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Cisplatin-induced kidney damage and the protective effect of bilberry (*Vaccinium myrtillus* L.): an experimental study

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Aim: Bilberry is a natural antioxidant and has been utilized as a free radical scavenger to reverse the toxic effects of oxidative stress. The aim of this study was to investigate the effect of bilberry on cisplatin-induced toxic effects in rat kidney.

Materials and methods: Twenty-one female Wistar-albino rats were randomly divided into 3 groups: group I (control), one dose of saline solution; group II (cisplatin), single dose of 7.5 mg/kg of cisplatin; group III (cisplatin + bilberry), single dose of 7.5 mg/kg of cisplatin and 10 days of bilberry treatment applied as 200 mg/kg of bilberry. Malondialdehyde (MDA) levels and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured. Histopathologic damage to the kidney tissue was analyzed via light microscopic examination.

Results: Severe necrosis, edema, hemorrhage, interstitial cell infiltration, vascular dilatation, glomerular atrophy, and tubular degeneration were observed in group II. SOD, CAT, and GPx activities were decreased and MDA levels were increased in the cisplatin group compared to the cisplatin + bilberry group, and the differences were statistically significant ($P < 0.05$). Kidney tissue damage was significantly higher in group II than in group III ($P < 0.05$).

Conclusion: Bilberry is efficient in the treatment of cisplatin-induced kidney damage.

Key words: Cisplatin, nephrotoxicity, rat, bilberry, protective effect

1. Introduction

Bilberry (*Vaccinium myrtillus* L.) is a natural substance. It serves as a main fruit ingredient in pies, jams, etc. Bilberry is known as a source of flavonoids. Flavonoids have intensive antioxidant activity (1). Bilberry has been utilized in the treatment of cancer and heart disease because of its free radical scavenging properties (2,3). The chemical substances in bilberry are known as anthocyanosides (4). Bilberry has been utilized in the treatment of intestinal, heart, kidney, and retina diseases (3,5).

Cisplatin [CDDP, cis-diamminedichloroplatinum(II)] is a potent antineoplastic drug used in the treatment of solid tumors. Clinicians should pay attention to the toxic effects of this drug (6,7). Cisplatin shows its cellular toxic effect directly. The platinum derivatives of the drug are responsible for the cytotoxicity (8). Cisplatin increases the lipid peroxidation in kidney tissue. The inevitable consequence of this reaction is the elevation of free radicals and reactive oxygen species (ROS). These free radicals, and especially elevated malondialdehyde (MDA) levels, lead to cellular damage (9,10).

Numerous studies have been performed to alter this cisplatin-induced nephrotoxicity. The chemoprotective agents utilized against cisplatin toxicity include selenium, quercetin, silymarin, simvastatin, etc. (11–14). We hypothesized that administration of bilberry could preserve the kidney tissue. Thus, the aim of this study was to investigate the protective effect of bilberry on cisplatin-induced toxic effects in rat kidney. There is no information about the effects of bilberry against cisplatin-induced nephrotoxicity.

2. Materials and methods

2.1. Chemical agents

Cisplatin was purchased from Sigma Chemical Co. (St Louis, MO, USA). Bilberry was purchased from a drugstore in Yozgat, Turkey.

2.2. Animals and experimental protocols

The study was reviewed and approved by the Ethical Committee of Çukurova University Medical Faculty. Twenty-one female adult Wistar-albino rats (weight range,

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150–220 g) were divided into 3 equal groups. In group I (control group), 1 mL of 0.9% NaCl saline solution was given intraperitoneally (ip). In group II (cisplatin group), a single dose of 7.5 mg/kg of cisplatin was administered ip. In group III (cisplatin + bilberry group), 7.5 mg/kg of cisplatin was administered ip, and 200 mg/kg of bilberry was given orally. Bilberry treatment was applied for 10 successive days starting 1 day before cisplatin administration. All the rats were weighed and killed. Then their kidneys were surgically extirpated.

2.3. Histologic examination

The kidneys were fixed in 10% formalin solution for 24 h and embedded in paraffin. The kidney tissues were sectioned at 5 μm and stained with hematoxylin-eosin. The sections were examined and photographed by using an Olympus BX-51 light microscope (Olympus Corp., Tokyo, Japan). Five microscopic fields were examined to assess the tissues. The severity of the changes observed were scored as follows: none (-), moderate (+), and severe (++).

2.4. Biochemical measurements

MDA levels were assessed by measuring thiobarbituric acid (TBA). The colorimetric absorbance was determined at 532 nm (15). Specific activity was presented as nmol/mg protein. Total SOD activity was determined according to the method described by Marklund and Marklund (16) by assaying the autoxidation and illumination of pyrogallol at 440 nm. Data are expressed as USOD/mg hemoglobin. CAT enzyme activity was measured according to the method described by Aebi (17). GPx activity was measured using H_2O_2 as substrate according to the method described by Paglia and Valentine (18) in absorbance at 340 nm. Data are presented as UGPx/mg hemoglobin. All of these were measured in kidney tissue.

2.5. Statistical analysis

The Statistical Package for the Social Sciences (SPSS 11.0; SPSS Inc., Chicago, IL, USA) version 11.0 was used for statistical analysis. The significance of differences was calculated using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P < 0.05$ was taken as statistically significant.

3. Results

The morphologic characteristics of the kidneys were assessed macroscopically. There was no difference between the groups in relation to the macroscopic features. Kidneys in the control group were normal in histopathologic appearance (Figures 1A and 1B). Treatment with bilberry

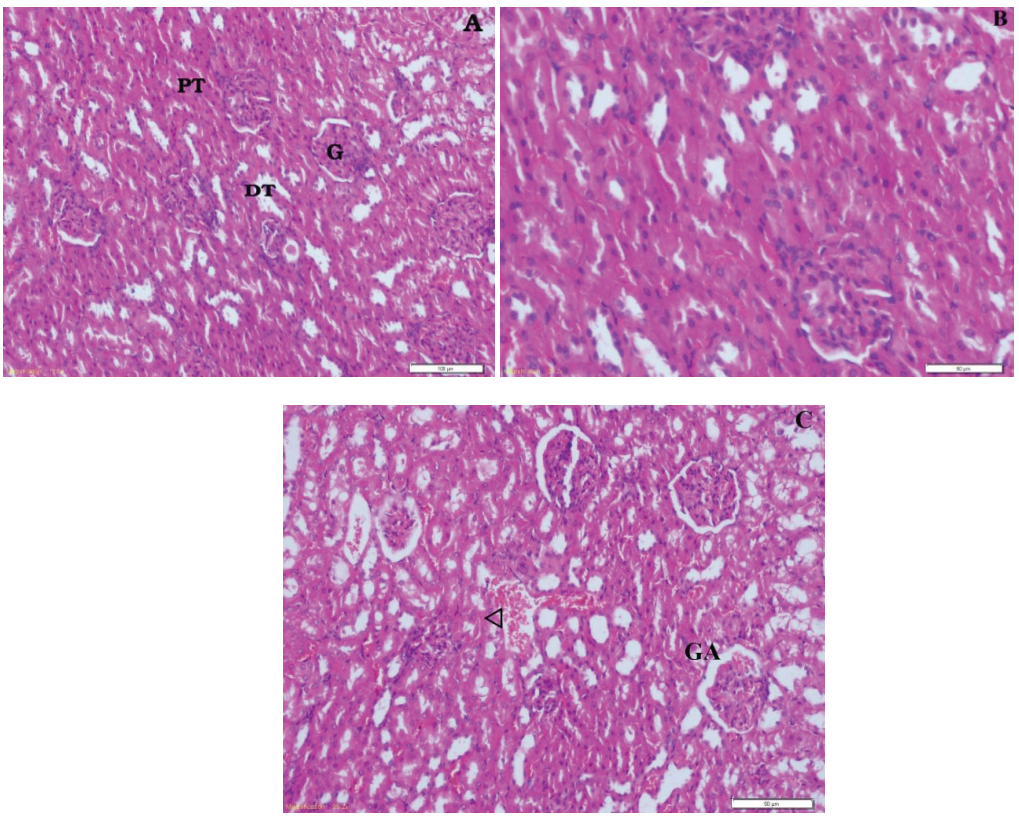

Figure 1. (A) Kidney section of control rats, (PT) proximal tubules, (DT) distal tubules, (G) glomerulus (×200). (B) No apparent pathology in control rats (×400), (C) glomerular atrophy (GA) vascular dilatation (▷) in kidney cisplatin + bilberry-treatment rats (×200).

effectively reduced the kidney tissue damage scores in the cisplatin + bilberry treatment group (Figure 1C).

Severe necrosis, edema, interstitial cell infiltration, and vascular dilatation were seen in group II (Figure 2A). Tubular degeneration and hemorrhage were massive in group II (Figure 2B). Glomerular atrophy and hemorrhage were observed in cisplatin-administered rats (Figure 2C). Cellular damage was higher in the cisplatin group than in the cisplatin + bilberry group. The histopathological changes were scored in Table 1 as follows: none (-), moderate (+), and severe (++).

The MDA level was significantly lower in group III than in group II ($P < 0.05$). SOD, CAT, and GPx activities decreased in the cisplatin group compared to the cisplatin + bilberry group, and the differences were statistically significant ($P < 0.05$) (Table 2). Body weight, kidney weight, and relative kidney weight of control and experimental rats are shown in Table 3.

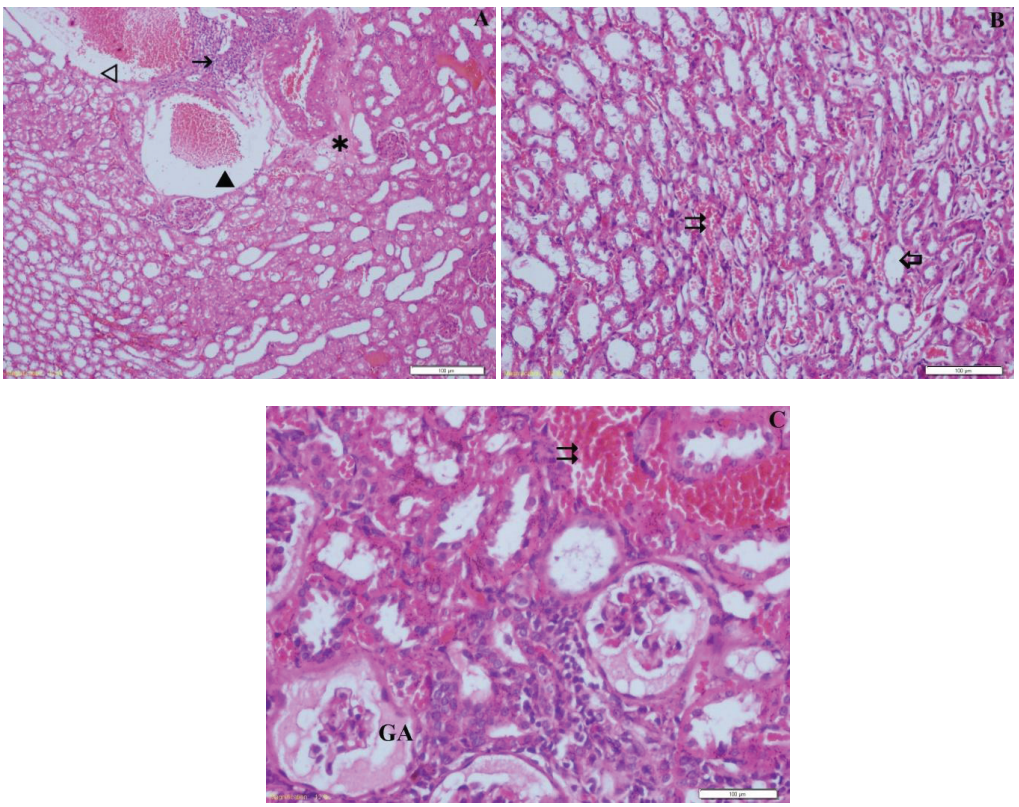

Figure 2. (A) Necrosis (▸), edema (*), interstitial cell infiltration (↑), and vascular dilatation (▹) in kidney cisplatin-treatment to rats (×200). (B) Tubular degeneration (⇪) and hemorrhage (↑ ↑) in kidney cisplatin-treatment rats (×200). (C) Glomerular atrophy (GA) and hemorrhage (↑ ↑) in kidney cisplatin-treatment rats (×400).

Table 1. Grading of the histopathological changes in kidney sections of rats exposed to cisplatin/cisplatin + bilberry. The features were scored as follows: none (-), moderate (+), and severe (++).

Groups	Necrosis	Edema	Hemorrhage	Interstitial cell infiltration	Vascular dilatation	Glomerular atrophy	Tubular degeneration
Control	-	-	-	-	-	-	-
Cisplatin (7.5 mg/kg)	++	++	++	++	++	++	++
Cisplatin + bilberry (7.5 mg/kg + 200 mg/kg)	-	-	-	-	+	+	-

Table 2. Effects of cisplatin treatment on superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities, and malondialdehyde (MDA) levels in Wistar rat kidneys (means ± SD).

Groups (each group, n = 7)	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	4.01 ± 0.13	54 ± 3.04	76 ± 1.08	130 ± 6.15
Cisplatin (7.5 mg/kg)	9.61 ± 0.26 [a]	19 ± 1.22 [a]	61 ± 1.12 [a]	47 ± 3.32 [a]
Cisplatin + bilberry (7.5 mg/kg + 200 mg/kg)	6.85 ± 0.27 [a,b]	34 ± 3.12 [a,b]	68 ± 1.27 [a,b]	86 ± 4.45 [a,b]

Table 3. Body weight, kidney weight, and relative kidney weight of control and experimental rats.

Groups	Body weight (g)			Absolute kidney weight (g)	Relative kidney g/100 g body weight
	Initial	Final	% Change		
Control	227 ± 5.78	231 ± 5.46	1.76 ± 0.69	1.95 ± 0.15	0.844 ± 0.13
Cisplatin	229 ± 3.67	218.8 ± 2.40[a]	–4.45 ± 1.01[a]	1.43 ± 0.18 [a]	0.656 ± 0.05[a]
Cisplatin + bilberry	228 ± 4.08	225 ± 3.52[a,b]	–1.32 ± 0.44 [a,b]	1.77 ± 0.09 [a,b]	0.790 ± 0.14[a,b]

Values are means ± SD for 7 rats in each group. Significance at $P < 0.05$.
[a]Comparison of control and other groups.
[b]Comparison of cisplatin-treated group with cisplatin and bilberry-treated groups.

4. Discussion

Many anticancer drugs have been shown to be teratogenic and carcinogenic in experimental systems. The therapeutic effects of cisplatin are significantly improved by dose escalation (19). The chemotherapeutic treatments used for cancers may damage organs such as the ovaries, liver, and kidneys (20,21). Several theories were asserted about cisplatin-induced nephrotoxicity. These mechanisms were lipid peroxidation, mitochondrial dysfunction, degradation of protein structure, and DNA injury (22–24). However, the real mechanism is not known. The platinum components of cisplatin bind to DNA. This process leads to DNA damage and cell death (8). Oxidative stress is another participating factor associated with cisplatin-induced renal damage. SOD, CAT, and GPx activities are increased in cellular membranes due to cisplatin-induced nephrotoxicity (25).

Quercetin is a derivative of a natural flavonoid. It exhibits strong antioxidant activity. Estela et al. (2006) performed a study on cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys (12). They reported that quercetin administration reversed histological and biochemical changes due to cisplatin. Subsequently, spirulina, erdosteine, simvastatin, grape seed, and taurine were studied as chemoprotective agents on cisplatin-induced nephrotoxicity (14,22,26–28). Bilberry has been used for the treatment of oxidative stress. Various theories have been proposed about the bilberry's mechanism of action. These include antioxidant activities, free radical scavenging properties, anti-inflammatory effects, and vasodilatory action (1,3,4). Therefore, bilberry could be beneficial for reducing the toxic renal damage due to cisplatin.

Both in vivo and in vitro studies indicate that cisplatin administration results in elevated oxygen free radicals (10,29). These oxygen free radicals cause multiple alterations in the cells and organs of animals that are exposed to cisplatin, which results in damage to organs (30). In the present study the protective effect of bilberry on cisplatin-induced toxic effects in rat kidney was studied. The findings of our study suggest that bilberry may be useful for reducing kidney damage due to cisplatin. Bilberry administered ip at 200 mg/kg preserved the renal tissue and reduced the histological and biochemical changes. Bilberry treatment resulted in a decrease in the level of tissue MDA and an increase in the activities of SOD, CAT, and GPx. This is the first study indicating the preventative effect of bilberry on cisplatin-induced nephrotoxicity. Tissue damage criteria such as degeneration, hemorrhage, interstitial cell infiltration, and vascular dilatation were less common in the cisplatin +

bilberry group than in the cisplatin only group. Although our results demonstrated the protective effect of bilberry on cisplatin-induced nephrotoxicity, large prospective randomized trials are required.

In conclusion, bilberry has a significant therapeutic benefit when administered with cisplatin treatment against reductions in body and kidney weight, nephrotoxicity, and oxidative stress. Bilberry may be used as a nutritional supplement to protect the kidney in cancer chemotherapy as an alternative medical resource.

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