Myelosuppression and Nephrotoxicity Induced by Cisplatin in Female Rats: The Role of Berberine Nanoparticles

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Abstract

Cisplatin (CDDP) is one of the most effective antineoplastic drugs used in chemotherapy, strategies to protect tissues against cisplatin nephrotoxicity is a clinical interest. This study aimed to evaluate the possible protective effect of berberine nanoparticles (BBR-NPs) against cisplatin-induced nephrotoxicity in female rats. Intraperitoneal (IP) injection of cisplatin (8 mg/kg) caused significant decrease in RBC, Hb, Hct, WBC and platelets. Also, cisplatin caused disturbances in kidney function as documented by a significant increase in urea, uric acid, creatinine and MDA, with significant decreases in the total protein, albumin, GSH and total thiol. TNF-α, caspase-3, IL-2, IL-6 and IL-1β were increased in cisplatin treated group. The histopathological changes in cisplatin group include degeneration and desquamation of tubular epithelial cells, hyaline cast formation, inflammatory cell infiltration and tubular dilation. Oral administration of BBR-NPs at a dose 1mg/kg/day for 30 days after cisplatin produced significant decrease in the levels of urea, uric acid, creatinine, TNF-α and caspase-3 as well as kidney MDA with a marked increase in total protein, albumin, GSH, total thiol and repairing the histopathological changes. Scanning microscope of RBC showed the protective effect of BBR-NPs against the different changes induced by CDDP. The present study suggested that the anti-oxidant and anti-inflammatory effects of BBR-NPs may prevent CDDP-induced nephrotoxicity via decreasing the oxidative stress, inhibiting the inflammation and apoptosis.

Keywords: Berberine Nanoparticles, Cisplatin, Nephrotoxicity, Female Rats.

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Introduction

Cisplatin (Cis-diaminedichloroplatinum (II), CDDP) is one of the most efficient antineoplastic agents. It is used to treat various types of cancers, including sarcomas, some carcinomas, lymphomas and germ cell tumors [1]. However, cisplatin can produce severe toxicity during chemotherapy including nausea, emesis, nephrotoxicity, myelosuppression and ototoxicity [2].

CDDP binds to DNA and forms intrastrand crosslinks and adducts, which results in the DNA conformation changes and impaired DNA replication [3]. Additionally, CDDP-mediated cytotoxicity includes mitochondrial damage, decreased ATP-ase activity and impaired cellular transport mechanisms [4]. Besides tumor cells, the cytotoxic activity of CDDP also targets normal somatic cells, particularly in the kidneys. Oxidative stress, inflammation, apoptosis and necrosis are suggested as the major mechanisms of CDDP-induced nephrotoxicity [5].

The use of nanotechnology in different branches of therapeutics has revolutionized the field of medicine where nanoparticles used for diagnostics, therapeutics and as biomedical tools for research [6]. The principle property of the nanoparticle is its small nature that allowing them to cross cellular membranes and avoid detection by the reticuloendothelial system and their high surface area to volume ratio can allow increased loading of therapeutics such properties makes nanoparticles desirable for diagnostic and therapeutic applications [7].

Berberine (BBR) is an isoquinoline quaternary alkaloid isolated from many types of medicinal plants [8]. Berberine has been considered to possess anti-inflammatory, antimicrobial, antiproliferative and apoptosis-inducing effects [9]. BBR has antioxidant effects and multiple pharmacological properties. It has been found to be used in the treatment of gastroenteritis, diarrhea, hyperlipidemia, obesity, fatty liver and coronary artery diseases, hypertension, diabetes, metabolic syndrome, polycystic ovary [10] and Alzheimer's disease [11]. BBR also plays a major role in the controlling and stopping cancer cell proliferation, migration and induces apoptosis in a variety of cancer cell lines [12].

Therefore, the present study aimed to investigate the possible protective effect of berberine nanoparticles (BBR-NPs) on cisplatin-induced nephrotoxicity in female rats.

2. Materials and Methods

2.1. Chemicals

Berberine (BBR) was purchased from Swanson Company. Chitosan (CS) was purchased from Oxford lab Chem. India. Cisplatin (CDDP) was purchased from Mylan Company.

2.2. Preparation of berberine nanoparticles (BBR-NPs)

Nanoparticles were formed suddenly upon incorporation of 6 ml of tripoly phosphate, aqueous solution (0.5 mg/ml) to 15 ml of the chitosan (CS) acidic solution 0.5 mg/ml containing a concentration of berberine 1mg/ml under magnetic stirring for 15 minutes. The physical properties of berberine nanoparticle were studied previously [13].

2.3. Experimental animals

Female adult Wistar albino rats (150±10g) were obtained from the animal Agriculture Faculty, Alexandria University, Egypt. The animals were acclimated under laboratory conditions (22-25°C, 12h light/dark cycle and relative humidity) for at least two weeks prior to the experiment. They were kept on a standard balanced laboratory diet and tap water *ad libitum*. The local committee approved the design of the experiments and the protocols were carried out according to the guidelines of the National Institutes of Health (NIH).

2.4. Experimental design:

Twenty-eight female adult Wistar albino rats were divided into four groups (7 each) as follows:

Control group: Received saline and stayed for 30 days.

CDDP group: Received CDDP as a single dose of 8 mg/kg (IP) [14] and stayed for 30 days.

CDDP+BBR-NPs group: Received CDDP as a single dose of 8 mg/kg (IP), then treated with BBR-NPs at a dose 1mg/kg/day for 30 days.

BBR-NPs group: Received BBR-NPs only at a dose 1mg/kg/day [15] for 30 days.

2.5. Preparation of blood and serum

Blood samples were collected from anesthetized rats into sterile tubes. The first part of blood was collected in EDTA tubes for the determination of hematological parameters (RBCs, Hb, Hct, PLts and WBCs). The second part was centrifuged at 3000 rpm for 15 min at 30°C for serum preparation. The sera were then collected and stored at -20°C for the determination of some biochemical parameters.

2.6. Preparation of kidney homogenate

The kidney tissues were quickly removed, washed with saline and cut into pieces. One gram of kidney was homogenized with 9 volumes of phosphate buffer (0.1M, pH 7.9) and then centrifuged at 3000 rpm for 15 minutes and the supernatant was saved for the determination of oxidative stress markers and antioxidant enzyme activities.

2.7. Determination of kidney function:

Urea and creatinine concentrations were analyzed by the method of Henry [16] and Patton & Crouch [17], respectively. Uric acid was determined by using kits from Biodiagnostic Co., Cairo, Egypt. Colorimetric methods of Gornall et al. [18] and Doumas et al. [19] were applied for the determination of total proteins and albumin, respectively. Globulin and alb/glob ratio were calculated.

2.8. MDA, GSH and total thiol assays:

The level of renal MDA was evaluated according to the procedure of Ohkawa et al. [20]. GSH and total thiol were determined according to Jollow et al. [21] and Sedlak and Lindsay [22], respectively.

2.9. Dtermination of EPO, IL-2, IL-6, IL-1β, caspase-3, P53 and TNF-α,:

A quantitative sandwich enzyme immunoassay technique (ELISA) kits were used for the determination of EPO (Cat. No. MBS703724), caspase-3 (Cat. No. MBS700575), P53 (Cat. No. MBS700880) and IL-2 (OmniKine™, Cat. No. OK-0207). Enzyme-Linked Immunosorbent Assay Kits for the quantitative measurement of rat IL-1β (Abcam Co. ab100767) and TNF-α (Abcam Co. ab46070) were used. IL-6 was estimated according to the method of Ferguson-Smith et al. [23].

2.10. DNA fragmentation Assay.

The amount of fragmented DNA was assayed according to Wu et al. [24]. The kidney tissues were homogenized in 10 volumes of a lysis buffer (pH 8.0) consisting of 5 mM Tris-HCl, 20 mM EDTA (Sigma) and 0.5% (w/v) t-octylphenoxypolyethoxyethanol (Triton X-100; Sigma). One-milliliter aliquots of each sample were centrifuged at 27,000 g for 20 mins to separate the intact chromatin (pellet) from the fragmented DNA (supernatant). The supernatant was decanted and saved, and the pellet was resuspended in 1 ml of Tris buffer

(pH 8.0) consisting of 10 mM Tris-HCI and 1 mM EDTA. The pellet and supernatant fractions were assayed for DNA content using a diphenylamine reaction. Optical density was measured at 620 nm. The results were expressed as a percentage of fragmented DNA divided by total DNA.

2.11. Scanning electron microscope (SEM):

Blood was collected in a clear microcentrifuge tube and fixed in 2.5% glutaraldhyde for 30 min at room temperature. After fixation the blood was centrifuged (750g at 4°C, 5 min). The cell pellet was washed twice and resuspended in distilled water. A thin film of the suspension was made on a clear coverslip and allowed to air dry. The coverslip was mounted on the brass stub with electroconducting point and then coated with gold in a fine coat ion sputter. The red blood cells were thoroughly examined. The SEM images were obtained by the use of a scanning electron microscope (Jeol Japan, JSM-5300) at 15 KV.

2.12. Histological studies:

The kidneys from control and treated groups were excised and allowed to fix at room temperature overnight in 10% formalin solution, then processed to be stained routinely with Haematoxylin and Eosin [25].

2.13. Statistical analysis:

Data were analyzed using SPSS version 22.0 software by One Way Anova and post hoc comparisons were carried out with Dunn's multiple comparison test. Differences with $P \leq 0.05$ were considered to be statistically significant.

3. Results

3. 1. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the hematological parameters:

RBC count, Hb content, Hct value, MCHC, MCH, MCV and platelets were all found to be significantly ($P \leq 0.05$) reduced in the CDDP group as compared with the control ones (Table 1). Leucopenia and lymphocytosis are the most prominent features of WBC in CDDP group in comparison with the control group. While, co-administration of BBR-NPs with CDDP provided a protective effect against these alterations.

Table (1): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the hematological parameters of experimental rats.

Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
RBCs ($\times 10^6$ /mm^3)	5.47±0.15^a	4.03±0.16^b	5.81±0.08^{ac}	6.23±0.17^d
Hb (g/dl)	12.91±0.51^a	8.57±0.74^b	11.18±0.58^c	12.73 ±0.19^{ad}
Hct (%)	34.58±1.32^a	23.23±1.21^b	31.57±0.14^c	33.93±1.52^{ad}
PLt ($\times 10^3$ /mm^3)	787.33±95.80^a	584.17±47.97^b	655.00±26.10^c	780.50±40.11^{ad}
WBC ($\times 10^3$ /mm^3)	5.45±0.25^a	3.77±0.19^b	6.90±0.84^c	6.03±0.42^d
LY (%)	57.67±11.61^a	72.53±8.44^b	58.23±3.08^c	54.33±3.74^d

MO (%)	**16.77±1.47 [a]**	**11.67±3.57 [b]**	**16.93±0.77a [c]**	**16.65±1.51[ad]**
GR (%)	**25.57±10.24 [a]**	**15.81±4.95 [b]**	**24.83±3.37[a c]**	**29.02±3.45[d]**

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0. 05.

3.2. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the kidney function:

The levels of uric acid, urea and creatinine were significantly enhanced in the CDDP-treated group while, EPO level was significantly (P≤0.05) declined as compared with the control rats. On the other hand, CDDP+BBR-NPs treated group showed significant (P≤0.05) decrease in the levels of urea, uric acid and creatinine as compared with CDDP group. The levels of these parameters in BBR-NPs treated group were more or less like the control ones.

Table (2): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the kidney function of experimental rats.

Parameter	**Experimental groups**			
	Control	**CDDP**	**CDDP+BBR-NPs**	**BBR-NPs**
Uric acid (mg/dl)	**1.51±0.06[a]**	**4.43±0.19[b]**	**2.15±0.18[c]**	**1.44 ±0.15[ad]**
Urea (mg/dl)	**34.33±1.38[a]**	**175.33±4.11[b]**	**42.41±4.15[c]**	**33.66±0.66[ad]**
Creatinine (mg/dl)	**1.01±0.02[a]**	**3.81±0.14[b]**	**2.10±0.13[c]**	**1.10±0.16[ad]**
EPO (ng/ml)	**8.89±0.24[a]**	**3.07±0.24[b]**	**6.34±0.11[c]**	**10.01±0.06[ad]**

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0.05.

3.3. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of total protein, albumin, globulin and alb/glob ratio:

Results arranged in table 3 showed that CDDP treatment caused a significant (P≤0.05) decrease in total protein, albumin, globulin and alb/glob ratio. Moreover, oral supplementation of BBR-NPs with CDDP resulted in a significant (P≤0.05) increment in these parameters.

Table (3): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of total protein, albumin, globulin and alb/glob ratio of experimental rats.

Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
Total protein (g/dl)	7.22±0.09[a]	6.28±0.8[b]	6.79±0.03[c]	7.54±0.30[ad]
Albumin (A) (g/l)	3.76±0.09[a]	2.76±0.07[b]	3.42±0.03[c]	3.87±0.99[ad]
Globulin (G) (g/l)	3.46±0.11 [a]	3.52±0.55 [b]	3.37±0.17 [c]	3.67±0.27 [ad]
A/G ratio	1.09±0.68 [a]	0.78±0.18 [b]	1.01±0.06 [c]	1.05±0.33 [ad]

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0.05.

3.4. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of MDA, GSH,

and T. thiol in the kidney:

In the rat kidney tissues, the cisplatin group showed a remarkable elevation in the levels of MDA, however, GSH and total thiol levels were significantly (P≤0.05) reduced (Table 4). The co-treatment of BBR-NPs with CDDP resulted in the reversal of these biochemical alterations when compared to the cisplatin group.

Table (4): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of MDA, GSH, and T. thiol in the kidney of experimental rats.

Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
MDA (pmol/mg protein)	7.20±0.39 [a]	15.31±0.25[b]	9.53±0.82[c]	6.27±0.26[d]
GSH (nmol/mg protein)	4.41±0.08 [a]	2.67±0.12[b]	3.59±0.07[c]	4.68±0.23 [ad]
T. Thiol (nmol/mg protein)	39.96±3.10 [a]	18.71±0.90[b]	31.44±1.52[c]	39.53±1.11[ad]

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0. 05.

5. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of IL-2, IL-6 and IL-1β:

Rats treated with CDDP (8 mg⁄kg) had significant elevations in IL-2, IL-6 and IL-1β. However, the combination of BBR-NPs with CDDP resulted in a significant (P≤0.05) reduction in these parameters as compared with CDDP alone.

Table (5): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of IL-2, IL-6 and IL-1β of experimental rats.

Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
IL-2 (pg/ml)	22.36±0.46^{a}	44.53 ±0.01^{b}	30.33±0.39^{c}	18.85 ±0.43^{ad}
IL-6 (pg/ml)	339.33±7.18^{a}	487.33 ±2.08^{b}	386.32±1.36^{c}	300.17±1.47^{ad}
IL-1β (pg/ml)	117.17±3.66^{a}	171.67±2.49^{b}	147.83±1.19^{c}	100.67±1.49^{ad}

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0.05.

3.6. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of caspase-3,

TNF-α and P53 in kidney:

CDDP (8 mg⁄kg) treatment caused significant elevations in Caspase-3, TNF-α and P53. On the other hand, the combination of BBR-NPs with CDDP revealed a significant decrease in these parameters as compared with CDDP alone.

Table (6): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the levels of Caspase-3, TNF-α and P53 in kidney of experimental rats.

Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
Caspase-3 (ng/ml)	9.78±0.26^{a}	27.31±0.12^{b}	15.5±0.24^{c}	8.97±0.07^{d}
TNF-α (pg/mg protein)	7.93±0.07^{a}	25.1±0.46^{b}	13.02±0.22^{c}	6.77±0.13^{d}
P53 (pg/mg protein)	71.03±0.51^{a}	131. 6±1.9^{b}	100.48±1.52^{c}	65.32±1.17^{d}

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0. 05.

3.7. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the DNA fragmentation of experimental rats.

CCDP treated group showed a significant (P≤0.05) increase in the level of DNA fragmentation as compared with the control group. In contrast, co-treateatment with BBR-NPs showed a significant (P≤0.05) improvmement in DNA with respect with CCDP group (Table 7).

Table (7): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the DNA fragmentation of experimental rats.

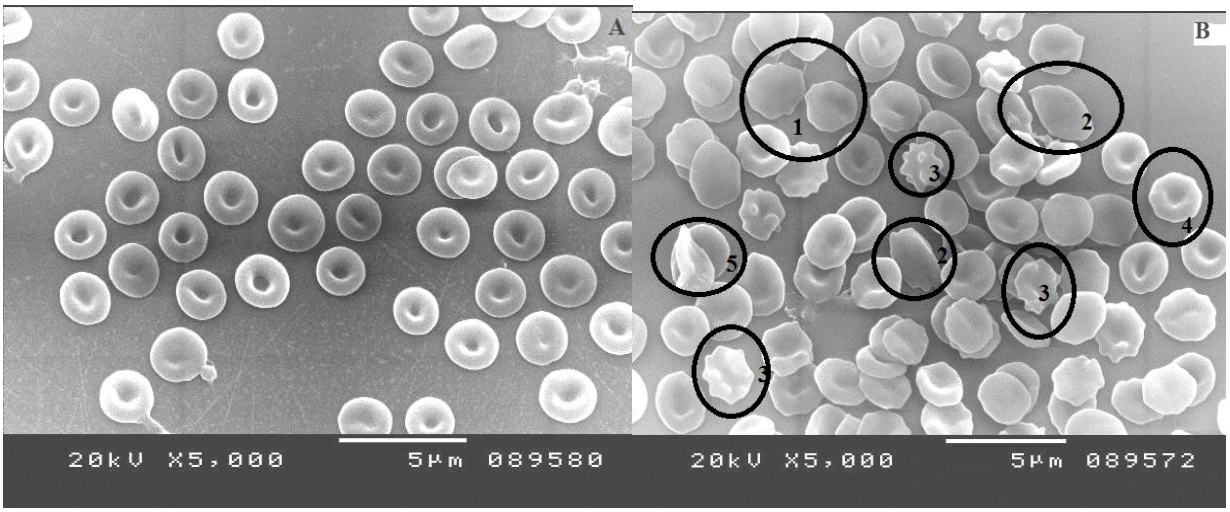
Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
DNA fragmentation (%)	9.94±0.12[a]	30.97 ±0.68[b]	17.27±0. 82[c]	7.61 ±0.34 [d]

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0. 05.

3.8. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the morphological pattern of RBC:

Cisplatin-induced morphological abnormalities in erythrocytes as documented by scanning electron microscopic observations as compared with control ones (Fig. 1A). These alterations include spherocytes, ovallocytes, echinocytes, Burr cells and scalloping (Fig. 1B). On the other hand, BBR-NPs treatment revealed an obvious improvement in the erythrocyte abnormalities as shown in figure 1C.



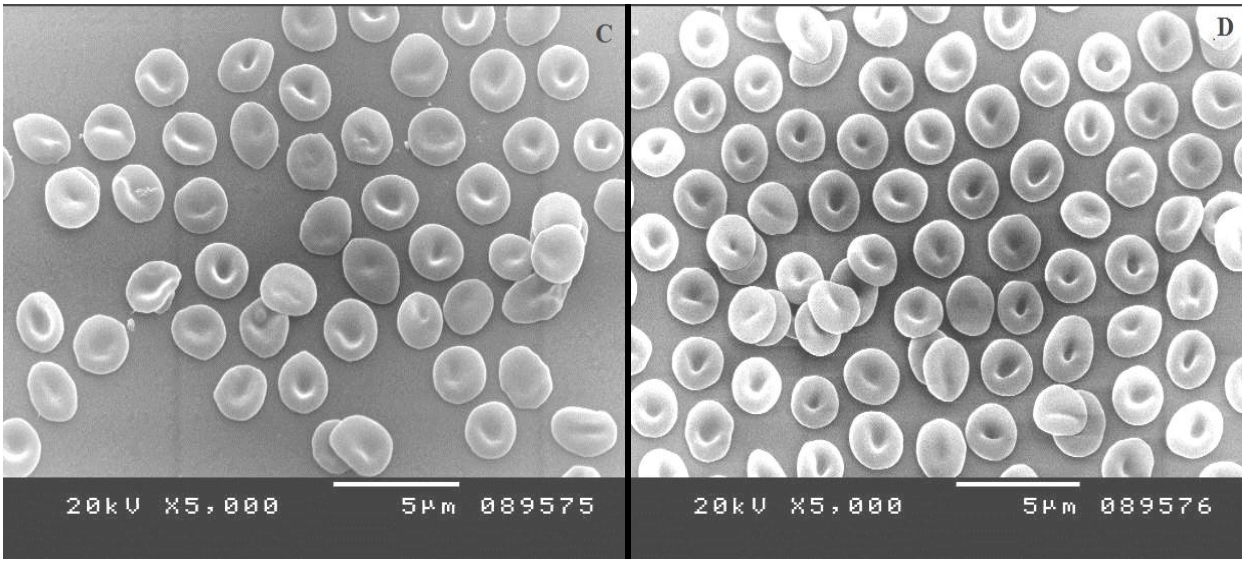


Fig. 1: Scanning electron micrographs (Bars=5 µm) of red blood cells from normal controls (A), CDDP (B) 1: Spherocytes 2: Ovallocytes 3: Echinocytes 4: Burr cells 5: Scalloping, CDDP+BBR-NPs (C) and BBR-NPs (D).

3.9. The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the diameter of RBCs of experimental rats.

Table 8 showed that CDDP group exhibited a significant (P≤0.05) decrease in the diameter of red blood cells compared to the control group. Supplementation of BBR-NPs caused significant (P≤0.05) increase in the diameter of red blood cells compared to CDDP group.

Table (8): The effects of CDDP, CDDP+BBR-NPs and BBR-NPs on the RBC diameter of experimental rats.

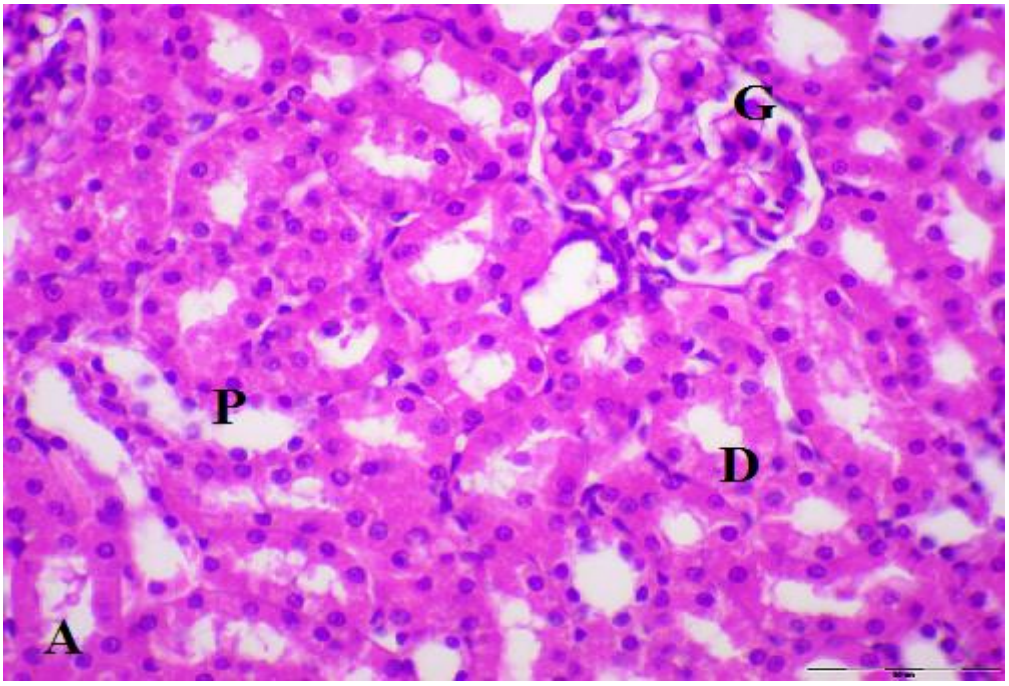
Parameter	Experimental groups			
	Control	CDDP	CDDP+BBR-NPs	BBR-NPs
Diameter (µm)	2.076±0.053^{a}	2.328 ±0.061^{b}	2.126±0.026^{c}	2.090±0.063^{ad}

Values are expressed as means±S. E., n=7 for each treatment group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P≤0. 05.

3.10. Histopathological studies:

Renal tissue sections from the control and BBR-NPs groups showed normal architecture (Figure 2A&D). Treatment with cisplatin caused a marked tubular and glomerular degenerative changes including desquamation, necrosis in epithelial cells, hyaline casts in the lumens of tubules and lymphocytic infilteration (Figure 2B). In contrast, treatment with BBR-NPs improved renal histopathological changes compared with the cisplatin group (Figure 2C).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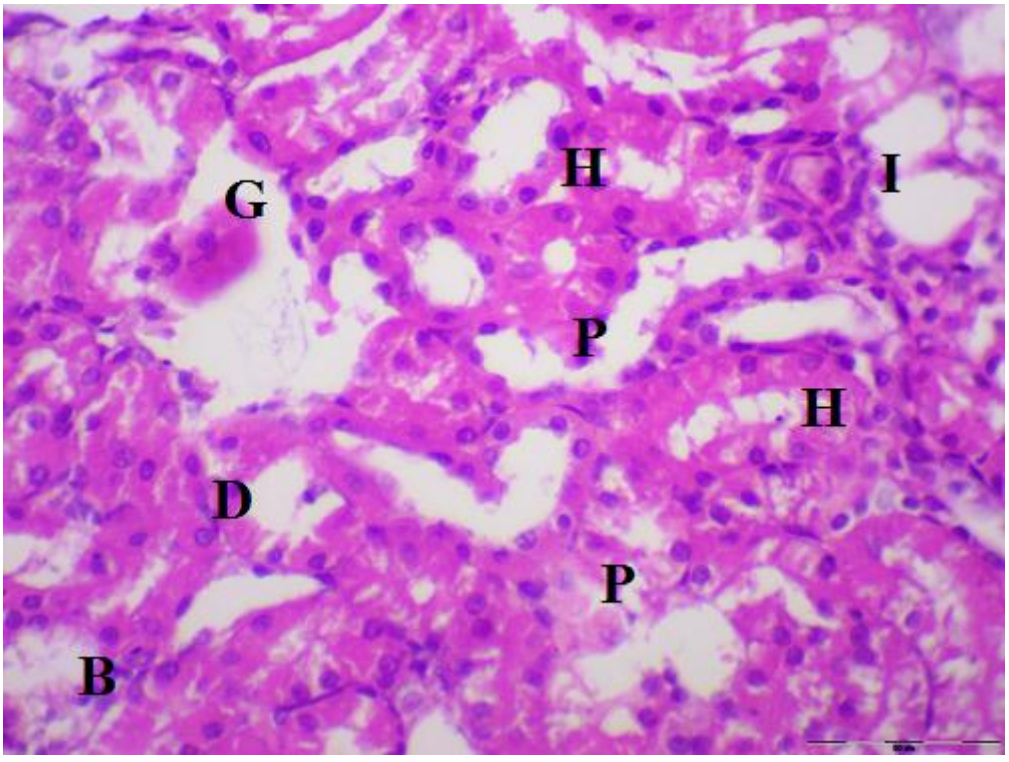
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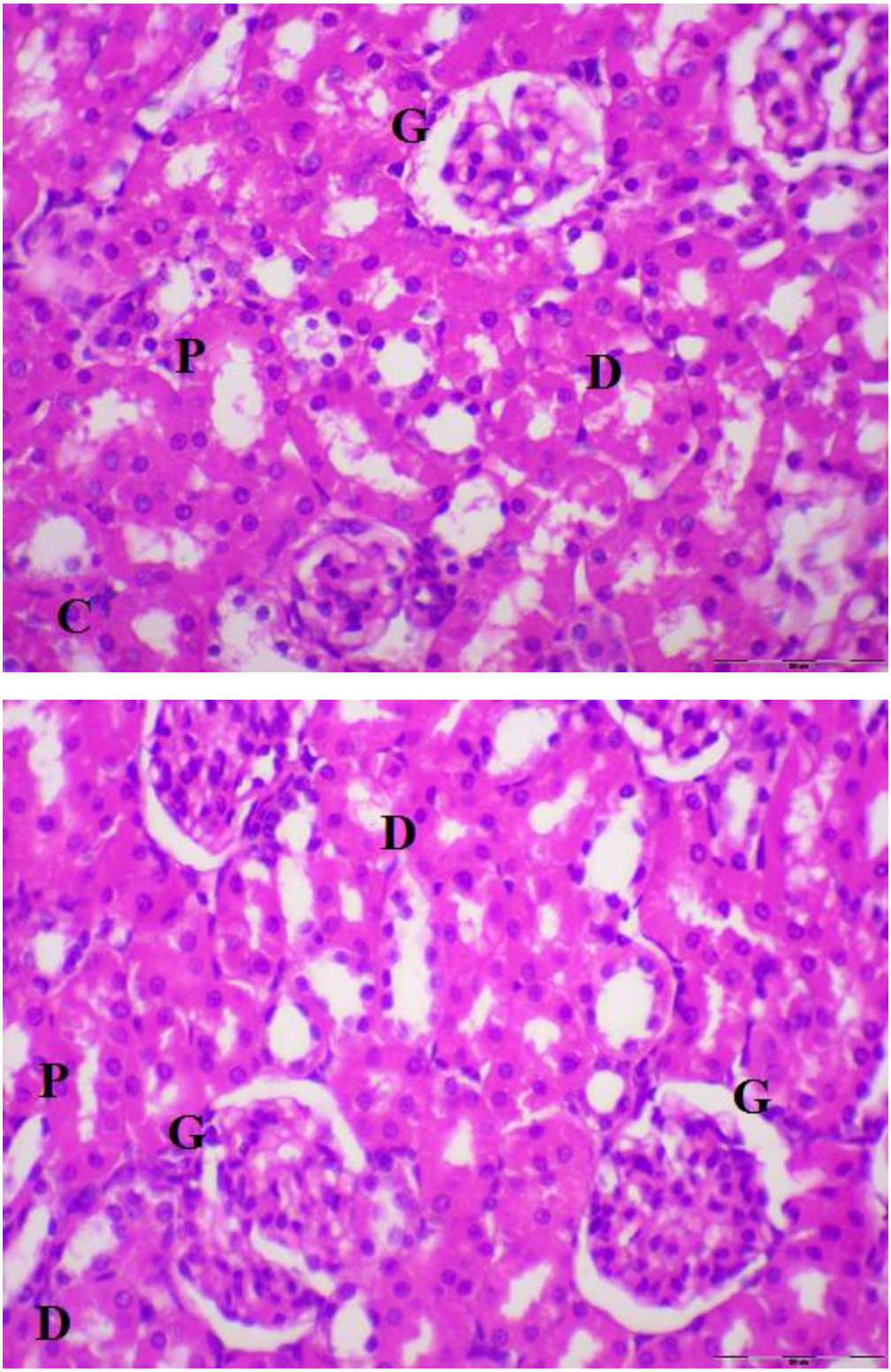

Fig. 2: T.S. in kidney of control and BBR-NPs treated rats (A&D) showed normal renal architecture, normal glomerular tuft (**G**), proximal convoluted tubule (P) and distal convoluted tubules (**D**): T.S. in kidney of cisplatin-treated rats (B) showed atrophied glomerular tuft (**G**) with wide space, necrosis of epithelial cells of proximal (**P**) and distal (**D**) tubules with hyaline casts (**H**) and lymphocytic infiltration (**I**). T.S. in kidney of Cisplatin+BBR-PRs treated rats (C) showed that the histological alterations were markedly reduced (H & E stain, x 400).

Discussion:

Hematopoietic system is considered as sensitive to investigate the adverse effects induced by chemotherapeutic agents. The present study revealed that cisplatin (CDDP) induced blood related toxicity such as myelosuppression, pancytopenia, anemia, thrombocytopenia and/or leucopenia. Thus, treatment with CDDP might induce anemic disorders due to suppression of hematopoietic tissues, disturbances in erythropoiesis and a defective iron metabolism (13, 26). CDDP accumulation in the renal tubular cells, leading to free radical production and lipid peroxidation, which is the main factor causing anemia by diminishing synthesis of erythropoietin due to a reduction of functional renal mass [27]. The abnormal shape of the RBC could be attributed to the interaction of CDDP with phospholipids located in the inner monolayer of the red blood cell membrane [28]. Moreover, it was demonstrated that CDDP induces oxidative stress injury in human thrombocytes and lymphocytes leads to apoptosis by affecting their life span [29]. Kim et al. [30] stated that the CDDP group showed leukopenia in approximately 57% of the normal group; in particular, lymphocyte counts were decreased in half that of the normal group. The present findings clearly showed that the BBR-NPs treatment protected the hematological parameters against CDDP induced hematotoxicity. These results came accordance with Chandirasegaran et al. [31] who reported that the treatment with berberine chloride notably protected the blood components against STZ induced diabetic rats.

Kidneys are the main route of CDDP excretion and proximal tubule cells serve as a primary site of CDDP accumulation [32]. In particular, nephrotoxicity associated with cisplatin remains a major problem [5, 33]. The exposure of tubular cells to cisplatin activates complex signaling pathways that lead to tubular cell injury and death. Meanwhile, a robust inflammatory response is stimulated, further exacerbating renal tissue damage. Cisplatin may also induce injury in renal vasculature and result in decreased blood flow and ischemic injury of the kidneys, contributing to a decline in glomerular filtration rate. These events, together, culminate in the loss of renal function triggering acute renal failure [34]. Single CDDP injection caused marked alterations in serum parameters, as is evident by increased urea, creatinine and uric acid. The present results were consistent with Alhoshani et al. [35] and Song et al. [36] who stated that cisplatin administration to rats induced nephrotoxicity associated with a significant increase in urea and creatinine compared to control group. The elevated serum urea, uric acid and creatinine levels induced by CDDP were significantly restored to their normal levels by the berberine treatment. The protective effect of berberine against nephrotoxicity can be attributed to its antioxidant and anti-inflammatory effects. [37]

Several mechanisms hypothesized to underlie CDDP-induced nephrotoxicity including, oxidative stress resulting in enhancing the production of ROS [38], inflammation and apoptosis pathways [39]. And then, ROS in turn damaged the mitochondria leading to cell death via apoptosis and necrosis inducing nephrotoxicity followed by an increased expression of proinflammatory mediators [40, 41]. CDDP treatment decreases kidney glutathione (GSH) and total thiol contents and increases lipid peroxidation, which is closely associated with the acute kidney injury caused by CDDP [42]. Thus, a combination of effective antioxidant agents may be an appropriate approach to minimiz the toxic side effects of CDDP with preserving its chemotherapeutic efficacy [43, 44]. In the present study, berberine treatment attenuated increases in MDA in kidney tissues and restored renal antioxidants including the levels of GSH and total thiol. The nephroprotective role of berberine could be attributed to its free radicals scavenging and strong antioxidant properties as reported by Abd El-Wahab et al. [45]. Berberine also improved glutathione, glutathione peroxidase and superoxide dismutase levels in diabetic mice [46]. Berberine acts as an antioxidant and free radical scavenger *in vitro* and an inhibitor of lipid peroxidation in diabetic mice livers [47]. BBR treatment improved blood BUN, creatinine and 24 h urinary protein in a streptozotocin-induced diabetic nephropathy rat model [48,49].

A number of inflammatory mediators, including TNF-α, IL-6, IL-1β, TGF-0058, MIP2, MCP1, were identified to be up-regulated in cisplatin-induced nephrotoxicity [50]. In the present study, CDDP increased the expression levels of IL-2, IL-6, IL-1β and TNF-α. in the kidneys of cisplatin-treated rats. Ramesh and Reeves [51] reported that nephrotoxicity caused up-regulation in TNF-α, IL-1β, macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), ICAM-1 and TGF-β. The present results showed that the BBR-

NPs supplementation improved the CDDP-induced increased in the expression levels of IL-2, IL-6, IL-1β and TNF-α that were in agreement with a previous report [52]. Yuna et al. [53] reported that cisplatin-induced cell death in HCT116 a human colorectal cancer cell line, it promoted by apoptosis via MST-1 in a p53-dependent manner. Nho et al. [54] stated that cisplatin-induced up-regulation of caspase-3/MST-1 pathway decreased by treatment of cynaroside in HK-2 cells. Mostafa et al. [55] cisplatin-induced elevation in renal tissue MPO, TNF-α, IL-1β, NF-kB and caspase-3, suggesting cisplatin-induced nephro-inflammation and apoptosis.

Several recent studies demonstrated amelioration of inflammatory response by berberine *in vitro and in vivo* [48] and also in patients with acute coronary syndrome [56]. Lu et al. [57] showed an inhibitory effect of berberine against neuroinflammatory response in BV-2 microglia through the activation of adenosine monophosphate-activated protein kinase signaling pathways. BBR-NPs act as antioxidant and anti-inflammatory and improves renal abnormality induced by several factors or chemotherapeutic agents like doxorubicin or cisplatin [58, 59]. Previously, it was evidenced that berberine suppressed the proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (47). Also, these results were in consistent with Zou et al. [9] who stated that berberine has anti-inflammatory activity as detected by the reduction in the proinflammatory cytokines such as TNF- α, IL-13, IL-6, IL-8 and IFN-γ.

Cisplatin and related platinum compounds bind to DNA to induce crosslinks and adducts, resulting in DNA damage and replication stress [41, 60]. The antiapoptotic activity of berberine has been demonstrated in renal proximal tubule cells in ischemia/reperfusion injured kidneys through the suppression of mitochondrial and endoplasmic reticulum stress [61]. In addition to apoptosis, berberine was also shown to induce autophagic cell death in several cancer cell lines [62].

The kidney damage caused by CDDP was accompanied by corresponding histopathological changes, such as degeneration and desquamation of tubular epithelial cells, hyaline cast formation, inflammatory cell infiltration and tubular dilation. These changes came accordance with Hosseinian et al. [63] who stated that CDDP treatment impairs kidney function and structure. Also, Abdel-Aziz et al. [64] showed that cisplatin caused tubular dilatation, cytoplasmic vaculation, tubular cells degeneration and tubules filled with protein casts. In contrast, the BBR-NPs treatment effectively amileorated the histopathological changes in kidney tissues, indicating that BBR-NPs confers protective effects against CDDP-induced acute kidney injury. Domitrovic´ et al. [47] reported that treatment with berberine dose-dependently reduced changes in the kidney structure.

Conclusion:

It was concluded that while CDDP caused deleterious nephrotoxic effects by causing severe damage to cells by suppressing antioxidant defense mechanism, however, these effects were ameliorated by dietary supplementation with BBR-NPs. Present study thus supports that BBR-NPs may be effective dietary supplementation to maximize the clinical use of CDDP in the treatment of various malignancies without nephrotoxic and other side effects.

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