013.248

39. El-Sayed RM, Abo El Gheit RE, Badawi GA. Vincamine protects against cisplatin induced nephrotoxicity via activation of Nrf2/HO-1 and hindering TLR4/IFN-gamma/CD44 cells inflammatory cascade. Life Science 2021; 272: 119224. https://doi.org/10.1016/j.lfs.2021.119224

40. Chen YF, Chang WH, Tsai HY, Lee MM, Wu TS. Bioactivities and action mechanisms of active compounds from Hottuynia cordata THUNB on human lung cancer cells. BioMedicine 2021; 11: 7. https://doi.org/10.37796/2211-8039.1219

41. D'Arcy MS, Pike CVS, Coussons PJ. A novel combined resveratrol/berberine phytochemotheraputic using the HePG2 cell line as a model for the treatment of hepatocarcinoma. Cell Biology International 2021; 45 (12): 2499-2509. https://doi.org/10.1002/cbin.11695

42. Khan MW, Zhao P, Khan A, Raza F, Raza SM et al. Synergism of cisplatin-oleanolic acid co-loaded calcium carbonate nanoparticles on hepatocellular carcinoma cells for enhanced apoptosis and reduced hepatotoxicity. International Journal of Nanomedicine 2019; 14: 3753-3771. http://doi.org/10.2147/IJN.S196651

Table 1. Primer sequences of target genes for RT-PCR analyses

target		primers
NF-κB	F	5’-GTA ACA GCA GGA CCC AAG GA-3’
	R	5’-AGC CCC TAA TAC ACG CCT CT-3’
p38	F	5’-ACT CAG ATG CCG AAG ATG AAC-3’
	R	5’-GTG CTC AGG ACT CCA TCT CT-3’
NLRP3	F	5′-TCT CAG CAC CAA CCA GAG CCT CAC-3′
	R	5′-CCA CGC ACA GCA GTC TGA CTC CAA-3′
Nrf2	F	5’-CTC GCT GGA AAA AGA AGT G-3’
	R	5’-CCG TCC AGG AGT TCA GAG G-3’
GAPDH	F	5’-GGA TGC AGG GAT GAT GTT C-3’
	R	5’-TGC ACC ACC AAC TGC TTA G-3’

Table 2. Effects of Coi upon feed intake (FI, g/mouse/day), water intake (WI, mL/mouse/day), initial body weight (IBW, g/mouse), final body weight (FBW, g/mouse), BW gain (g), liver weight (LW, g) and kidney weight (KW, mg) of normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Values are mean ± SD, n = 8. The same superscript letters on the same row indicate statistical similarity between groups, p >0.05. Different superscript letters indicate statistical differences between groups, p <0.05.

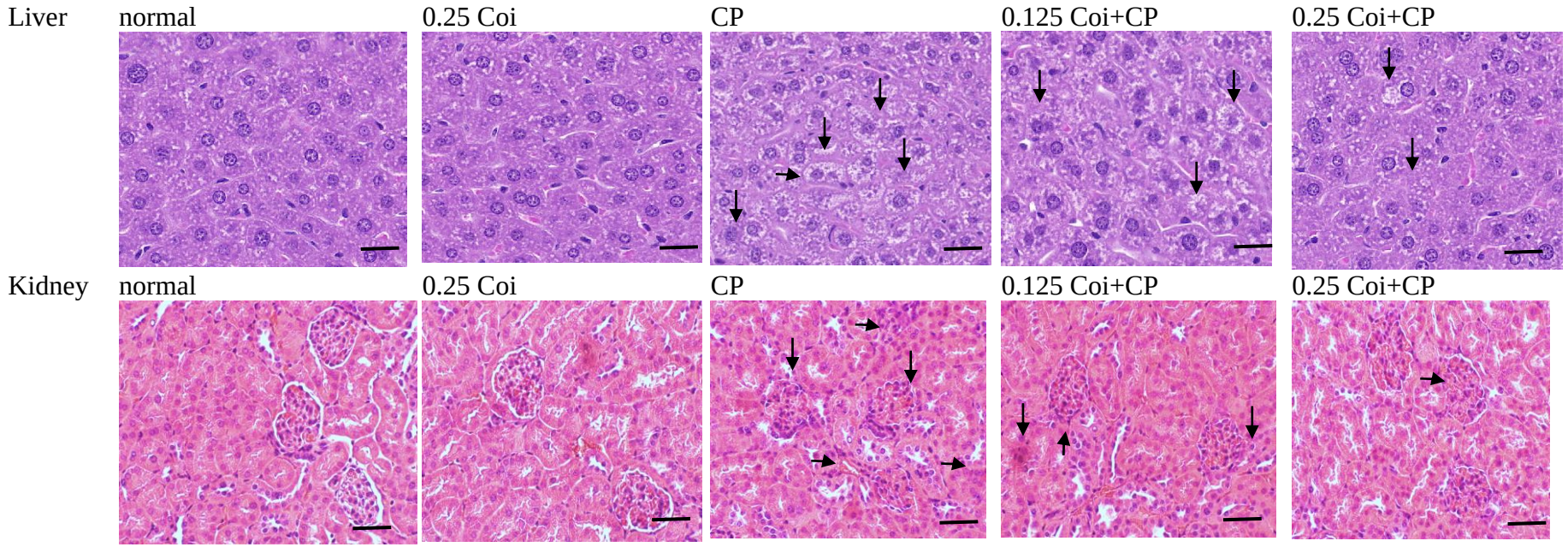
	Normal	0.25 Coi	CP	0.125 Coi+CP	0.25 Coi+CP
FI	2.4 ± 0.7^{a}	2.5 ± 0.4^{a}	2.2 ± 0.3^{a}	2.3 ± 0.5^{a}	2.6 ± 0.6^{a}
WI	2.3 ± 0.6^{a}	2.0 ± 0.5^{a}	2.1 ± 0.4^{a}	2.2 ± 0.6^{a}	2.2 ± 0.5^{a}
IBW	22.5 ± 1.1^{a}	23.0 ± 0.7^{a}	22.7 ± 0.9^{a}	22.8 ± 0.8^{a}	22.6 ± 0.5^{a}
FBW	25.6 ± 0.5^{b}	25.9 ± 0.9^{b}	23.6 ± 0.6^{a}	24.1 ± 1.0^{a}	24.5 ± 0.3^{a}
BW gain	3.1 ± 0.3^{c}	2.9 ± 0.8^{c}	0.9 ± 0.5^{a}	1.3 ± 0.3^{a}	1.9 ± 0.4^{b}
LW	1.45 ± 0.08^{a}	1.41 ± 0.1^{a}	1.69 ± 0.07^{b}	1.61 ± 0.11^{b}	1.49 ± 0.06^{a}
KW	464 ± 13^{a}	470 ± 9^{a}	495 ± 12^{b}	475 ± 10^{a}	472 ± 8^{a}

Table 3. Effects of Coi upon activity of ALT (IU/L) and AST (IU/L), level of BUN (mmol/L) and Cr (μmol/L) in plasma, and GFR (mL/min) in normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Values are mean ± SD, n = 8. The same superscript letters on the same row indicate statistical similarity between groups, p >0.05. Different superscript letters indicate statistical differences between groups, p <0.05.

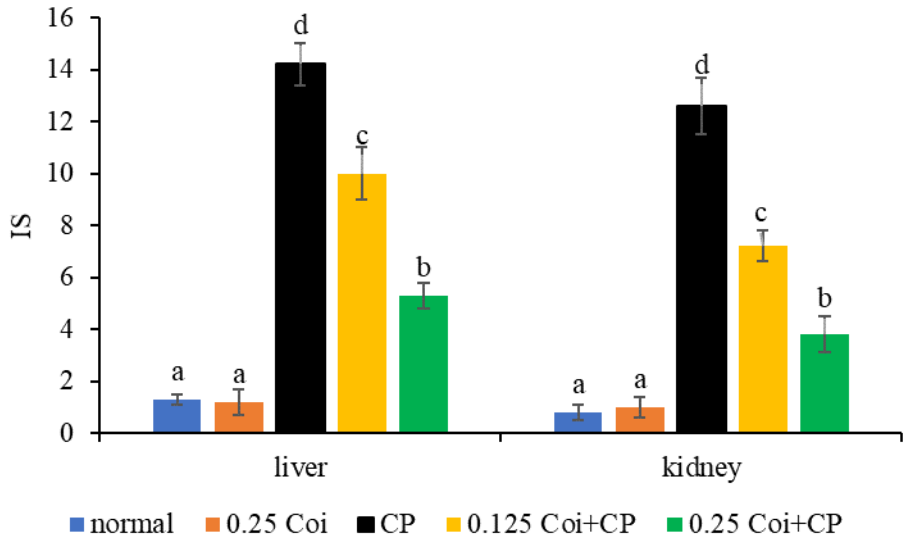
	normal	0.25 Coi	CP	0.125 Coi+CP	0.25 Coi+CP
ALT	20 ± 4^{a}	19 ± 3^{a}	109 ± 10^{d}	84 ± 9^{c}	53 ± 5^{b}
AST	21 ± 2^{a}	18 ± 5^{a}	121 ± 8^{d}	92 ± 6^{c}	64 ± 7^{b}
BUN	14.5 ± 1.0^{a}	14.1 ± 0.7^{a}	134.7 ± 12.1^{d}	102.5 ± 9.2^{c}	60.3 ± 6.8^{b}
Cr	28.9 ± 1.5^{a}	27.2 ± 1.3^{a}	177.8 ± 11.5^{d}	129.3 ± 8.2^{c}	87.1 ± 10.4^{b}
GFR	88.5 ± 2.4^{d}	90.6 ± 3.1^{d}	40.2 ± 5.3^{a}	55.9 ± 4.4^{b}	70.3 ± 4.0^{c}

Figure 1. Histological analysis of hepatic and renal injury. A: Representative images (400 X) of liver and kidney sections. B: IS value from normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Scale bar = 50 μm. Arrows indicated the sites of injury. IS values are mean ± SD, n = 8. The same superscript letters among bars indicate statistical similarity between groups, p >0.05. Different superscript letters indicate statistical differences between groups, p <0.05.

A.



1 B.


16
14
12
10
8
6
4
2
0
IS
a
a
d
c
b
a
a
d
c
b
liver
kidney
normal
0.25 Coi
CP
0.125 Coi+CP
0.25 Coi+CP

2

3

4

5

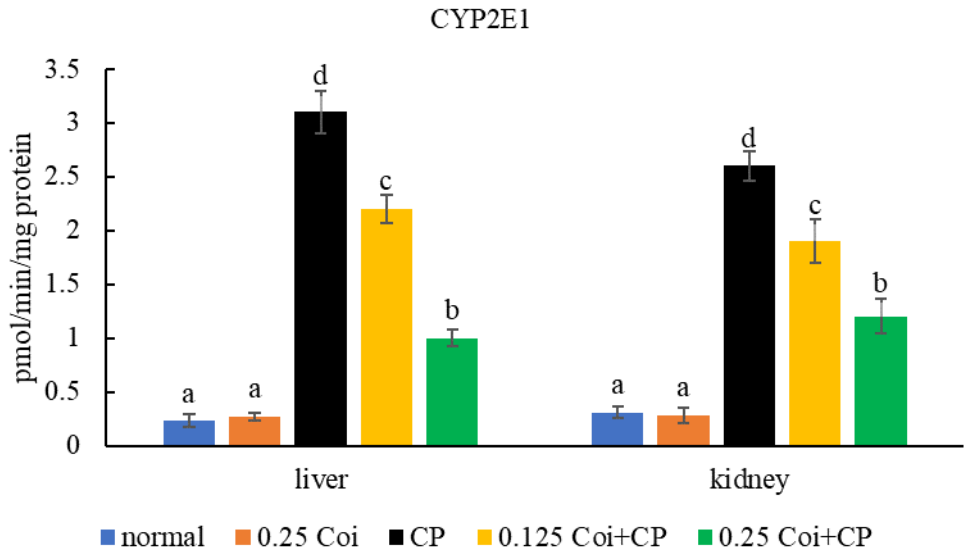
Table 4. Effects of Coi upon hepatic and renal levels of ROS (RFU/mg protein), NO (μmol/mg protein), GSH (nmol/mg protein), and activity (U/mg protein) of GPX and GR in normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Values are mean ± SD, n = 8. The same superscript letters on the same row indicate statistical similarity between groups, $p > 0.05$. Different superscript letters indicate statistical differences between groups, $p < 0.05$.

	normal	0.25 Coi	CP	0.125 Coi+CP	0.25 Coi+CP
Liver					
ROS	0.19 ± 0.07^{a}	0.21 ± 0.06^{a}	1.41 ± 0.13^{d}	1.03 ± 0.09^{c}	0.62 ± 0.10^{b}
NO	0.41 ± 0.05^{a}	0.47 ± 0.08^{a}	1.68 ± 0.14^{d}	1.27 ± 0.13^{c}	0.89 ± 0.07^{b}
GSH	12.5 ± 0.6^{d}	12.7 ± 0.9^{d}	6.2 ± 0.6^{a}	8.0 ± 0.5^{b}	10.2 ± 1.0^{c}
GPX	18.0 ± 0.6^{c}	18.3 ± 0.8^{c}	10.4 ± 1.0^{a}	12.8 ± 0.7^{b}	14.0 ± 1.1^{b}
GR	1.42 ± 0.14^{d}	1.50 ± 0.11^{d}	0.71 ± 0.08^{a}	0.97 ± 0.1^{b}	1.21 ± 0.05^{c}
Kidney					
ROS	0.14 ± 0.05^{a}	0.12 ± 0.04^{a}	1.58 ± 0.19^{d}	1.12 ± 0.12^{c}	0.73 ± 0.07^{b}
NO	0.54 ± 0.07^{a}	0.51 ± 0.05^{a}	1.77 ± 0.15^{d}	1.35 ± 0.09^{c}	0.96 ± 0.10^{b}
GSH	12.4 ± 0.8^{d}	12.0 ± 0.6^{d}	5.9 ± 0.7^{a}	7.6 ± 0.9^{b}	9.8 ± 0.5^{c}
GPX	16.8 ± 1.4^{c}	17.1 ± 0.9^{c}	9.6 ± 1.1^{a}	12.3 ± 0.8^{b}	13.1 ± 1.0^{b}
GR	1.35 ± 0.16^{d}	1.42 ± 0.12^{d}	0.67 ± 0.05^{a}	0.89 ± 0.07^{b}	1.18 ± 0.06^{c}

Table 5. Effects of Coi upon hepatic and renal levels (pg/mg protein) of IL-1beta, TNF-alpha and PGE_2 in normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Values are mean ± SD, n = 8. The same superscript letters on the same row indicate statistical similarity between groups, $p > 0.05$. Different superscript letters indicate statistical differences between groups, $p < 0.05$.

	normal	0.25 Coi	CP	0.125 Coi+CP	0.25 Coi+CP
Liver					
IL-1beta	13 ± 2^{a}	11 ± 4^{a}	375 ± 29^{d}	261 ± 22^{c}	157 ± 17^{b}
TNF-alpha	18 ± 5^{a}	16 ± 3^{a}	416 ± 20^{d}	294 ± 12^{c}	187 ± 13^{b}
PGE_2	549 ± 23^{a}	527 ± 39^{a}	1975 ± 84^{d}	1520 ± 102^{c}	966 ± 71^{b}
Kidney					
IL-1beta	11 ± 3^{a}	14 ± 4^{a}	651 ± 51^{d}	448 ± 35^{c}	276 ± 23^{b}
TNF-alpha	15 ± 2^{a}	16 ± 5^{a}	733 ± 60^{d}	558 ± 42^{c}	326 ± 25^{b}
PGE_2	606 ± 19^{a}	614 ± 24^{a}	2583 ± 110^{d}	1849 ± 68^{c}	1021 ± 94^{b}

Figure 2. Effects of Coi upon hepatic and renal activities of CYP2E1 and COX-2 in normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Values are mean ± SD, n = 8. The same superscript letters among bars indicate statistical similarity between groups, p >0.05. Different superscript letters indicate statistical differences between groups, p <0.05.



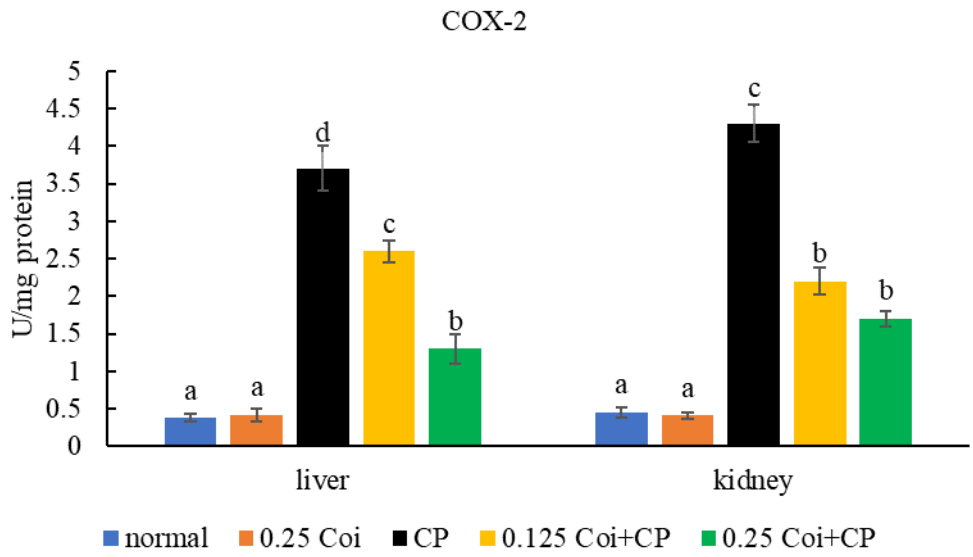
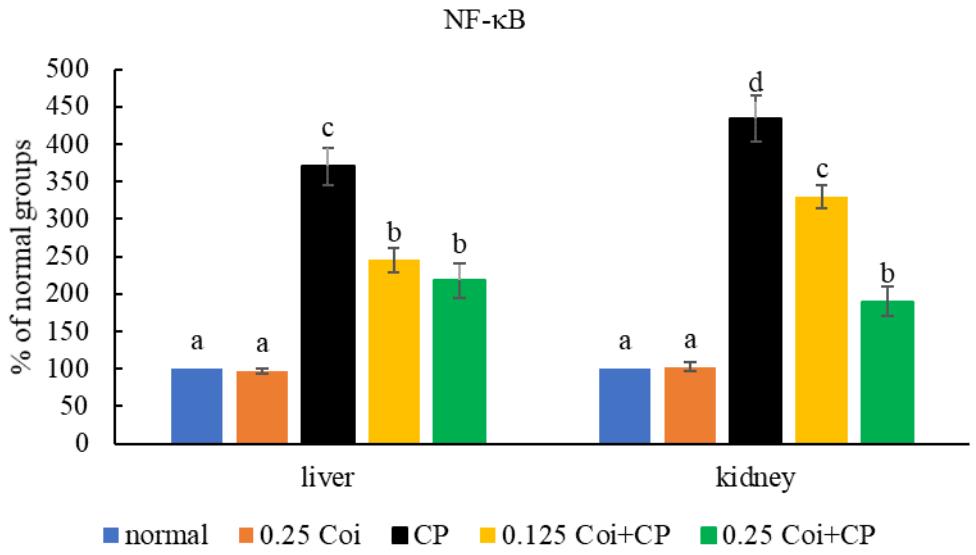
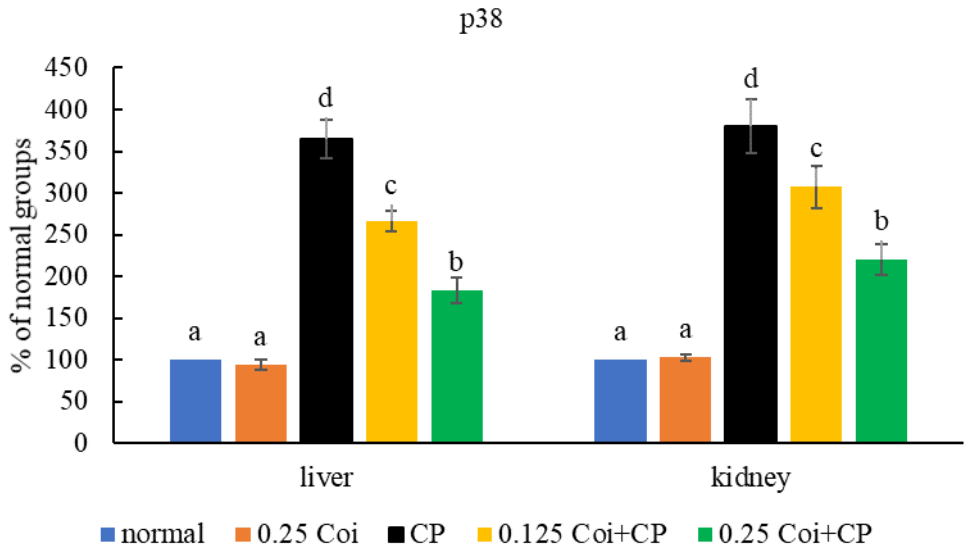
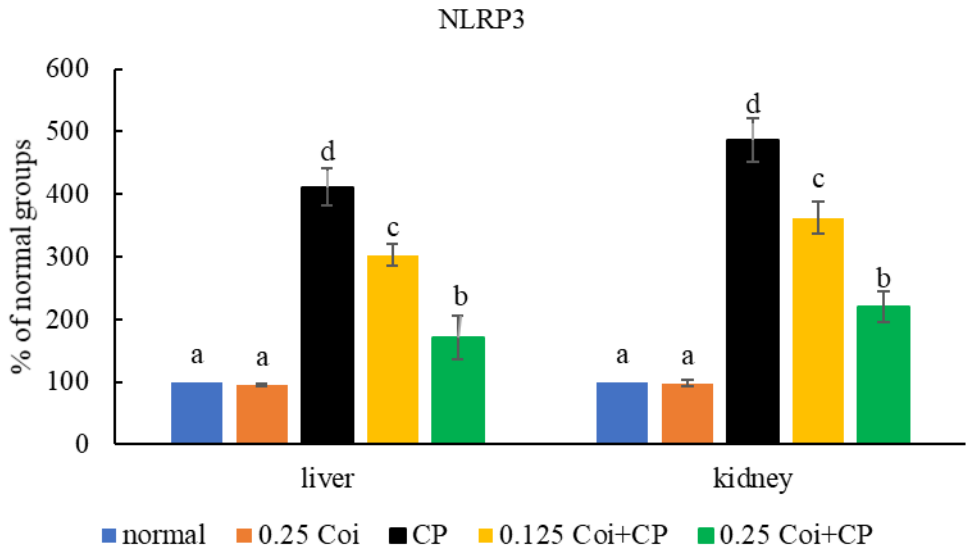


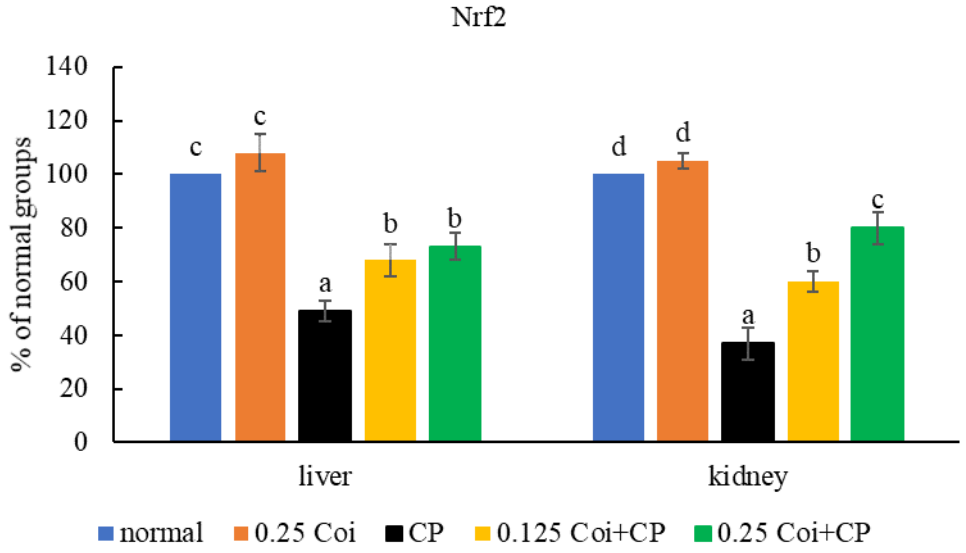
Figure 3. Effects of Coi upon hepatic and renal mRNA expression of NF-κB, p38, NLRP3 and Nrf2 in normal mice, 2-wk 0.25% Coi treated mice (0.25 Coi), CP administrated mice (CP), and mice with 2-wk 0.125 or 0.25% Coi treatment and followed by CP administration (0.125 Coi+CP, 0.25 Coi+CP). Values are mean ± SD, n = 8. The same superscript letters among bars indicate statistical similarity between groups, p >0.05. Different superscript letters indicate statistical differences between groups, p <0.05.






NLRP3
% of normal groups
600
500
400
300
200
100
0
a
a
d
c
b
a
a
d
c
b
liver
kidney
normal
0.25 Coi
CP
0.125 Coi+CP
0.25 Coi+CP

1


Nrf2
% of normal groups
140
120
100
80
60
40
20
0
c
c
a
b
b
d
d
a
b
c
liver
kidney
normal
0.25 Coi
CP
0.125 Coi+CP
0.25 Coi+CP

2

3

Figure 4. Effects of Coi upon the viability of HepG2 and A549 cells. Cells were treated by Coi at 0 (normal groups), 2, 4, 8, 16 or 32 μM at 37 °C for 48 h. Viability was measured at time 48 h. Values are mean ± SD, n = 5.

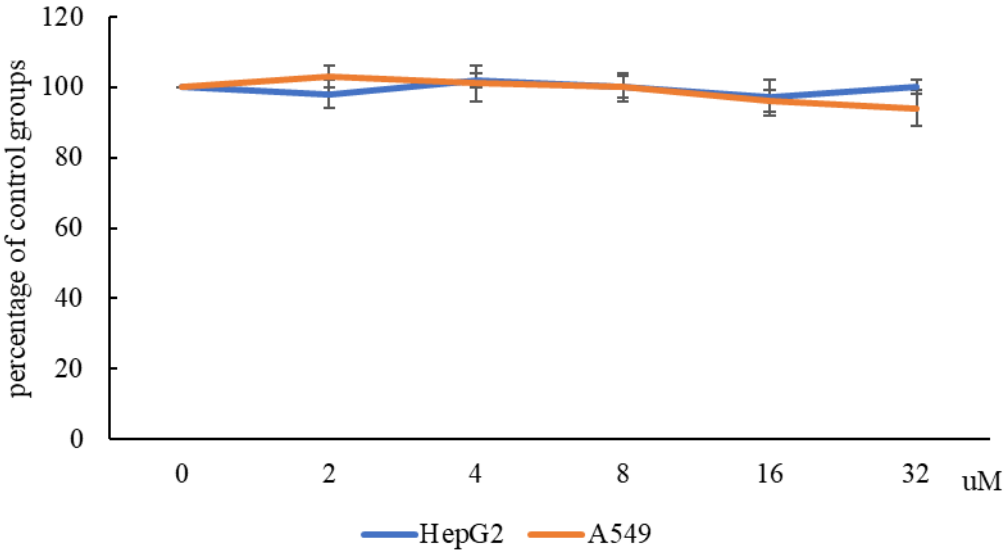


Figure 5. Effects of Coi upon the inhibitory effects of CP. HepG2 and A549 cells (10^5/mL) were treated with Coi (32 μM), CP (5 μM), or Coi (32 μM) plus CP (5 μM) at 37 °C for 48 h. Normal groups were cells had no CP or Coi. Cell viability was measured at 0 and 48 h. Values are mean ± SD, n = 5. The same superscript letters among lines indicate statistical similarity between groups, p >0.05. Different superscript letters indicate statistical differences between groups, p <0.05.