Research Article

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Nephroprotective effects of eriocitrin via alleviation of oxidative stress and DNA damage against cisplatin-induced renal toxicity

Sisplatin kaynaklı Renal Toksisitesine Karşı Oksidatif Stres ve DNA Hasarının Hafifletilmesi İle Eriocitrinin Nefroprotektif Etkileri

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Abstract

Background: Cisplatin, a common anti-neoplastic drug used in the medical industry for cancer treatment has shown adverse nephrotoxic effects. This research targets to demonstrate the protective measure of eriocitrin, a bioactive flavonoid, against cisplatin-induced renal toxicity in rats.

Materials and methods: Rats of normal control and model groups were treated with saline whereas experimental groups received oral administration of eriocitrin (25 and 50 mg/kg b.w.) for 10 days and a single intraperitoneal (i.p.) injection of cisplatin (8 mg/kg b.w.) was given on the 7th day for all except normal control group. Blood serum, urine, and kidney tissue samples were collected for analysis.

Results: Cisplatin-induced rats demonstrated significant renal toxicity and damage. Eriocitrin dose-dependently reversed the effects by decreasing the proteinuria in urine, and urea, creatinine, lipid peroxidation, nitric oxide (NO)

and pro-inflammatory cytokine levels (TNF- α , IL-1 β) in serum. The tissue levels of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were increased, whereas the levels of tissue DNA fragmentation and serum PARP-1 and Caspase-3 were reduced against model group. Histopathological modulations were supporting the protective effect of eriocitrin.

Conclusion: Eriocitrin has significant nephroprotective effects against cisplatin-induced renal toxicity by alleviating oxidative stress, preventing apoptosis and DNA damage.

Keywords: Eriocitrin; DNA fragmentation; Apoptosis; Cisplatin; Nephrotoxicity.

Öz

Amaç: Medikal endüstride kanser tedavisi için sık kullanılan bir anti-neoplastik ilaç olan sisplatinin nefrotoksik yan etkileri gösterilmiştir. Bu çalışmanın amacı, bioaktif bir flavonoid olan eriositrinin ratlarda sisplatin kaynaklı renal toksisiteye karşı koruyucu etkilerini göstermektir.

Gereç ve Yöntem: Kontrol ve model gruplara salin uygulaması yapılırken, deney grubuna 10 gün boyunca oral eriositrin (25 ve 50 mg/kg vücut ağırlığı) verilmiştir. Kontrol grubu hariç tüm gruplara 7. günde tek doz intraperitoneal (i.p.) sisplatin (8 mg/kg vücut ağırlığı) uygulaması yapılmıştır. Serum, idrar ve böbrek dokusu örnekleri analiz için toplanmıştır.

Bulgular: Sisplatin verilen ratlarda anlamlı düzeyde renal toksisite ve hasar gösterildi. Eriocitrin, doz bağımlı olarak idrarda proteinüriyi azaltarak, serumda üre, kreatinin, lipid peroksidasyonu, nitrik oksit (NO) ve pro-inflamatuar sitokin (TNF- α , IL-1 β) düzeylerini azaltarak sisplatin etkisini geri çevirdi. İndirgenmiş glutatyon

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(GSH), superoksit dismutaz (SOD) ve katalazın (CAT) doku düzeyleri artarken, dokuda DNA fragmantasyonu ile serum PARP-1 ve Kaspaz-3 düzeyleri model gruba göre azalmıştı.

Histopatolojik modülasyonlar eriositrinin koruyucu etkisini desteklemektedir.

Sonuç: Eriositrin sisplatin kaynaklı renal toksisiteye karşı oksidatif stresi hafifleten, apoptozisi ve DNA hasarını engelleyen nefroprotektif etkilere sahiptir.

Anahtar kelimeler: Eriositrin; DNA fragmantasyonu; apoptozis; sisplatin; nefrotoksiste.

Introduction

Renal toxicity or nephrotoxicity could arise due to several factors, including adverse drug effects incurred during medical treatments. Similarly, nephrotoxicity is induced by cisplatin, a common anti-neoplastic drug used in the medical industry for cancer treatment [1]. Cisplatin is commonly used for the treatment of malignant tumors in various body parts but was proven to have adverse toxicity in multiple organs especially brain and kidney [2]. The nephrotoxic manifestation of cisplatin is demonstrated with pathological changes in the kidney, increased inflammatory reactions and oxidative stress, DNA damage and cellular apoptosis [3, 4]. Kidney becomes the most vulnerable organ to toxicity caused by cisplatin because of its function for excretion of xenobiotics. The use of cisplatin in medical industry has been limited due to the extreme adverse effect, yet it is still widely in use for cancer treatment [5]. This is because cisplatin does have remarkable curative properties against malignant tumors. Cancer patients who are treated with cisplatin does show improvement in the initial stages, but continual administration of cisplatin leads to unfavorable results [6]. Although cisplatin fights the tumor, it also causes glomerular filtration rate to decrease drastically, therefore restricting the survival rate of patients [2]. To overcome this problem, antioxidants are currently in highlights to prevent cisplatin from damaging the kidney without impairing its antineoplastic effect [7].

Natural antioxidant compounds have been proven to possess nephroprotective effect against oxidative stress and inflammation induced by toxicants [8]. The effects of antioxidant compounds with therapeutic potentials have been documented and numerous flavonoids are reported to possess protective effects against renal toxicity [9, 10]. Eriocitrin is a flavonoid constituent of lemon and citrus fruits [11]. This bioactive compound

has been reported with antioxidant, oxidative stress prevention in diabetic model, protective effect against hepatic steatosis and lipid-regulation against cholesterol [12, 13]. Lemon and citrus fruits having eriocitrin as their major polyphenolic constituent are known to possess anti-inflammatory, nephroprotective, neuroprotective, cardioprotective and other pharmacological properties [14, 15]. Therefore this research targets to demonstrate the protective measure of eriocitrin against cisplatin-induced renal toxicity in rats. This research will be the first to report the nephroprotective effects of eriocitrin, hence it will stand as a reference for future clinical research on this phenolic compound.

Materials and methods

Drugs preparation

Cisplatin (15663-27-1) and eriocitrin (94113) were purchased from Sigma Aldrich (St. Louis, MO, USA). Stock solution of cisplatin was prepared in pure distilled water, diluted in 0.9% saline and stored in 4°C until use. Eriocitrin was prepared freshly in distilled water for each administration.

Animals and grouping

Thirty male Wistar albino rats weighing approximately 140 g–180 g were obtained from the animal housing facility of Shandong University. The rats were acclimatized for 1 week before the experiment at $25 \pm 2^\circ\text{C}$, with adequate ventilation and humidity ($55 \pm 5\%$) and supplied with water and standard rodent feed ad libitum. Ethical approval for the experiment was provided by the Animal Healthcare and Ethical Committee of Jinan Central Hospital affiliated to Shandong University with approval reference number JXC20180915. For the experimental procedure, the rats were divided into five groups with six animals ($n=6$) in each group. The whole experiment was carried out in the biochemical and histopathological laboratories of Jinan Central Hospital affiliated to Shandong University for approximately 8 months beginning in May 2018.

Experimental procedure

The experimental procedure was done following the methods of Abdel-Daim et al. [16]. with slight

modifications. Rats of normal control group were orally administered with saline for 10 days. Nephrotoxic model group rats were orally administered with saline for 10 days, with a single i.p. injection of cisplatin (8 mg/kg b.w.) on the 7th day to induce renal toxicity. The animals of first dose experimental group were orally administered with 25 mg/kg b.w. of eriocitrin for 10 days and a single i.p. injection of cisplatin (8 mg/kg b.w.) on the 7th day. Animals of second dose experimental group received oral administration of 50 mg/kg b.w. eriocitrin for 10 days and a single i.p. injection of cisplatin (8 mg/kg b.w.) on the 7th day. Drug control group animals were subjected to oral administration of eriocitrin alone at 50 mg/kg b.w. for 10 days. All the animals were placed in metabolic cages for 24 h urine collection prior to end of experiment for proteinuria determination. On the 11th day, rats were anesthetized with isoflurane to obtain blood serum through retro orbital puncture. Blood samples were allowed to clot for 30 min and centrifuged at 3000 rpm for 15 min. The aliquots obtained were stored and later subjected to biochemical analysis. Upon blood collection, rats were immediately sacrificed by cervical dislocation and kidneys were excised and cleansed with cold isotonic NaCl (0.9%) and distilled water. A portion of kidney tissue was fixed in 10% neutral buffered formalin for histopathological studies. The remaining kidney tissues were homogenized in cold sodium phosphate buffered saline (5 mM) and centrifuged at 5000 rpm for 30 min to obtain aliquots for biochemical studies. The aliquots were used for determination of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and DNA fragmentation.

Biochemical assays

Determination of renal biomarkers; proteinuria in urine, urea, creatinine and albumin levels in the serum were done using colorimetric assays using commercial kits (Shimadzu CL-7200, Japan) following the method of Abdel-Daim et al. [16]. Oxidative stress markers such as lipid peroxidation (MAK085), reduced glutathione (GSH)(ab239709, Abcam, USA), superoxide dismutase (SOD)(19160), catalase (CAT)(219265) and nitric oxide (NO)(482650) production by measuring stable NO metabolites (nitrate/nitrite ions) using Griess reagent in the kidney tissues were determined by colorimetric assays using commercial diagnostic kits (Sigma Aldrich Inc., St. Louis, MO, USA) & (Merck Millipore, Darmstadt, Germany), following the protocols of Busari et al. [8] and Basu et al. [17]. The absorbance readings were measured

using UV-vis spectrophotometer (Shimadzu UV-1900, Japan).

ELISA analysis

The levels of serum pro-inflammatory cytokines TNF- α and IL-1 β together with activities of apoptosis marker enzymes (Poly-(ADP ribose) polymerase (PARP-1), Caspase-3 and percentage of DNA fragmentation in tissue were measured using ELISA assay of commercially available diagnostic kits (R&D Systems, Minneapolis, MN) according to the manufacturer's protocols. All the assays were similarly performed according to the methods of Abdel-Daim et al. [18] and Shukla et al. [19].

Histopathological examination

Kidney tissues fixed in 10% neutral buffered formalin were trimmed, dehydrated with gradient alcohol and xylene, embedded in paraffin wax and sliced into 5 μ m sections. The tissue sections were stained with hematoxylin and eosin (H&E), viewed under light microscope and photographed to identify the pathological changes due to cisplatin-induced toxicity.

Statistical analysis

All data were expressed as mean $\pm$ standard error of mean. Statistical significance ($p < 0.05$) was determined using SPSS software version 22.0 for windows (Chicago, USA) with one-way analysis of variance to compare means followed with Tukey's multiple comparison analysis between groups.

Results

Effect of eriocitrin on renal biomarkers

The levels of proteinuria in urine, and serum levels of urea, creatinine and albumin did not show any abnormality in normal control and eriocitrin alone administered control group. Cisplatin-induced nephrotoxic model group showed significant difference ($p < 0.05$) in all the serum biomarker levels against control group (Table 1). Administration of eriocitrin dose-dependently altered ($p < 0.05$) the serum biomarker levels towards normal against cisplatin-induced renal toxicity.

Table 1: Nephroprotective effects of eriocitrin on urine and serum levels of renal biomarkers in cisplatin-induced nephrotoxicity.

	Urea (U/L)	Proteinuria (U/L)	Creatinine (U/L)	Albumin (U/L)
Normal control	33.25 ± 0.56	64.29 ± 0.28	0.75 ± 0.08	2.89 ± 0.07
Cisplatin model group	155.36 ± 1.23 ^a	247.36 ± 1.16 ^a	3.69 ± 0.10 ^a	1.36 ± 0.09 ^a
Eriocitrin (25 mg/kg b.w.) + cisplatin	88.25 ± 0.65 ^b	115.29 ± 0.82 ^b	1.84 ± 0.04 ^b	1.93 ± 0.05 ^b
Eriocitrin (50 mg/kg b.w.) + cisplatin	59.36 ± 0.82 ^{b,c}	91.76 ± 0.98 ^{b,c}	1.12 ± 0.07 ^{b,c}	2.33 ± 0.03 ^{b,c}
Eriocitrin alone (50 mg/kg b.w.)	44.12 ± 0.34 ^{b,c}	69.36 ± 0.85 ^{b,c}	0.82 ± 0.06 ^{b,c}	2.71 ± 0.05 ^{b,c}

The results are given as mean ± SEM for six rats (n = 6). Significant difference against normal control group is symbolized as ^a(p < 0.05), whereas significant difference against cisplatin-induced nephrotoxic model group is symbolized as ^b(p < 0.05), significant difference against eriocitrin (25 mg/kg b.w.) group is symbolized as ^c(p < 0.05).

Effect of eriocitrin on renal oxidative stress markers

Administration of cisplatin significantly induced oxidative stress (p < 0.05) in nephrotoxic model group compared to control group with an increase in malondialdehyde (MDA) formation due to lipid peroxidation, reduced levels of GSH, SOD, CAT and elevated NO production (Table 2). Eriocitrin was able to reduce oxidative stress dose-dependently (p < 0.05) against the renal toxicity induced by cisplatin. Eriocitrin alone treated control group did not differ significantly from the normal control group.

Effect of eriocitrin on pro-inflammatory cytokines

Pro-inflammatory cytokines TNF-α and IL-1β were at normal levels in the serum of control group and eriocitrin alone administered group. The serum levels of TNF-α and IL-1β for cisplatin-induced nephrotoxic model group were significantly high (p < 0.05) as compared with normal control group (Table 3). The serum levels of pro-inflammatory cytokines were dose-dependently reduced (p < 0.05) in eriocitrin administered groups against cisplatin-induced nephrotoxicity.

Effect of eriocitrin on DNA fragmentation and apoptosis markers

Normal control group and eriocitrin alone treated control group did not express any abnormal levels in the percentage of DNA fragmentation and also apoptosis marker enzymes PARP-1 and Caspase-3. The nephrotoxic model group administered with cisplatin showed significant increase (p < 0.05) in the percentage of DNA fragmentation and significantly high activities (p < 0.05) of PARP-1 and Caspase-3 enzymes (Table 4). In contrast, administration of eriocitrin dose-dependently prevented DNA fragmentation and reduced the activities (p < 0.05) of apoptosis marker enzymes against cisplatin-induced DNA damage and apoptosis.

Effect of eriocitrin on histopathological changes

Histopathological results of the normal control and eriocitrin alone administered control group were having completely normal architecture (Figure 1). Cisplatin-induced renal toxic model group showed derangements in the renal pathology with dilated tubules, loss of epithelial cell borders and atrophy. These pathological changes were

Table 2: Nephroprotective effects of eriocitrin on renal oxidative stress markers in cisplatin-induced nephrotoxicity.

	MDA (nmol/mg protein)	GSH (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	NO (μmol/mg protein)
Normal control	15.97 ± 0.67	9.51 ± 0.90	7.43 ± 1.23	6.71 ± 0.75	165.19 ± 1.99
Cisplatin model group	57.94 ± 1.55 ^a	3.35 ± 0.98 ^a	3.48 ± 0.31 ^a	2.44 ± 0.47 ^a	448.22 ± 9.48 ^a
Eriocitrin (25 mg/kg b.w.) + cisplatin	33.54 ± 1.41 ^b	5.81 ± 0.57 ^b	5.3 ± 0.31 ^b	4.35 ± 0.32 ^b	277.88 ± 10.86 ^b
Eriocitrin (50 mg/kg b.w.) + cisplatin	25.21 ± 1.76 ^{b,c}	7.99 ± 0.68 ^{b,c}	5.92 ± 0.12 ^{b,c}	5.39 ± 0.26 ^{b,c}	208.87 ± 7.62 ^{b,c}
Eriocitrin alone (50 mg/kg b.w.)	17.42 ± 0.73 ^{b,c}	8.66 ± 0.85 ^{b,c}	6.46 ± 0.37 ^{b,c}	6.11 ± 0.06 ^{b,c}	176.42 ± 2.44 ^{b,c}

The results are given as mean ± SEM for six rats (n = 6). Significant difference against normal control group is symbolized as ^a(p < 0.05), whereas significant difference against cisplatin-induced nephrotoxic model group is symbolized as ^b(p < 0.05), significant difference against eriocitrin (25 mg/kg b.w.) group is symbolized as ^c(p < 0.05).

Table 3: Protective effects of eriocitrin on pro-inflammatory cytokines in cisplatin-induced nephrotoxicity.

	TNF- α (pg/mL)	IL-1 β (pg/mL)
Normal control	29.25 $\pm$ 0.89	20.37 $\pm$ 1.02
Cisplatin model group	68.28 $\pm$ 1.06 ^a	57.24 $\pm$ 1.08 ^a
Eriocitrin (25 mg/kg b.w.) + cisplatin	47.42 $\pm$ 1.19 ^b	34.58 $\pm$ 1.89 ^b
Eriocitrin (50 mg/kg b.w.) + cisplatin	34.95 $\pm$ 1.68 ^{b,c}	26.53 $\pm$ 0.58 ^{b,c}
Eriocitrin alone (50 mg/kg b.w.)	30.15 $\pm$ 0.31 ^{b,c}	21.47 $\pm$ 0.35 ^{b,c}

The results are given as mean $\pm$ SEM for six rats (n = 6). Significant difference against normal control group is symbolized as ^a(p < 0.05), whereas significant difference against cisplatin-induced nephrotoxic model group is symbolized as ^b(p < 0.05), significant difference against eriocitrin (25 mg/kg b.w.) group is symbolized as ^c(p < 0.05).

Table 4: Protective effects of eriocitrin on DNA damage and apoptosis markers in cisplatin-induced nephrotoxicity.

	DNA fragmentation (%)	PARP-1 (%)	Caspase-3 (%)
Normal control	100.19 $\pm$ 0.12	100.19 $\pm$ 0.12	100.04 $\pm$ 0.12
Cisplatin model group	542.48 $\pm$ 6.60 ^a	388.00 $\pm$ 9.05 ^a	219.85 $\pm$ 8.70 ^a
Eriocitrin (25 mg/kg b.w.) + cisplatin	273.25 $\pm$ 4.45 ^b	238.44 $\pm$ 3.00 ^b	166.65 $\pm$ 6.56 ^b
Eriocitrin (50 mg/kg b.w.) + cisplatin	189.99 $\pm$ 7.76 ^{b,c}	167.43 $\pm$ 2.49 ^{b,c}	167.43 $\pm$ 2.49 ^b
Eriocitrin alone (50 mg/kg b.w.)	135.52 $\pm$ 2.55 ^{b,c}	135.05 $\pm$ 2.74 ^{b,c}	124.24 $\pm$ 0.51 ^{b,c}

The results are given as mean $\pm$ SEM for six rats (n = 6). Significant difference against normal control group is symbolized as ^a(p < 0.05), whereas significant difference against cisplatin-induced nephrotoxic model group is symbolized as ^b(p < 0.05), significant difference against eriocitrin (25 mg/kg b.w.) group is symbolized as ^c(p < 0.05).

Figure 1: Histopathological changes in kidney due to administration of eriocitrin against cisplatin-induced nephrotoxic rat model. Normal control group and eriocitrin control group demonstrate common renal architecture; Cisplatin-induced nephrotoxic model group show signs of cell atrophy, dilated tubules and loss of boundary in endothelial cells (changes indicated by black arrow); Eriocitrin treated groups show protective features on the renal morphology against cisplatin-induced toxicity with mild loss of boundary (changes indicated by black arrow). H&E at 100 $\times$ magnification.

of malignancies has shown side effects on kidney toxicity profile of patients [3]. Elimination of cisplatin during chemotherapy occurs mostly through kidneys compared to intestinal excretion. Due to high accumulation of cisplatin in kidney, administration of cisplatin at non-toxic blood levels causes renal toxicity [20]. As a management strategy, supplementation of antioxidants to attenuate the toxic effect of cisplatin has been promising for the past years [7]. From the results obtained, it was found that eriocitrin was able to prevent oxidative stress induced by cisplatin in rats. Elevated levels of renal biomarkers; urea, proteinuria and creatinine in the nephrotoxic model group can be related to degradation of glomerular function and membrane permeability in kidney. Moreover, low serum albumin level in the model group explains the degradation and decreased synthesis of albumin due to toxicity of cisplatin. Administration of eriocitrin altered the levels of cisplatin-induced renal biomarkers similar to the results of El-Shen et al. [21]. Cisplatin induces nephrotoxicity by accumulating in the lining epithelium of tubules in kidney which triggers a complex reaction of oxidative stress, inflammation and apoptosis. Cisplatin induced oxidative stress is based on formations of cisplatin-DNA adducts, disallowing DNA replication, causing production of free radicals [22]. Reactive oxygen species (ROS) formed during cisplatin-induced renal toxicity is assumed to be generated during entrance of cisplatin into renal tubular cells, creating positively charged electrophiles upon hydrolysis. These electrophiles accumulate in negatively charged mitochondria, causing inactivity of respiratory complexes, leading to ROS formation. Another process of ROS formation is due to reduction of GSH and antioxidant enzymes by cisplatin [20]. Oxidative stress levels in the nephrotoxic model group were demonstrated by declined levels of GSH, SOD and CAT along with an increase of lipid peroxidation. Similar to the results of Abdel-Daim et al. [16], eriocitrin remarkably altered the effects of oxidative stress induced by cisplatin through regulation of GSH and antioxidant enzymes to prevent oxidization of cell membranes.

Inflammatory reactions caused by cisplatin were observed in the nephrotoxic model group as the ELISA assay found that serum levels of pro-inflammatory cytokines TNF- α and IL-1 β along with the serum nitric oxide concentrations were elevated. This shows the antioxidant regulation was disturbed in the kidney tissues similar to the findings of Sooriyaarachchi et al. [23] and Adil et al. [10]. Administration of eriocitrin mitigated the inflammatory response induced by cisplatin by suppressing the pro-inflammatory cytokines and NO levels.

Prevention of cell membrane oxidation and preservation of mitochondrial integrity can be related to the mitigating effects of eriocitrin against oxidative stress and inflammation. The renal morphology of cisplatin-induced toxic model group was in accordance to the biochemical results which showed severe damage to the glomerulus along with dilated tubules and degenerated cells. These observations were significantly altered in eriocitrin treated groups where the renal morphology was well preserved. These results were in agreement with the results of Canayakin et al. [24]. Cisplatin-induced apoptosis and DNA damage in the model group was proven through the apoptotic enzyme reactions and DNA fragmentation results. It was found that PARP-1 and Caspase-3 activities were increased in the nephrotoxic model group. PARP-1 is a nuclear enzyme known to be involved in DNA repair mechanism and is associated with Caspase-3 during an apoptotic reaction, where the latter cleaves PARP-1 to prevent recruitment of the enzyme to DNA repair site. Therefore, apoptosis of cell takes place with the help of Caspase-3. High percentage of DNA fragmentation in the nephrotoxic model group can be related to the high activities of PARP-1 and Caspase-3. Eriocitrin was able to protect the renal cells through prevention of apoptosis and DNA damage similar to the results of Shukla et al. [19] and Kim et al. [25].

Recent studies on oral administration of eriocitrin reported the anti-inflammatory potentials on experimental colitis in mice model [26] and ameliorative effect on hepatic steatosis in diet-induced zebrafish [13]. Another in vitro study on hepatocellular carcinoma cells evidenced anti-proliferative effect of eriocitrin [27]. These studies have common findings with the results obtained in this study such as anti-inflammatory effect of eriocitrin by suppression of pro-inflammatory cytokines, anti-apoptotic effect on normal cells, and DNA damage prevention. The dose-dependent nephroprotective results of eriocitrin suggest that 50 mg/kg b.w. dose of eriocitrin can be used for further studies on pre-clinical trials on similar disease models. The eriocitrin alone treated control group results additionally provide evidence on the safety of the compound at 50 mg/kg b.w. dose. Limitations of this study are that the specific mechanisms or pathways involved in the anti-apoptotic and nephroprotective of eriocitrin were not elucidated. Moreover, due to limited resources, a study on curative effects (post-treatment) of eriocitrin on cisplatin-induced renal toxicity could not be conducted. Future studies are recommended for curative effects of eriocitrin on cisplatin-induced nephrotoxicity along with the mechanisms and pathways involved. Overall, the findings of this study provides evidence that eriocitrin has the

ability to prevent oxidative stress, inflammatory reaction and apoptosis in cisplatin-induced nephropathy in rats through regulation of antioxidants and alleviation of cellular membrane and DNA-damage.

Conclusion

The findings of this research prove that eriocitrin has anti-apoptotic and nephroprotective effects against cisplatin-induced nephrotoxicity via alleviation of oxidative stress and DNA-damage. It can be suggested that eriocitrin could function as a chemopreventive adjuvant to be administered along with cisplatin during malignant cancer treatments. Clinical validations are required to proof the effectiveness of eriocitrin against nephrotoxicity in cisplatin-treated cancer patients.

Conflicts of interest: Authors have no potential conflicts of interests to declare regarding this study.

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