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Research Article

The Effects of *Cornus mas* Hydro-Methanolic Extract on Cisplatin-Induced Nephrotoxicity in Rats

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ABSTRACT

Background: Cisplatin (Cis) is a chemotherapeutic agent and nephrotoxicity is a serious adverse effect of the drug. This study investigated the protective effects of *Cornus mas* fruit hydro-methanolic extract (CME) on cisplatin-induced nephrotoxicity in a rat model.
Methods: Forty Wistar rats were divided into the control group, CME group, CME 300 + Cis group, CME 700 + Cis group, and Cis group. After the intervention samples were taken for biochemical and histopathological analysis.
Results: The CME analysis showed considerable total antioxidant and total phenol contents. The blood serum urea and creatinine increased ($p < 0.01$) in the Cis group rats. In the Cis + CME groups, the parameters were not significantly different from that of the control group. There were no significant differences in the renal activities of SOD, GPx, CAT, and TAC between the Cis-treated and control groups ($p > 0.05$). The renal MDA levels of the Cis and CME 300 + Cis groups decreased significantly in comparison to the control and the CME groups ($p < 0.01$ and $p < 0.05$, respectively). In the histopathological examination, mild to severe degeneration was observed in the Cis group. Despite the presence of mild degeneration, they were less severe in the Cis + CME groups, and maintained a better morphology when compared to the Cis group ($p < 0.01$).
Conclusion: The results showed renoprotective effects of CME against Cis-induced nephrotoxicity in rats.

Introduction

Cisplatin (cis-diamminedichloroplatinum II) is one of the most effective and widely used chemotherapeutic agents for treating malignant tumours in testis, ovary, breast, lung, cervix, and many other organs.[1-3] The therapeutic effects of cisplatin are dose-dependent,[4] but cisplatin-induced nephrotoxicity is one of the serious adverse effects that limit its clinical utility at high doses.[5-7] After administration of cisplatin, about 34% of patients develop renal dysfunction followed by acute kidney injury, which is associated with high morbidity and mortality.[3,8] The mechanism underlying cisplatin-induced nephrotoxicity is complex and not fully clear. The renal dysfunction is involved in mitochondrial oxidative stress, inflammation, and tubular necrosis.[5,9-11]

It is well known that medicinal plants can be seen as a main source of new chemical substances with potential therapeutic effects and there has been a shift towards the therapeutic evaluation of herbal products in recent years. Cornelian cherry (*Cornus mas* L.), a medicinal plant, is a member of the Cornaceae species.[12,13] The plant is found in parts of central and southern Europe as well as in western Asia, including the northern forests of Iran (East Azerbaijan and Qazvin provinces).[13,14] It is a small tree or medium-to-large deciduous shrub that grows up to 5–12 m.[13] Cornelian cherries are typically single-seeded, olive-shaped red fruits. They are sweet-sour and 10–23 mm long. The fruits may be consumed fresh or used to produce jam, stewed fruit, compote, or syrup. The fruits are rich in sugar, organic acids, oxalic acid, tannins, anthocyanins (delphinidin-3-glucoside, cyanidin-3-rhamnoglucoside, cyanidin-3-glucoside, cyanidin-3-galactoside, and pelargonidin-3-galactoside), phenols, flavonoids, and other antioxidants. Fresh cornelian cherry fruits contain B1, B2, E vitamins, folic acid, and twice as much vitamin C than oranges. Cornelian cherries have high contents of K and Mg, and low contents of Na, Cu, Mn, Fe, and Zn; their levels of toxic elements are negligible.[12-16] The cornelian cherry fruits have been used for treating sore throat, digestion problems, diarrhoea,

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intestinal inflammation, fever, malaria, measles, cholera, chickenpox, anaemia, rickets, liver (hepatitis A), kidney stones, and kidney and bladder infections in traditional and conventional medicine. The fruit flesh and the seed oil have been successfully used to cure difficult-to-heal wounds, stomach ulcers, and colitis. It was also reported to have anti-allergic and anti-microbial activities.[13,17]

Cornus mas (*C. mas*) can be an interesting natural source for decreasing systemic and tissue oxidative stress.[15] This experimental study was designed to investigate cisplatin-induced nephrotoxicity in rats and the possible protective effects of *C. mas* hydro-methanolic fruit extract (CME) against it by using biochemical and histopathological parameters.

Materials and Methods

Extraction

The *C. mas* fruits were obtained from the suburbs of Kaleibar (East Azerbaijan, Iran). The fruits were washed and the fruit plant parts were separated manually and air-dried, protected from direct light, and then turned into a coarse powder by a blender. Later, 500 g of the powder was extracted by methanol (Merck, Germany) and distilled water mixture (7:3) at 25 ± 2°C. The mixture was then filtered through 0.45 µ pore size filters and the solvent was completely removed by a rotary vacuum evaporator (Hidolf, Germany) at 40°C. Finally, the *CME* was frozen and stored in a deep freezer (-80°C) until use.[13]

Animals

Forty male Wistar rats (200 ± 20 g) were obtained from Pasteur Institute (Karaj, Iran). The animals were maintained under 22 ± 2°C, 50–70% humidity, and a 12/12 h light/dark cycle. Water and food were provided *ad libitum*. The rats were adapted to the conditions described above for one week before beginning the study. All protocols were approved by the Animal Research Ethics Committee of Tabriz University of Medical Sciences (certificate no.: 5-4-9723) and were performed in accordance with the related guidelines.

Experimental design

The rats were randomly divided into the following five groups (n = 8): Placebo control group (orally received distilled water daily by gavage needle for 16 days and an IP injection of sterile distilled water on Day 11), CME group (orally received 700 mg/kg CME daily for 16 days and an IP injection of sterile distilled water on Day 11), CME 300 + Cis group (orally received 300 mg/kg CME daily for 16 days and an IP injection of 5 mg/kg Cis. on Day 11), CME 700 + Cis group (orally received 700 mg/kg CME daily for 16 days and an IP injection of 5 mg/kg Cis on Day 11), and the Cis group (orally received distilled water daily for 16 days and an IP injection of 5 mg/kg Cis on Day 11).[18-20]

At the end of the experimental period, blood samples were obtained by cardiac puncturing method under anaesthetic condition and were centrifuged at 2,000 g and 4°C for 10 minutes. All the rats were sacrificed and the kidney tissues were excised. The right kidney of the rats were placed in 10% neutral buffered formalin for 24 hours and embedded in paraffin for histopathological study. The left kidney tissues were homogenized in ice-cold KCl (10 mM) phosphate buffer (1.15%) with EDTA: pH 7.4 and centrifuged at 10,000 g and 4°C for 20 minutes. The supernatant was used for measuring superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA). The blood serum samples and kidney tissue homogenate supernatants were stored in a -80°C freezer until use.

Biochemical analyses

The urea, creatinine (Cr), uric acid (UA), total protein, and albumin of the blood serum samples were determined by commercial kits (Pars Azmun, Karaj, Iran). The supernatant superoxide dismutase (SOD), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) were determined by commercial kits (Randox, Italy). The catalase activities of the supernatants were assayed by Cayman kit (USA). The malondialdehyde (MDA) contents of the supernatants were analysed by the barbituric acid method. The automated Abbott biochemistry analyser (Alcyon 300, USA), after calibrating and validating the biochemical tests, was used for performing the tests. All the supernatants' results were divided and normalized with the supernatants' protein contents, which were assessed by a commercial kit (Parsazmun, Iran) and expressed as per milligram tissue protein.

Histopathological evaluation

Histopathological evaluation was performed on the kidney tissues. The paraffin-embedded specimens were cut into five-micrometre-thick sections and stained with hematoxylin and eosin for examination under a light microscope (BH-2; Olympus, Tokyo, Japan). The score of the severity of kidney damage was semi-quantitatively assessed on the basis of the following parameters: necrosis, inflammation (mononuclear cells infiltration), urine space reduction (cellular changes), and focal haemorrhage. The microscopic score of each parameter was calculated separately for each tissue sample, as also for the sum of the scores given to each criterion. The scores were given as absent (0), slight (1), moderate (2), and severe (3) for each criterion. The maximum score noted was 12.

Analyses of CME

The antioxidant properties of the CME was assayed by the DPPH assay method[21] and RC50 (50% reduction capacity) was expressed as mg/ml. The control material, quercetin RC50, was 4 µg/ml. The total phenolic equivalent was determined by using Folin-Ciocalteu reagent, estimated using the gallic acid standard curve, and expressed as mg of gallic acid equivalents (GAE) per gram of extract.[22] The sum total of flavonoids was determined as described by Vador *et al.* (2012) using the spectrophotometric method.[21,23]

Table 1. Composition of Cornus mas hydro-methanolic fruit extract (CME) (average of three measurements).

Sample	Antioxidant activity (RC50; µg/ml)	Total phenolic content (mg GAE/g extract)	Total flavonoid (%)
CME	246.2 ± 0.2	131.5 ± 0.3	0.07 ± 0.01

Table 2. Effects of *Cornus mas* hydro-methanolic fruit extract (CME) on rats' blood serum biochemical parameters following cisplatin treatment (n = 8).

Group	Urea (mg/dl)	Inine(mg/dl)	Uric acid(mg/dl)	Protein(g/dl)	Albumin(g/dl)
Control	39.5 ± 3.1	0.27 ± 0.07	2.73 ± 1.09	7.70 ± 0.41	3.25 ± 0.18
CME	40.5 ± 6.5	0.31 ± 0.06	2.15 ± 0.43	7.57 ± 0.59	3.23 ± 0.15
CME 300 + Cis	46.7 ± 10.0	0.47 ± 0.14	3.03 ± 1.34	7.72 ± 0.69	3.15 ± 0.19
CME 700 + Cis	64.8 ± 23.4	0.65 ± 0.28	1.94 ± 0.53	7.40 ± 0.39	3.10 ± 0.22
Cis	98.2 ± 43.8*+	1.28 ± 0.60*+	2.47 ± 1.10	7.45 ± 0.63	3.07 ± 0.25

Results are the means ± 1SD.
* significantly different at $p < 0.01$ when compared to the control group
+ significantly different at $p < 0.01$ when compared to the CME-treated rats

Statistical analyses

Statistical analyses were performed using SPSS (version 20) for Windows (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance followed by Tukey post-hoc multiple comparative tests were used to compare the inter-group parameters for normally distributed data. The data were expressed as mean ± 1 standard deviation (SD). The statistical analyses of the histopathological evaluation of the groups were carried out by the Chi-square test. P-values less than 0.05 and 0.01 were considered statistically significant.

Results

The antioxidant properties, polyphenol, and flavonoid contents of CME

The CME's composition in terms of poly-phenolic and flavonoid compounds, and antioxidant properties are represented in Table 1.

Effect of CME on serum biochemical parameters

The effect of cisplatin administration on the serum concentration of urea, creatinine, uric acid, protein, and albumin are presented in Table 2. The administration of cisplatin significantly increased the level of urea and creatinine ($P < 0.01$). The changes of uric acid, protein, and albumin were not significant compared to the control group ($p > 0.05$). The treatment of CME significantly reversed the changed levels of urea and creatinine.

Effect of CME on renal MDA and antioxidant enzymes activity

Effect of CME on renal MDA and antioxidant enzymes activity has been listed in Table 3. There were no significant differences in the activities of SOD, GPx, CAT, and TAC between the Cis-treated and the control groups ($p > 0.05$). The kidney tissue TAC level of CME 300 + Cis group was significantly ($p < 0.05$) higher than the TAC of the control and the CME groups. The renal GPx activities of the CME 300 + Cis and the CME 700 + Cis groups were significantly ($p < 0.05$) lower than the CME group. Surprisingly, the renal MDA levels of the Cis and CME 300 + Cis-treated groups significantly decreased compared to the control and the CME groups ($p < 0.01$ and $p < 0.05$, respectively).

Histopathological findings

In histopathological examination of the kidney, no pathologic changes were observed in the control group. In the cisplatin-treated rats, there were mild to severe tubular necrosis, mononuclear cell infiltration, haemorrhage, and urine space reduction as compared to the control and the CME groups (Figure 1). In rats treated with CME + Cis, though mild tubular degeneration and necrosis were present (Figure 2), these were less severe, and glomeruli and tubular showed better morphology when compared to the Cis group (Table 4). In the kidney tissues of CME-treated rats, mild tubular degeneration was observed (Figure 3).

Discussion

In the current study, we induced nephrotoxicity in rats by a single dose of Cis injection after five days, while the rats of the test groups received oral CME 11 days before and five days after the injection. The findings showed the effects of Cis administration on the blood serum's biochemical parameters related to renal function and structure that were reversed by co-administering CME and Cis. Our results did not show any significant alteration by Cis administration on renal antioxidant enzymes, including SOD, GPx, catalase, and the total antioxidant capacity.

Table 3. Effects of *Cornus mas* hydro-methanolic fruit extract (CME) on kidney antioxidant enzymes in rats following cisplatin treatment (n=8).

Group	SOD	GPx	CAT	TAC	MDA
Control	37.1 ± 8.4	0.104 ± 0.029	24.3 ± 8.4	0.155 ± 0.007	4.60 ± 0.84
CME	40.7 ± 5.3	0.135 ± 0.034	31.9 ± 3.3	0.154 ± 0.003	4.10 ± 1.05
CME 300 + Cis	39.1 ± 2.9	0.072 ± 0.028+	30.4 ± 4.5	0.169 ± 0.008*+	2.48 ± 0.93**+
CME 700 + Cis	37.9 ± 3.9	0.075 ± 0.022+	26.7 ± 12.5	0.167 ± 0.011	3.62 ± 0.56
Cis	42.0 ± 9.3	0.0936 ± 0.042	28.0 ± 11.5	0.163 ± 0.007	2.52 ± 0.82**+

GPx (u/mg protein), Glutathione peroxidase; SOD (u/mg protein), Superoxide dismutase; CAT (nmol/min/mg protein), Catalase; MDA (nmol/mg protein), Malondialdehyde; TAC (mmol Trolox equivalent/mg protein) Total Antioxidant Capacity. Results are the means ± 1SD.
* and ** significantly different at $p < 0.05$ and $p < 0.01$ (respectively) when compared to the control group.
+Significantly different at ($p < 0.05$) when compared to the CME-treated rats

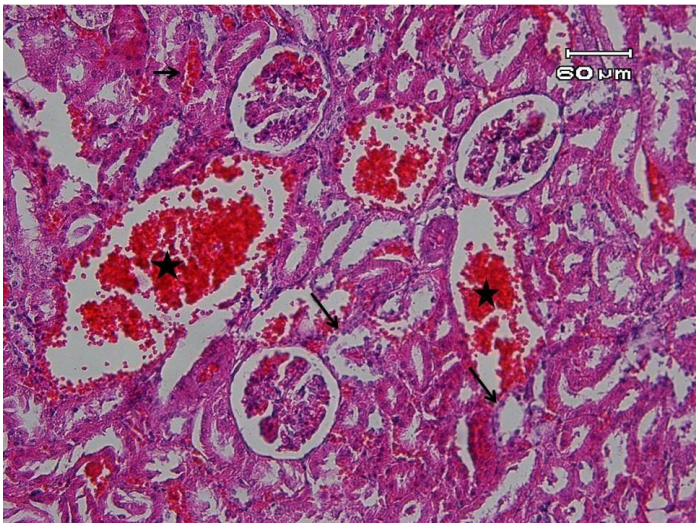

Figure 1. Severe renal lesions including congestion (short arrow), focal haemorrhage (asterisk), and tubular necrosis (long arrows) caused by cisplatin in rats (H&E).

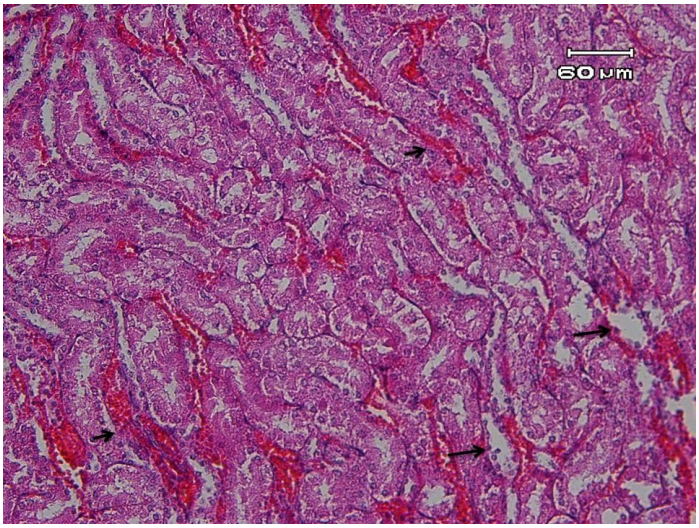

Figure 2. Moderate renal lesions including congestion (short arrows) and tubular necrosis (long arrows) caused by cisplatin and *Cornus mas* (700 mg/kg) in rats (H&E).

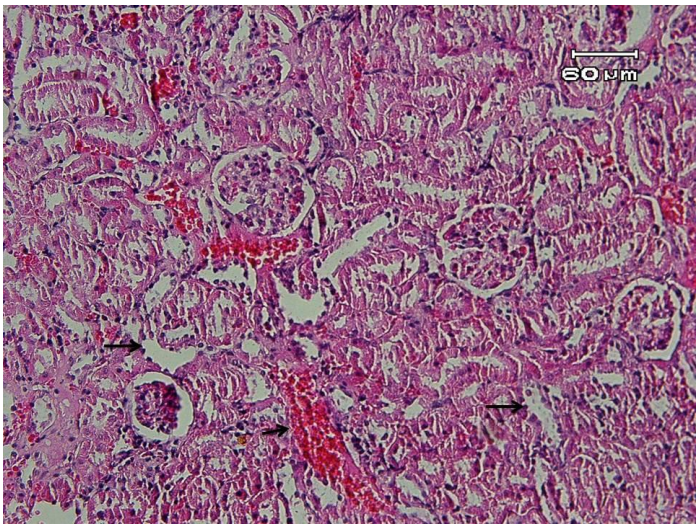

Figure 3. Mild renal lesions including congestion, focal haemorrhage (short arrow) and tubular necrosis (long arrows) caused by *Cornus mas* (700 mg/kg) in rats (H&E).

Table 4. Histopathological changes index in the kidney of the control and the treated groups (n = 8).

Parameter	Control	CME	CME 300 + Cis	CME 700 + Cis	Cis
Tubular necrosis	0.00	0.33 ± 0.51	0.83 ± 0.75	0.50 ± 0.54	1.33 ± 0.51**
Inflammation	0.00	0.17 ± 0.40	0.50 ± 0.54	0.50 ± 0.54	1.00 ± 0.81*
Urine space reducing	0.00	0.33 ± 0.51	1.17 ± 0.75**	0.17 ± 0.40	1.33 ± 0.51**
Haemorrhage	0.00	0.67 ± 0.51	1.00 ± 0.63*	0.50 ± 0.54	1.67 ± 0.51**
Total pathologic changes	0.00	1.50 ± 0.54 (p = 0.068)	3.50 ± 1.51** (p < 0.001)	1.67 ± 0.81 * (p = 0.035)	5.33 ± 1.03** (p = 0.000)

CME: *Cornus mas* extract; Cis: Cisplatin. The results are expressed as means ± of scores. The results are the means ± 1SD. * and ** significantly different in comparison to the control group ($p < 0.05$ and $p < 0.01$, respectively).

Surprisingly, the renal MDA levels were significantly decreased in the Cis and the CME 300 + Cis-treated groups in comparison to the control and the CME groups. We found that the levels of SOD, GPx, CAT and TAC were not statistically different in cisplatin group in compare with control group. The defensive reaction of the cells against cisplatin may be one of the possible explanations of these results.[24] Some previous experimental studies showed that the administration of cisplatin might cause an unexpected increase in the levels of the enzymes.[25,26]

In our study, the histopathological examination of kidney showed mild to severe degeneration in cisplatin-treated rats as compared to the control and the CME groups that were reversed and improved by CME administration in the CME and Cis co-administrated groups. The improvement effect was dose-dependent in the range of at least 300–700 mg/kg CME.

Renal histopathological degenerations induced by Cis have been reported earlier by several studies that are in line with our results.[1,27,28] The renoprotective effect of CME on carbon tetrachloride-induced nephrotoxicity in rats has also been reported by Haghi *et al*..[29] The protective effects of *C. mas* extract on CCl4-induced hepatotoxicity in rats,[4] neuroprotective effect of *C. mas* on the brain tissue of Wistar rats,[3] glycaemic and insulin control effects of *C. mas* in Type 2 diabetic adult patients,[2] and lipid-modifying and anti-inflammatory effects of *C. mas*[30] have been proved by previous studies.

Nephrotoxicity due to the drugs is usually associated with their affinity to kidneys, accumulation in the renal cortex, and kinetics of the drug-trapping process. After cisplatin administration, approximately 34% patients develop renal dysfunction manifested by lower glomerular filtration rate and higher serum creatinine, resulting in acute kidney injury. Renal tubular cell death is a common feature of cisplatin nephrotoxicity. In *in vivo* cisplatin nephrotoxicity models, both necrotic and apoptotic cell death are identified.[3] Cisplatin has multiple cellular targets[1] and the Cis-induced nephrotoxicity mechanisms are not exactly known. Cisplatin is freely filtered in the glomeruli, and it is taken up by renal tubular cells reaching the proximal tubular inner medullae and outer cortices. The mechanisms of cisplatin-induced nephrotoxicity are complex. Several mechanisms including oxidative stress, DNA damage, and inflammatory responses have been suggested to be associated with cisplatin-induced nephrotoxicity.[31,32] Some previous investigations suggests that Cis accumulates in the mitochondria of kidney epithelial cells and induces reactive oxygen species (ROS) synthesis. The antioxidants act positively on oxidative stresses in cisplatin-induced nephrotoxicity.[4]

The present results showed a considerable sum of antioxidant and phenolic contents of CME. Although these findings demonstrated the protective effects of CME on serum urea and creatinine as well as the histopathological changes in kidney, the changes in renal antioxidant enzymes were not significant, which suggests other or complex protective mechanisms. The CME has considerable vitamin C content.[12-16] In some previous studies, oral administration of vitamin C showed remarkable protection against kidney damage and histopathological changes induced by cisplatin, lead, and nickel in rats.[33-35]

More investigations are needed to explain the underlying mechanisms of the protective effects that remain unexplained. The small sample size and limited CME dose range are limitations of the current study.

Conclusion

Our results showed a considerable sum of antioxidant and phenolic contents of CME. The findings also showed noticeable renoprotective effects of CME against Cis-induced nephrotoxicity in rats. More studies are needed to investigate mechanisms underlying the nephrotoxicity effect of cisplatin and also the protective effects of CME on that.

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Conflict of interests

The authors claim that there is no conflict of interest.

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