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RESEARCH ARTICLE

An Investigation of the Protective Effect of *Rhododendron luteum* Extract on Cisplatin-Induced DNA Damage and Nephrotoxicity and Biochemical Parameters in Rats

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ABSTRACT

The nephrotoxicity mechanisms caused by cisplatin (CP), which is used in the treatment of different types of cancer, include several processes, such as DNA damage, apoptosis, inflammation, and oxidative stress. This study investigated the protective effects of *Rhododendron luteum* leaf extract (RLE) in a rat model of CP-induced nephrotoxicity. Dimethyl sulfoxide extract of RLE was prepared, and its contents were clarified using liquid chromatography-mass spectrometry (LC-MS-MS). Thirty female Wistar Albino rats were divided into five different groups and sacrificed on the seventh day. Biochemical and histopathological analyses were then performed. The presence of quercitrin, quinic acid, chlorogenic acid, protocatechic acid, isoquercitrin, and quercetin was detected in the RLE. While CP significantly increased the levels of oxidative stress, inflammation, and apoptosis markers in the rat kidney tissue and blood samples, treatment with RLE significantly reversed the levels of these parameters in a dose-dependent manner. Total antioxidant status (TAS) values and glutathione (GSH) levels increased significantly in the serum samples from the treatment group compared to the CP group, while blood urea nitrogen (BUN), creatinine, oxidative stress index (OSI), tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA) and total oxidant status (TOS) values decreased significantly. GSH levels increased significantly in the treatment group compared to the CP group, while TNF-α, caspase-3 (Casp-3), 8-hydroxy-2'-deoxyguanosine (8-OHdG), OSI, TOS, MDA levels, and damage scores decreased significantly at histopathological examination. In conclusion, RLE exhibited strong protective effects against nephrotoxicity and oxidative damage caused by CP in rats.

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INTRODUCTION

Cancer is one of the greatest public health problems worldwide (Demir *et al*., 2018). Numerous chemotherapeutic agents are used in cancer treatment, including cisplatin (CP), which causes various side-effects, such as nephrotoxicity (Jacob and Lavakumar, 2016). The mechanisms involved in the nephrotoxicity caused by CP are complex and include several processes, such as DNA damage, apoptosis, inflammation and oxidative stress (Elsherbiny *et al.*, 2016). Herbs have represented an important part of traditional medicine since ancient times. The medicinal value of plants used for medicinal purposes generally depends on their phytochemical contents. The most important of these phytochemicals are alkaloids, flavonoids, tannins, and phenolic compounds (Padmavathi,

2013). Phenolic compounds, secondary metabolites of plants, can exhibit numerous medicinal properties (anti-inflammatory, antioxidant, antimicrobial, anticancer, anti-aging effects, etc.). Phenolic compounds or natural products containing these are therefore used as potential agents for the treatment and prevention of such as aging, diabetes, cardiovascular diseases and cancer (Pandey and Rivzi, 2009; Li *et al*., 2014). *Rhododendron luteum*, a natural product, is a member of the Ericaceae family, from the genus *Rhododendron*, which consists of more than 800 species. Members of the genus *Rhododendron* genus are used in the treatment of several diseases, such as diarrhea, spasm, arthritis, eczema, bronchitis, hypertension, and gonorrhea (Li *et al*., 2014; Demir *et al*., 2016; Demir *et al*., 2018; Turan *et al*., 2022). The extract obtained from *R. luteum* flowers and leaves is reported to possess powerful antioxidant properties, to exhibit selective cytotoxicity against colon and liver cancer cells and to inhibit various enzymes, such as acetylcholinesterase, butyrylcholinesterase, tyrosinase and α-glucosidase (Demir *et al*., 2016; Demir *et al*., 2018; Mahomoodally *et al*., 2020).

The present study investigated the *in-vivo* protective effect of *R. luteum* leaf extract (RLE), with its powerful antioxidant properties, against damage such as DNA injury, increased oxidative stress, apoptosis, inflammation, and nephrotoxicity caused by CP.

MATERIALS AND METHODS

Chemicals: CP, H_3PO_4, TBA, acetic acid, 1,1,3,3-tetramethoxypropane, dimethyl sulfoxide (DMSO), HCl, and H_2SO_4 were purchased from Sigma (St. Louis, MO, USA). Pierce BCA Protein Assay kits were obtained from Thermo Scientific (Waltham, MA, USA), SOD, CASP3, TNF-α 8-OHdG and GSH ELISA kits from USCN (Wuhan, China), and TAS-TOS analysis kits from Rel Assay (Gaziantep, Türkiye).

Extract preparation: *R. luteum* leaves collected from Artvin province in Türkiye were dried at room temperature and converted into powder (IKA, Staufen, Germany). One gram of powder was then mixed with 20 mL of DMSO and left to incubate for 24 h at 45°C with continuous shaking (Shell Lab, Cornelius, USA) at 150 rpm. The resulting supernatant was then lyophilized (Xianou-12N, China). In the experimental stages, the extracts were dissolved with DMSO (Demir *et al*., 2016).

LC-MS/MS analysis: Chromatographic separation was achieved on an Inertsil C18 column (100 mm x 2.1 mm, 2 µm). The column temperature was kept at 35ºC throughout the analysis. The mobile phase consisted of water (A, 10 mM ammonium formate-0.1% formic acid) and methanol (B). The applied gradient profile was 5-20% B (0-10 min), 20% B (10-22 min), 20-50% B (22-36 min), 95% B (36-40), and 5% B (consisting of 40-50 min). The injection volume was 4 µL and the flow rate 0.25 mL/min. The optimal ESI parameters for mass spectrometry are 350ºC interface temperature, 250ºC DL temperature, 400ºC heatsink temperature, and nebulizer and desiccant gas (N_2) flow rates of 3 L/min and 15 L/min, respectively (Yilmaz *et al*., 2018).

Experimental design: Eight-week-old female Wistar Albino rats weighing 200-250 g were used in the study. The experiments were compliant with the World Medical Association Declaration of Helsinki, and the animal experiment was approved by the Karadeniz Technical University animal experiments local ethics committee. All rats were housed under optimum conditions (appropriate ventilation system, relative humidity 50-60%, light cycle 12-h light/12-h dark, and temperature 23±2ºC). The rats were given water *ad libitum* and pellet feed (Palipoch *et al*., 2013).

The rats were divided into five groups and housed in cages with six animals in each group;

Group I, the control group, received 0.5 mL saline solution intraperitoneally (i.p.) for six days.

Group II, the CP group, received 0.5 mL CP (7.5 mg/kg) on the first day and saline for the next five days.

Group III, the DMSO group, received DMSO for six days.

Group IV, the RL-1 group, received CP (7.5 mg/kg) on the first day and RLE (12.5 mg/kg) for five days.

Group V, the RL-2 group, received CP (7.5 mg/kg) on the first day and RLE RL (25 mg/kg) for five days (Jamshidzadeh *et al*., 2016; Kukner *et al*., 2016). On the seventh day, deep anesthesia was applied to all the experimental group rats, and blood and kidney tissues were collected.

Biochemical analysis: One hundred milligrams of tissue sample was homogenized at 9500 rpm in 2 mL of phosphate buffer (pH: 7.4) (IKA, Staufen, Germany). The protein content of the samples was measured using a commercial kit (Thermo Scientific Pierce BCA Protein Assay Kit, Rockford, lL, USA). The malondialdehyde (MDA) levels of the tissue samples were determined according to the method developed by Mihara and Uchiyama (1978). 1,1,3,3-tetramethoxypropane was used as a standard, and tissue MDA levels were expressed as nmol/ mg protein. Tissue total antioxidant status (TAS) and total oxidant status (TOS) values were determined using commercial colorimetric kits (Rel Assay Diagnostics, Gaziantep, Turkey) according to the producer's recommendations. The TOS/TAS ratio was used as the oxidative stress index (OSI) and was calculated using the formula (Demir *et al*. 2020).

$$\text{OSI (arbitrary unit)} = \frac{\text{TOS (µmol hydrogen peroxide equivalent/L)}}{\text{TAS (µmol trolox equivalent/L)}} \times 100$$

Superoxide dismutase (SOD), GSH, 8-OHdG, caspase-3, and TNF-α levels were measured using ELISA kits (USCN, Wuhan, ChinaThe creatinine and blood urea nitrogen values in serum samples were determined using an autoanalyzer (Beckman Coulter AU5800, Brea, CA, USA).

Histological analysis: Kidney tissues were subjected to routine histological tissue processing. Serial sections 5 µm in thickness were then taken from the paraffin-embedded blocks. (Leica RM 2255, Leica Instruments, Nussloch, Germany). The slides were dyed with hematoxylin-eosin (H&E) and appraised under a light microscope (Olympus BX -51; Olympus Co., Tokyo, Japan) and photographed (Olympus DP 71 Olympus Co., Japan). Degeneration of the Bowman's space and/or glomeruli, proximal and distal

tubule degeneration and/or cast formation, and vascular congestion and/or interstitial edema were determined as three indicative criteria of kidney damage. Each preparate was scored between 0 and 3 (0: no damage, 1: mild damage, 2: moderate damage, 3: severe damage) for each criterion, with a total score of 0-9 (Iseri *et al*., 2007).

Statistical Analysis: Normality distribution of the study data was evaluated using the Kolmogorov-Smirnov test. Non-normally distributed data were first evaluated using Kruskal-Wallis analysis of variance and then with the Mann-Whitney-U test. Data were expressed as median and 25-75% quartiles (interquartile range). p values <0.05 were considered statistically significant.

RESULTS

LC-MS/MS findings: The LC-MS/MS analysis results are given in Fig. 1. Protocatechuic acid (0.122 mg/g extract), quinic acid (28.014 mg/g extract), chlorogenic acid (2.003 mg/g extract), isoquercitrin (1.829 mg/g extract), quercitrin (1.449 mg/g extract), and quercetin (0.027 mg/g extract) were determined in the DMSO extract.

Biochemical findings: The biochemical findings of the tissue samples are shown in Table 1. MDA, TOS, OSI, caspase-3, TNF-α, and 8-OHdG levels increased significantly in tissue samples from the CP group compared to the control group (P<0.05). Tissue MDA, caspase-3, and TNF-α levels were significantly lower in the RLE groups compared to the CP group (P<0.05). Tissue GSH levels were significantly lower in the CP group compared to the control group (P<0.05). Significant increases were also observed in the RLE groups compared to the CP group (P<0.05).

The biochemical findings of the blood samples are shown in Table 2. MDA, TOS, OSI, 8-OhdG, BUN, and creatinine levels increased significantly in serum samples from the CP group compared to the control group (P<0.05). Serum TOS, OSI, BUN, and creatinine levels were significantly lower in the RLE groups compared to the CP group (P<0.05). While serum TAS and GSH levels decreased significantly in the CP group compared to the control group (P<0.05), they were significantly higher in the RLE groups (P<0.05).

Histological findings: Histopathological images from the experimental groups are shown in Fig. 2. The glomeruli exhibited a normal structure in sections from the Control Group (A) and the DMSO Group (B), and no pathological finding was observed. Degeneration of tubules (double arrow), caste formation in tubule lumen (four arrows), apoptotic cells and bodies (chevrons), vascular congestion (arrowhead) (H&E X400) were observed in kidney sections from CP Group II (C), CP+12.5 mg/kg/day Group (D), and CP+25 mg/kg/day (E) (H&E X400). Histological damage scores for the kidney tissue samples from the experimental groups are given in Table 3.

Damage scores in the CP and RLE groups were significantly higher compared to those in the control group (P<0.05), while damage scores in the RLE groups were significantly lower than in the CP group (P<0.05).

DISCUSSION

Although the mechanisms underlying the toxic effect of CP, an agent widely used in chemotherapy, are not fully understood, one possible mechanism may involve an increase in oxidative stress through increased ROS (Elsherbiny *et al*., 2016). Increasing ROS cause lipid oxidation by affecting the fatty acids in the cell membrane. MDA levels rise as a result, while antioxidant enzymes such as SOD, CAT and GSH are inhibited. ROS have a short half-life, making direct measurements difficult. The measurement of oxidative stress markers is therefore generally preferred (Hassan *et al*., 2017; Yuluğ *et al*., 2019). *R. luteum*, popularly known as yellow rhododendron, has been reported to exhibit anticancer and powerful antioxidant properties in the flower and leaf (Demir *et al*., 2016; Demir *et al*., 2018; Turan *et al*., 2022). The present study represents the first report of the effect of *R. luteum* on MDA, SOD, GSH, TAS, TOS, and OSI levels in an experimental kidney injury model.

Quinic acid (28,014 mg/g extract), protocatechuic acid (0.122 mg/g extract), chlorogenic acid (2,003 mg/g extract), isoquercitrin (1,829 mg/g extract), quercitrin (1,449 mg/g extract), and quercetin (0,027 mg/g extract) were determined in the RLE in this study by means of phytochemical analysis using the LC-MS-MS method. Mahomoodally *et al*. (2020) analyzed the content of R. *luteum* extracts obtained using different solvents and determined the presence of catechin and its derivatives, caffeoyl quinic acids, myricetin, and quercetin glycosides (Mahomoodally *et al*., 2020). Yesil and Akgul identified isoquercetin in their study of the main components of *R. luteum* leaves (Yesil and Akgul, 2022). In another study, performed LC-ESI-MS/MS characterization of the fractions obtained from *R. luteum* leaves, and identified five hydroxybenzoic acids, four hydroxycinnamic acids, 12 flavonoid aglycones, and seven flavonoid glycosides, with quercetin, one of these flavonoids, being detected in significant amounts. The present study is also compatible with other publications reporting that quercetin and its glycosides are commonly found in *Rhododendron* species (Oyko *et al*., 2022).

Our scan of the literature revealed no previous studies of the effect of *Rhododendron* species on nephrotoxicity. Consistent with previous studies, TOS and MDA levels in serum and tissue samples increased significantly in the CP group compared to the control group in this study. However, in the *R. luteum* groups, MDA and TOS levels decreased significantly, in a dose-dependent manner, compared to the CP group. The significant increase in TOS levels in the CP group compared to the control group suggests that oxidative damage contributes to CP-induced nephrotoxicity. A comparison of TAS values in tissue samples revealed no significant difference between the groups. This may be due to the intraperitoneal administration of *R. luteum*. TAS values in serum samples increased in the RLE groups compared to CP group. OSI values calculated using TAS and TOS in tissue and serum samples decreased significantly in the RLE groups compared to the CP group. Levels of SOD, one of the antioxidant enzymes, increased significantly in tissue samples from the CP and RLE groups compared to the control group. We attribute the increase in the CP group to

Table 1: Biochemical parameter results in tissue samples

	Control	DMSO	CP (7.5 mg/kg)	*CP* (7.5 mg/kg) *R. luteum* (12.5 mg/kg/day)	*CP* (7.5 mg/kg) *R. .luteum* (25 mg/kg/day)
MDA	17.5	18.2	20.4[a]	17.5[b]	17.8[b]
(nmol/mg protein)	(16.2-19.2)	(17.3-19.1)	(18.1-21.7) *([a]p=0.025)*	(14.8-18.1) *([b]p=0.013)*	(16.3-18.7) *([b]p=0.039)*
TOS	14.6	15.5	18.6[a]	16	13.8[b]
(μM H_2O_2 equivalent)	(13.6-16.5)	(15.1-15.7)	(16.0-21.5) *([a]p=0.037)*	(13.9-16.7)	(11.4-18.1) *([b]p=0.042)*
TAS	0.80	0.62	0.57	0.54	0.67
(mM trolox equivalent)	(0.55-0.93)	(0.54-0.74)	(0.51-0.69)	(0.45-0.68)	(0.51-0.78)
OSI	2.00	2.50	3.50[a]	2.70	2.10[b]
(arbitrary unit)	(1.53-2.90)	(2.10-2.75)	(2.30-4.00) *([a]p=0.036)*	(2.23-3.60)	(1.58-2.98) *([b]p=0.041)*
SOD	0.052	0.083	0.133[a]	0.133[a]	0.094[a]
(ng/mg protein)	(0.038-0.071)	(0.072-0.090)	(0.087-0.280) *([a]p=0.016)*	(0.097-0.160) *([a]p=0.006)*	(0.086-0.117) *([a]p=0.016)*
Caspase-3	0.27	0.39	0.53[a]	0.34[b]	0.29[b]
(ng/mg protein)	(0.18-0.47)	(0.32-0.41)	(0.49-0.62) *([a]p=0.006)*	(0.22-0.41) *([b]p=0.016)*	(0.21-0.34) *([b]p=0.004)*
TNF- α	102.7	102	167.7[a]	106.9[b]	69.3[b]
(pg/mg protein)	(34.7-127.5)	(59.1-109)	(147.1-190.1) *([a]p=0.004)*	(58.8-124.7) *([b]p=0.004)*	(40.9-115.5) *([b]p=0.006)*
GSH	3.53	4.30	2.48[a]	9.08[b]	5.33[b]
(μg/mg protein)	(3.07-7.46)	(3.94-5.17)	(2.11-3.00) *([a]p=0.025)*	(3.04-11.1) *([b]p=0.01)*	(3.15-11.5) *([b]p=0.016)*
8-OHdG	68.9	62.8	95.9[a]	85.1	72.9[b]
(pg/mg protein)	(62.2-72.0)	(56.5-72.4)	(87.9-118.2) *([a]p=0.004)*	(69.7-99.8)	(57.5-84.3) *([b]p=0.01)*

Data were expressed as median and 25%-75% quartiles (interquartile range (IQR). P<0.05 was considered statistically significant. [a] Significantly different compared with the negative control group (P<0.05), [b] Significantly different compared with the CP group (P<0.05).

Table 2: Biochemical parameter results in serum samples

	Control	DMSO	CP (7.5 mg/kg)	*CP* (7.5 mg/kg) *R. luteum* (12.5 mg/kg/day)	*CP* (7.5 mg/kg) *R. luteum* (25 mg/kg/day)
MDA	1.30	1.35	3.55[a]	1.51	1.47[b]
(nmol/mL)	(1.11-1.80)	(1.28-1.48)	(1.68-5.70) *([a]p=0.016)*	(1.22-2.27)	(1.18-1.82) *([b]p=0.037)*
TOS	14.8	13.7	28.2[a]	14.8[b]	16.7[b]
(μM H_2O_2 equivalent)	(7.15-17.2)	(12.5-17.7)	(23.2-35.1) *([a]p=0.004)*	(12.6-23.0) *([b]p=0.016)*	(14.0-25.4) *([b]p=0.025)*
TAS	0.98	0.99	0.73[a]	0.99[b]	1.17[b]
(mM trolox equivalent)	(0.88-1.08)	(0.91-1.24)	(0.61-0.80) *([a]p=0.004)*	(0.88-1.11) *([b]p=0.006)*	(0.89-1.30) *([b]p=0.041)*
OSI	1.46	1.29	4.18[a]	1.53[b]	1.65[b]
(arbitrary unit)	(0.78-1.82)	(1.14-1.78)	(2.77-5.49) *([a]p=0.004)*	(1.15-2.26) *([b]p=0.01)*	(1.15-2.44) *([b]p=0.01)*
SOD	0.45	0.36	0.19	0.35	0.40
(ng/mL)	(0.26-1.11)	(0.22-0.67)	(0.095-0.61)	(0.13-0.54)	(0.36-0.56)
Caspase-3	1.59	1.60	6.90	2.29	1.81
(ng/mL)	(1.21-1.65)	(1.46-1.80)	(0.97-12.8)	(0.87-5.30)	(0.58-4.94)
TNF-α	34.1	30.7	134.1	42.0	30.3[b]
(pg/mL)	(13.8-48.4)	(25.4-45.0)	(37.0-333.3)	(23.2-67.3)	(15.2-44.4) *([b]p=0.025)*
GSH	213.9	212.3	175.0[a]	205.4[b]	210.9[b]
(μg/mL)	(196.5-217.2)	(208.1-219.3)	(164.2-179.0) *([a]p=0.004)*	(199.5-235.9) *([b]p=0.004)*	(188.4-228.5) *([b]p=0.004)*
8-OHdG	1086.9	1077.2	2563.2[a]	1805.2	1510.2
(pg/mL)	(771.7-1407.4)	(780.9-1266.7)	(1838.9-2741.3) *([a]p=0.004)*	(745.8-3052.8)	(952.8-2351)
BUN	17.5	19.0	255.5[a]	186[a,b]	20.0[b,c]
(mg/dL)	(16.0-21.0)	(17.0-21.0)	(235-280) *([a]p=0.0001)*	(166-212) *([a]p=0.0001) ([b]p=0.002)*	(14.0-35.0) *([b]p=0.0001) ([c]p=0.0001)*
Creatinine	0.285	0.320	3.46[a]	2.49[a,b]	0.251[b,c]
(mg/dL)	(0.270-0.350)	(0.170-0.370)	(3.40-3.79) *([a]p=0.0001)*	(1.35-3.23) *([a]p=0.0001) ([b]p=0.002)*	(0.210-0.350) *([b]p=0.0001) ([c]p=0.0001)*

Data were expressed as median and 25%-75% quartiles (interquartile range (IQR). P<0.05 was considered statistically significant. A, Significantly different compared with the negative control group (P<0.05), b, Significantly different compared with the CP group (P<0.05). c, Significantly different (P<0.05) compared with the R. luteum 12.5 mg/kg/day group.

Table 3: Histological damage scores in kidney tissue samples

	Control	DMSO	CP (7.5 mg/kg)	*CP* (7.5 mg/kg) *R. luteum* (12.5 mg/kg/day)	*CP* (7.5 mg/kg) *R. luteum* (25 mg/kg/day)
Damage scores	3.00	3.50	9.00[a]	7.50[a, b]	7.00[a, b]
	(2.00-3.25)	(3.00-4.00)	(9.00-9.00) *([a]p=0.002)*	(6.25-8.00) *([a]p=0.003) ([b]p=0.002)*	(6.00-7.00) *([a]p=0.003) ([b]p=0.002)*

Data were expressed as median and 25%-75% quartiles (interquartile range (IQR). P<0.05 was considered statistically significant. a, Significantly different compared with the negative control group (P<0.05), b, Significantly different compared with the CP group (P<0.05).

the antioxidant defense mechanism. When the levels of GSH, another antioxidant enzyme, were compared in tissue and blood samples, significant increases were observed in the R. luteum groups compared to the CP group. No previous studies have been conducted with *R. luteum* extract in this area, and similar results were obtained in studies involving natural product extracts and phenolic compounds. The previous study investigated the protective effect of quercetin on cisplatin nephrotoxicity and reported a decrease in kidney tissue MDA levels and an increase in GSH levels in groups receiving quercetin, a finding consistent with the results of the present study (Sanchez-Gonzalez *et al*., 2011). In another study, examined the protective effects of myricetin against CP-induced nephrotoxicity in mice and observed that MDA levels decreased in the myricetin group, while GSH levels increased (Hassan *et al*., 2017). The results of the our study were compatible with the active ingredients of *R. luteum* described in the literature. The damage caused by CP was reduced by *R. luteum* leaf extract, an effect we attributed to the secondary metabolites detected in the plant content.

CP causes DNA double helix damage by forming cross-links in DNA (Goodsell, 2006). The most familiar of the base mutations occurring in DNA is known as 8-OHdG, and this mutation is used for the quantification of DNA damage (Kroese and Scheffer, 2014; Ozcan *et al*. 2015). 8-OHdG levels were measured to determine DNA damage in blood and tissue samples in the present study,

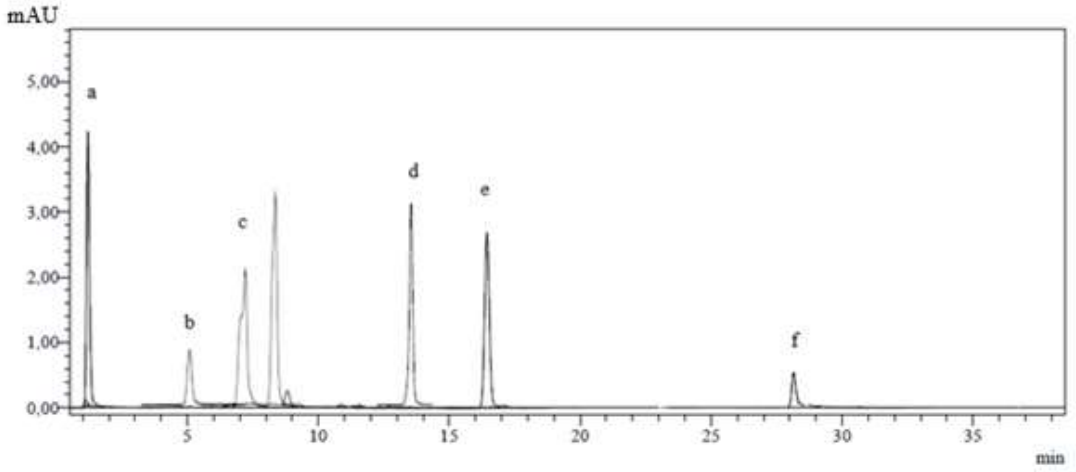

Fig. 1: Quantitative determination of 37 phytochemicals (mg analyte/g extract) was performed in DMSO of leaf parts of *R. luteum.* Compounds obtained from *R. luteum* leaves were a: Quinic acid (28.014 mg/g extract), b: Protocatechuic acid (0.122 mg/g extract), c: Chlorogenic acid (2.003 mg/g extract), d: Isoquercitrin (1.829 mg/g extract), e: Quercitrin (1.449 mg/g extract), f: Quercetin (0.027 mg/g extract).

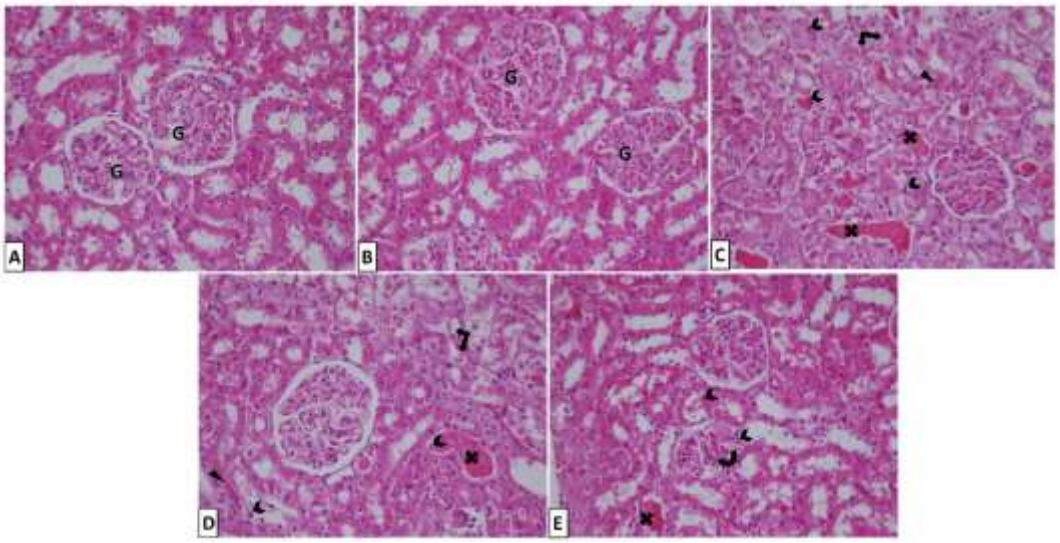

Fig. 2: Hematoxylin-eosin stained histopathological images of kidney tissue samples Control group (A), DMSO group (B), CP group (C), CP+12.5 mg/kg/day (D) and CP+25 mg/kg/day (E). Glomeruli (G), Tubular degeneration (double arrow), caste formation in the tubule lumen (four arrows), apoptotic cells and bodies (chevrons), vascular congestion (arrowhead), Bowman's space and degeneration in the glomeruli (bow).

and these increased significantly in the CP group compared to the control group. While no significant difference was observed between the CP and RLE groups in the serum samples the 8-OHdG level in tissue samples from the 25 mg/kg *R. luteum* group decreased significantly compared to the CP group ($P<0.05$). Ozyurt and co-workers, investigated the protective properties of quercetin on radiation-induced DNA damage in rat bladder and kidney tissues. Those authors reported that 8-OHdG levels increased in the radiation group, but decreased in the group in which quercetin was administered together with radiation, a finding consistent with our results (Ozyurt *et al*., 2014). The protective effects achieved occur due to the active substances in *R. luteum* and are compatible with the results in the previous literature.

The nephrotoxicity mechanism of CP is generally attributed to oxidative stress and DNA damage. CP is also capable of inducing the release of pro-inflammatory cytokines (Sharma *et al*., 2017). In the present study, TNF-α levels increased in the CP group compared to the control group, and were lower in the RLE groups than in the CP group. Hassan and co-workers analyzed the protective effect of myricetin on CP-induced nephrotoxicity. Those authors reported that TNF-α, an inflammatory marker, increased in the CP group and decreased in the group in which myricetin was used with CP (Hassan *et al*., 2017). In another study, lipopolysaccharide-induced inflammation was created in the RAW264.7 cell line, and the anti-inflammatory properties of *Rhododendron molle* leaf extract were then investigated. The authors reported that TNF-α levels decreased significantly in the groups receiving *R. molle*, and that *R. molle* exhibited powerful anti-inflammatory activity (Luye *et al*., 2020). Faddah *et al*. (2012) investigated the role of quercetin on nephrotoxicity and reported that TNF-α levels decreased after quercetin treatment, a finding consistent with our own results. The molecular mechanism involved the protective role of quercetin on nephrotoxicity was examined by Peng and co-workers (Peng *et al*., 2021). We think that that the inflammation caused by the use of CP is reduced by the active substances we detected in *R. luteum*, with its known antioxidant properties.

Caspases play a role in both apoptotic pathways. In the intrinsic apoptosis pathway, caspase-9 is activated, and caspase-dependent apoptosis begins. In the extrinsic pathway, apoptosis is initiated by caspase-8 activity as a result of ligand binding to the death receptor (Pabla and Dong, 2008). In the present study, serum caspase-3 levels in the CP group increased significantly compared to those in the control group. Tissue caspase-3 levels decreased significantly in the treatment groups compared to the CP group. Previously, it has been found that the protective effect of myricetin on CP-induced nephrotoxicity. Those authors reported that levels of caspase-3, one of the apoptosis markers, increased in the CP group and decreased in the group in which myricetin was used with CP (Hassan *et al*., 2017). In the light of the results from the previous literature, we concluded that the regulation of apoptotic markers is caused by the active substances detected in our RLE.

Nephrotoxicity, one of the most important side-effects of CP, causes a rapid deterioration in kidney functions and increases in plasma urea and creatine levels (Jacob and Lavakumar, 2016). In the present study, BUN and creatine levels in serum samples decreased significantly in the RLE groups compared to the CP groups. The previous study investigated the effect of *Rhododendron arboreum* on inflammatory parameters and reported that BUN and creatine levels increased in the lipopolysaccharide group and decreased in the group receiving *R. arboreum* (Ahmad *et al*., 2020). In another study, determined that quercetin exhibited a protective effect on rats with gentamicin-induced nephrotoxicity (Pinar *et al*., 2020). Our result showed that BUN and creatine levels, which increased with the use of CP, decreased with the use of *R. luteum* extract, a finding in accordance with the previous literature. We think that the quercetin detected in our content analysis may have contributed to this.

Histological examination in this study revealed damage in the kidney tissues from the CP-applied group. This damage decreased significantly in the treatment groups. Sanchez-Gonzalez *et al*. (2011) examined the protective effect of quercetin on CP nephrotoxicity and reported a decrease in kidney tissue damage in the groups administered quercetin (Sanchez-Gonzalez *et al*., 2011). Cetinavci *et al.* (2022) performed a histological examination of the effects of quercetin on kidney damage caused by CP. Those authors reported decreased kidney tissue damage in the groups administered quercetin, a finding consistent with the results of the present study (Cetinavci *et al*., 2022). The potential nephroprotective effect of *Rhododendron groenlandicum* in a diet-induced obese mouse model was investigated in another study, which concluded that impaired kidney functions due to obesity could be improved by means of *R. groenlandicum* (Li *et al*., 2016). Quercetin, which is contained in RLE, has been found to have a renoprotective effect by reducing kidney tissue cytotoxicity in a STZ-induced diabetes model (Gomes *et al*., 2014). Mangiferin, a flavonoid contained in *Mangifera indica*, has been reported to improve CP-induced nephrotoxicity and to reduce the induction of oxidative stress (Sadhuchan *et al*., 2018) Another study evaluating the effect of curcumin on CP-induced nephrotoxicity in rats concluded that this could be used to reduce chemotherapy-induced nephrotoxicity (Ugur *et al*., 2015). The results in the literature are consistent with our own study data.

Conclusions: In conclusion, the results of current study show, for the first time in the literature, that RLE may potentially protect against CP-induced nephrotoxicity. Our results also suggest that the suppression of oxidative stress, inflammation, and apoptosis by RLE reduced CP-induced kidney damage. *R. luteum* may be regarded as a potential resource for obtaining novel and promising therapeutic agents against CP-induced nephrotoxicity.

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Authors contribution: IT and DC designed the experiments. IT, SD and AM analyzed the data. DC, FÇ, NTA, GK, and YA contributed reagents/materials/analysis tools. IT, DC and SD wrote and revised the paper. All the authors read and approved the final version of the manuscript.

REFERENCES

Ahmad A, Wali AF, Rehman MU, *et al*., 2020. Therapeutic potential of Rhododendron arboretum polysaccharides in an animal model of lipopolysaccharide-ınflicted oxidative stress and systemic inflammation. Molecules 25:6045.

Cetinavci D, Elbe H, Taslidere E, *et al*., 2022. Effects of Quercetin on Cisplatin-Induced Renal Damage in Wistar Albino Rats. Nam Kem Med J 10:219-24.

Demir S, Kazaz IO, Aliyazicioglu Y, *et al.,* 2020. Effect of ethyl pyruvate on oxidative state and endoplasmic reticulum stress in a rat model of testicular torsion. Biotech Histochem 95:317–22.

Demir S, Turan I and Aliyazicioglu Y, 2016. Selective cytotoxic effect of Rhododendron luteum extract on human colon and liver cancer cells. JBUON 21:883-88.

Demir S, Turan I and Aliyazicioğlu Y, 2018. Cytotoxic effect of Rhododendron luteum leaf extract on human cancer cell lines. KSU J Agric Nat 21:950-56.

Elsherbiny NM, Eladl MA and Al-Gayyar MM, 2016. Renal protective effects of arjunolic acid in a cisplatin-induced nephrotoxicity model. Cytokine 77:26-34.

Faddah LM, Baky NAA, Al-Rasheed NM, *et al*., 2012. Role of quercetin and arginine in ameliorating nano zinc oxide-induced nephrotoxicity in rats. BMC Complement Med Ther 12:1-14.

Gomes IBS, Porto ML, Santos MCLFS, *et al*., 2014. Renoprotective, anti-oxidative and anti-apoptotic effects of oral low-dose quercetin in the C57BL/6J model of diabetic nephropathy. Lipids Health Dis 13:184

Goodsell DS, 2006. The Molecular Perspective: Cisplatin. Oncologist 2006:316–17

Hassan SM, Khalaf MM, Sadek AS, *et al*., 2017. Protective effects of apigenin and myricetin against cisplatin-induced nephrotoxicity in mice. Pharm Biol 55:766-74.

Iseri S, Ercan F, Gedik N, *et al*., 2007. Simvastatin attenuates cisplatin-induced kidney and liver damage in rats. Toxicology 230:256-64.

Jacob JRS and Lavakumar S, 2016. Nephroprotective effect of ethanolic extract of abutilon indicum root in gentamicin induced acute renal failure. Int J Basic Clin Pharmacol 5:841-45.

Jamshidzadeh A, Heidar R, Golzar T, *et al*., 2016. Effect of Eisenia foetida extract against cisplatin-ınduced kidney ınjury in rats. J Diet Suppl 13:551–59.

Kukner A, Ilter G, Soyler G, *et al.*, 2016. The Effect of Rhododendron Honey on Mice Liver Tissue. Int J Morphol 34:842-47

Kroese LJ and Scheffer PG, 2014. 8-hydroxy-2'-deoxyguanosine and cardiovascular disease: a systematic review. Curr Atheroscler Rep 16:452.

Li A, Li S, Zhang Y, *et al.,* 2014. Resources and biologigal activites of natural polyphenols. Nutrients 6:6020-47.

Li S, Brault A, Villavincencio SM, *et al*., 2016. Rhododendron groenlandicum (Labrador tea), an antidiabetic plant from the traditional pharmacopoeia of the Canadian Eastern James Bay Cree, improves renal integrity in the dietinduced obese mouse model. Pharm Biol 54:1998-2006.

Luye Z, Jin Z, Liangfang D, *et al*., 2020. The anti-inflammatory properties of Rhododendron molle G. Don leaf extract in LPS-induced RAW 264.7. Chem Biodiversity 17:1-18.

Mahomoodally MF, Sieniawska E, Sinan KI, *et al*., 2020. Utilisation of Rhododendron luteum sweet bioactive compounds as valuable source of enzymes inhibitors, Antioxidant, and Anticancer Agents. Food Chem Toxicol 135:1-9.

Mihara M and Uchiyama M, 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271-78.

Oyko L, Olech M and Nowark R, 2022. LC-ESI-MS/MS characterization of concentrated polyphenolic fractions from Rhododendron luteum and their anti-ınflammatory and antioxidant activities. Molecules 27:1-16.

Ozcan O, Erdal H and Cakirca G, 2015. Oxidative stress and its impacts on intracellular lipids, proteins and DNA. J Clin Exp Invest 6:331-36

Ozyurt H, Cevik O, Ozgen Z, *et al*., 2014. Quercetin protects radiation-induced dna damage and apoptosis in kidney and bladder tissues of rats. Free Radic Res 48:1247-55.

Pabla N and Dong Z, 2008. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 73:994–1007.

Padmavathi M, 2013. Drug delivery system in nano greens. Int J Herbal Med 1:56-60.

Palipoch S, Punsawad C, Chinnapun D, *et al*., 2013. Amelioration of cisplatin-induced nephrotoxicity in rats by curcumin and α-tocopherol. Trop J Pharm Res 12:973.

Pandey KB and Rivzi SI, 2009. Plant polyphenols as dietary antioxidants in human healty and disease. Oxid Med Cell Longev 2:270-78.

Peng X, Dai C, Zhang M, *et al*., 2021. Molecular mechanisms underlying protective role of quercetin on copper sulfate-induced nephrotoxicity in mice. Front Vet Sci 7:1-13.

Pinar N, Karatas Y, Gonlusen G, *et al*., 2020. Effects of Quercetin and Coenzyme Q10 on gentamicin-induced nephrotoxicity in rats. Cukurova Med J 45:251-56

Sadhuchan P, Saha S, Dutta S, *et al*., 2018. Mangiferin ameliorates cisplatin induced acute kidney injury by upregulating Nrf-2 via the activation of PI3K and exhibits synergistic anticancer activity with cisplatin. Front Pharmacol 9:638.

Sanchez-Gonzalez PD, Lopez-Hernandez FJ, Perez-Barriocanal F, *et al*., 2011. Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. Nephrol Dial Transplant 26:3484–95.

Sharma S, Joshi A and Hemalatha S, 2017. Protective effect of Withania coagulans fruit extract on cisplatin-induced nephrotoxicity in rats. Phcog Res 9:354-61.

Ugur S, Ulu R, Dogukan A, *et al*., 2015 The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. Ren Fail 37:332-36.

Turan I, Demir S, Yaman SÖ, *et al*., 2022. An investigation of the antiproliferative effect of *Rhododendron luteum* extract on cervical cancer (HeLa) cells via nrf2 signaling pathway. Nutr Cancer 74:1882-93.

Yesil T and Akgul Y, 2022. Major components of Rhododendron luteum leaves. Nat Prod Res 1-5 DOI: 10.1080/14786419.2022.2055015

Yilmaz MA, Altun MY, Demirtas I, *et al*., 2018. A comprehensive LC–MS/MS method validation for the quantitative investigation of 37 fingerprint phytochemicals in Achillea species: A detailed examination of A. coarctata and A. monocephala. J Pharm Biomed Anal 154:413–24.

Yuluğ E, Türedi S, Karagüzel E, *et al*., 2014. The short term effects of resveratrol on ischemia-reperfusion injury in rat testis. J Pediatr Surg 49:484-89.