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### Research Article

## Protective Effect of *Barringtonia racemosa* Ethyl Acetate Fraction against Cisplatin-Induced Nephrotoxicity in Rats

Rachna B. Patel<sup>1</sup>, Umesh B. Mahajan<sup>1</sup>, Sameer N. Goyal<sup>2</sup>, Sanjay J. Surana<sup>3</sup>, Chandragouda R. Patil<sup>1</sup>, Kalpesh R. Patil<sup>1\*</sup>

<sup>1</sup>Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-425405, District Dhule, Maharashtra, India

<sup>2</sup>Shri Vile Parle Kelavani Mandal's Institute of Pharmacy, Dhule-424001, Maharashtra, India

<sup>3</sup>Department of Pharmacognosy, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-425405, District Dhule, Maharashtra, India

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### ABSTRACT

Cisplatin is a major antineoplastic drug for the treatment of solid tumors. Nephrotoxicity is a dose-limiting side effect associated with the clinical use of cisplatin. The present study was executed to determine whether bartogenic acid, containing fraction of *Barringtonia racemosa* fruits (BREAf) possess a nephroprotective effect against cisplatin-induced nephrotoxicity in rats. Furthermore, the study was also aimed to explore the mechanisms underlying this effect of BREAf. The BREAf was orally administered at the doses of (2, 5, and 10 mg/kg) for five consecutive days following single-dose administration of cisplatin (5 mg/kg, i.p.). Treatment of animals with cisplatin resulted in significant body weight changes, oxidative stress, elevated levels of serum biomarkers, and histological alterations in the kidney architecture. The BREAf administration reduced relative body weight and organ weight changes in cisplatin-treated rats. The BREAf exhibited a nephroprotective effect through the significant reduction of the cisplatin-induced rise in the serum creatinine and blood urea nitrogen levels, as well as, renal levels of malondialdehyde (MDA) the makers of lipid peroxidation. Additionally, the treatment with BREAf resulted in increased renal levels of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase activity. Histopathological examination established the nephroprotective effect of BREAf. In conclusion, the anti-oxidant and anti-inflammatory effects of BREAf have an important role underlying its nephroprotective effect.

### INTRODUCTION

Cisplatin is a platinum compound and chemotherapeutic agent effective against wide variety of cancer.<sup>[1,2]</sup> Although higher doses of cisplatin suppress cancer, chemotherapy with cisplatin is associated with various organ toxicities.<sup>[2]</sup> Cisplatin-induced nephrotoxicity is a limiting factor in the clinical use of this potential chemotherapeutic agent.<sup>[3,4]</sup> Drug-induced nephrotoxicity is an extremely common condition that is responsible for a variety of pathological effects on the kidneys. Nephrotoxicity refers to characteristics of structural and functional abnormalities in the renal structures that occur in patients following exposure to the toxicants.<sup>[5]</sup>

Acute renal failure has been reported after a single dose of cisplatin.<sup>[4,6]</sup> Cisplatin accumulates in the proximal and distal convoluted tubules and promotes the kidney damage. Renal impairment is manifested by increased serum creatinine and blood urea nitrogen levels.<sup>[3,7]</sup> Several mechanisms, like DNA damage, oxidative stress, apoptosis, and inflammation are involved in cisplatin-induced nephrotoxicity.<sup>[4,8,9]</sup>

Cisplatin targets the proximal tubule and decreases the glomerular filtration rate and causes subsequent renal failure.<sup>[10]</sup> Anti-oxidants like GSH, SOD, and catalase (CAT) help to scavenge the reactive oxygen species (ROS). Anti-cancer drug, cisplatin produces ROS in kidney epithelial

\*Corresponding Author: Dr. Kalpesh R. Patil

Address: Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education and Research, Karvand Naka, Shirpur-425405, District Dhule, Maharashtra, India

Email ✉: [kalpeshpharma20@gmail.com](mailto:kalpeshpharma20@gmail.com)

Tel.: +91-9850875021

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cells and diminishes anti-oxidant enzyme activity by reducing the intracellular concentrations of GSH.<sup>[10,11]</sup> Thus, depletion of anti-oxidant mechanisms, increased production of ROS, and renal accumulation of lipid peroxide products have been proposed as important mechanisms behind cisplatin-induced nephrotoxicity.<sup>[3,12]</sup>

Several studies have demonstrated the protective role of natural anti-oxidants against cisplatin-induced nephrotoxicity.<sup>[13]</sup> *B. racemosa* is a mangrove plant belongs to the Lecythidaceae family and used in traditional Indian medicine. Various parts of this plant are used in asthma, cough, control of blood pressure, and skin diseases.<sup>[14,15]</sup> *B. racemosa* contains several biologically active phytoconstituents, amongst which bartogenic acid, a natural pentacyclic triterpenoid, is well known for its biological properties, including  $\alpha$ -glucosidase inhibitor, anti-arthritis, and anti-cancer.<sup>[16-18]</sup> It has also been reported that various parts of *B. racemosa* exhibit many pharmacological effects, like anti-oxidant,<sup>[14,15,19]</sup> anti-inflammatory,<sup>[20]</sup> anti-fungal,<sup>[21]</sup> anti-cancer,<sup>[22]</sup> analgesic,<sup>[23]</sup> anti-tuberculosis,<sup>[24]</sup> and anti-diarrhoea activity.<sup>[25]</sup> To our knowledge, there are no reports on the protective role of *B. racemosa* or bartogenic acid against anti-cancer drug-induced nephrotoxicity. Therefore, the present study was undertaken to evaluate the nephroprotective effect of bartogenic acid containing fraction of *B. racemosa* fruits (BREAF) against cisplatin-induced renal toxicity in rats.

## MATERIALS AND METHOD

### Animals

Experimental procedures involving the use of laboratory animals were approved by the Institutional Animal Ethics Committee (IAEC) of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, District Dhule, India (Reg. No. 651/PO/ReBi/S/02/CPCSEA) constituted under the "Prevention of Cruelty to the Animals Act, 1960." All the experiments were carried out according to the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Healthy Sprague Dawley rats of either sex (180–230 grams) were used in the present study. Rats were housed in polypropylene cages under the standard laboratory condition at  $22 \pm 2^\circ\text{C}$ , and 12 hours (light:dark) cycle with free access to water, and commercially available standard pellet feed (Nutrimix Std-1020) obtained from Nutrivet Life Sciences, Pune, India.

### Plant Material

*B. racemosa* Roxb. (Lecythidaceae) fruits, collected from the sea coast of Maharashtra, were purchased from the local vendor. The specimen was authenticated by the taxonomist, and the specimen was deposited for future reference. An authentic marker of bartogenic acid was supplied by

Dr. Mangala Gowri, Senior Scientist, Indian Institute of Chemical Technology, Hyderabad, Telangana, India.

### Extraction and Isolation of BREAF

The BREAF was isolated according to the earlier reported methods with some modification.<sup>[16,20]</sup> Briefly, the dried and powdered fruits of *B. racemosa* were defatted with petroleum ether, followed by cold maceration with methanol at room temperature for 3 days. The methanol extract was subsequently fractionated using ethyl acetate. Ethyl acetate extract was loaded on silica gel column ( $3 \times 90$  cm), using ethyl acetate as mobile phase. Elution was monitored by thin-layer chromatography (TLC) of each fraction using methanol as a solvent. The fractions having similar TLC patterns were combined to get a triterpenoids enriched fraction called BREAF. Part of this fraction (BREAF) was subjected to chromatography on pre-coated reverse-phase silica gel plate (Merck) using acetonitrile:water (85:15 v/v) as eluent. High-performance thin-layer chromatography (HPTLC) of fraction revealed three spots, including one blue spot ( $R_f = 0.68$ ) approaching to bartogenic acid marker. The composition of BREAF was confirmed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis, as described previously.<sup>[20]</sup>

### Chemicals

Cisplatin (CAS Number: 15663-27-1) were purchased from Sigma Aldrich, USA. Creatinine kit, urea/ blood urea nitrogen (BUN) kit, albumin kit, and protein kit were obtained from Accurex Biomedical Pvt. Ltd., Mumbai, Agappe Diagnostics Ltd., Mumbai, Beacon Diagnostic Pvt. Ltd., Navsari, India, and Span Diagnostic Ltd., Surat, India, respectively. 5-5-dithio bis-(2-nitrobenzoic acid) and thiobarbituric acid were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Other chemicals and solvents used in the fractionation and chromatographic separations were of analytical grade.

### Experimental Protocol

Rats were randomly divided into five groups, each consisting of six animals ( $n = 6$ ). Group I (normal): control group of rats received single dose of 1 mL saline i.p. and tween-80 solution p.o. for 5 days. Group II: cisplatin control group of animals was treated with single dose of cisplatin 5 mg/kg i.p. and tween-80 solution p.o. for 5 days. Group III: cisplatin + BREAF (2) group of rats received single dose of cisplatin 5 mg/kg i.p. and BREAF (2 mg/kg p.o.) for 5 days. Group IV: cisplatin + BREAF (5) group of animals received single dose of cisplatin 5 mg/kg i.p. and BREAF (5 mg/kg p.o.) for 5 days. Group V: cisplatin + BREAF (10) group of rats received single dose of cisplatin 5 mg/kg i.p. and BREAF (10 mg/kg p.o.) for 5 days.

The BREAF was dissolved in tween-80, and administered orally at the dosage of 2, 5, and 10 mg/kg



to respective groups for 5 days. On the fourth day, animals were kept in metabolic cages for urine collection. Prior to urine collection, blood sample was collected through retro-orbital puncture, and serum was separated. On the 5th day, animals were sacrificed by an overdose of anesthetics, and the spleen, thymus, and kidney were isolated for biochemical estimation and histopathology study. The dosage of BREAF (2, 5, and 10 mg/kg p.o.) were selected based on our preliminary experiments and other earlier reports estimating no toxicity even at higher dosages.<sup>[20]</sup> The dose of cisplatin (5 mg/kg i.p.) was selected according to earlier studies that demonstrated significant nephrotoxicity in rats.

### Observational Parameter

Body weight of rats was recorded before the administration of cisplatin and at the end of the experiment. Percentage (%) change in body weight was calculated. After scarification of rats on the 5th day, the organs like kidney, spleen, and thymus were harvested, and weighed immediately after the isolation.

### Biochemical Estimations

At the termination of the experiment, blood samples were analyzed for markers of renal function. Creatinine, albumin, alkaline phosphatase (ALP), and BUN levels were estimated using commercially available kits and according to the manufacturer's instructions. Creatinine estimation was done by using alkaline picrate method. Albumin was estimated by using bromocresol green method. Determination of albumin in serum is based on a binding behavior of albumin with 3',3',5',5'-tetrabromom-cresol-sulfonephthalein in acidic medium (pH 4.2). Alkaline phosphates were estimated by p-nitrophenyl phosphate method. Urea and urea nitrogen estimation was done by using urease/ glutamate dehydrogenase method. This method is based on the preliminary hydrolysis of urease, followed by analytical processes that quantities the ammonium ion.<sup>[3,26-28]</sup>

### Oxidative Stress Parameter

#### Preparation of Renal Homogenate

Immediately after the scarification, the kidney from each rat was dissected, rinsed with isotonic saline, and weighed. Kidney tissue was minced, and the homogenate was prepared with 10% (w/v) phosphate-buffered saline (0.1 mol/L, pH 7.4) using a homogenizer. Homogenate was used to estimate MDA and reduced GSH.

#### Determination of Lipid Peroxidation (LPO)

The LPO was measured as MDA content, according to the earlier method.<sup>[29]</sup> Briefly, the assay mixture containing 0.5 mL homogenate and 3 mL of thiobarbituric acid (0.6% w/v) was heated in boiling water bath for 45 minutes. Subsequently, the mixture was cooled

immediately in ice bath, and 4 mL of n-butanol was added to the mixture. It was vortex and centrifuged at 5,000 rpm for 10 minutes. Absorbance of organic layer was recorded at 535 nm, and results were expressed as a percentage of control.

#### Determination of Reduced GSH

Reduced GSH in the kidney tissue was estimated according to the previous method with some modifications.<sup>[30]</sup> The homogenate (0.75 mL) was precipitated with 4% sulfosalicylic acid (0.75 mL). Sample was centrifuged at 1,200 g for 15 minutes at 4°C. The assay mixture consisted of 0.5 mL supernatant and 4.5 mL of 0.001 mol/L of 5-5-dithio bis-(2-nitrobenzoic acid) (in 0.1 mol/L phosphate buffer, pH 8.0). The yellow color developed was read immediately at 412 nm, using a microplate reader (BioTek, USA).<sup>[31]</sup>

#### Determination of SOD

The SOD is estimated by the reported method.<sup>[32]</sup> Briefly, the reaction mixture containing 100 µL of each of the 500 mM/L of Na<sub>2</sub>CO<sub>3</sub>, 1 mM/L of ethylenediaminetetraacetic acid (EDTA), 240 µM/L of nitro blue tetrazolium (NBT), 0.3% trion X-100, 25 µL of 10 mM/L of hydroxylamine and 25 µL of the sample was thoroughly mixed. The absorbance was recorded at 560 nm at 30 seconds and 210 seconds.<sup>[33]</sup>

#### Determination of Catalase Activity

Catalase activity was assayed according to the method of Luck,<sup>[34]</sup> in which the breakdown of hydrogen peroxide is measured at 240 nm. Briefly, the assay mixture consisted of 3 mL of hydrogen peroxide phosphate buffer ( $1.25 \times 10^{-2}$  H<sub>2</sub>O<sub>2</sub> mol) and 0.05 mL supernatant of kidney homogenate (10%). The change in absorbance was recorded at 240 nm using microplate reader. Enzyme activity was calculated using the millimolar coefficient of H<sub>2</sub>O<sub>2</sub>.<sup>[35]</sup>

#### Histopathological Analysis

At the end of the experiment, animals were sacrificed after the 24 hours urine collection. Paraformaldehyde-fixed kidney tissues were dehydrated in ascending graded series of alcohol and embedded in paraffin. Kidney tissue specimens were cut into slices of 5 µm thickness using microtome, followed by staining with hematoxylin and eosin, according to routine staining protocols. The histological sections were examined with a light microscope.<sup>[3,13]</sup>

#### Statistical Analysis

Results are expressed as mean ± SEM, and statistical significance of difference in the central tendencies of treatment groups was determined by one-way ANOVA followed by Dunnett's multiple comparison test.  $p < 0.05$  was considered statistically significant.



## RESULTS

### BREAF contains Bartogenic Acid

The BREAF on HPTLC showed an intense blue spot ( $R_f = 0.68$ ) matching with bartogenic acid marker. LC-ESI-MS analysis showed molecular ion peak at  $m/z$  517.5 [M-H]<sup>-</sup>, corresponding to molecular formula  $C_{30}H_{46}O_7$ , which is in congruence with previously available data of bartogenic acid.<sup>[20]</sup> HPTLC and LC-ESI-MS analysis revealed that the chief constituent of BREAF was bartogenic acid.<sup>[20]</sup>

### BREAF significantly altered the Body and Organ Weights in Treated Animals

Single intraperitoneal injection of cisplatin (5 mg/kg) caused the notable weight loss in the cisplatin-control group as compared with the normal control group on 5th day ( $p < 0.001$ ). However, these changes were significantly ( $p < 0.001$ ) prevented by BREAF at the dosage of 2, 5, and 10 mg/kg, p.o. (Fig. 1A). The relative kidney, spleen, and thymus weights in BREAF (2, 5, and 10 mg/kg, p.o.) treated animals were significantly ( $p < 0.001$ ) reduced as compared with organ weights of animals in cisplatin-control group (Figs 1A to D).

### BREAF demonstrated favorable Effects on Renal Functions

Serum creatinine and BUN levels were measured in all the animals to evaluate the protective effect of BREAF on renal function. Elevated levels of creatinine and BUN indicate acute nephrotoxicity. However, elevated alkaline phosphatase is the hallmark of organ toxicity. Following injection of cisplatin (5 mg/kg i.p.), the serum levels of creatinine, BUN, and alkaline phosphatase were found to increase significantly ( $p < 0.001$ ), as compared

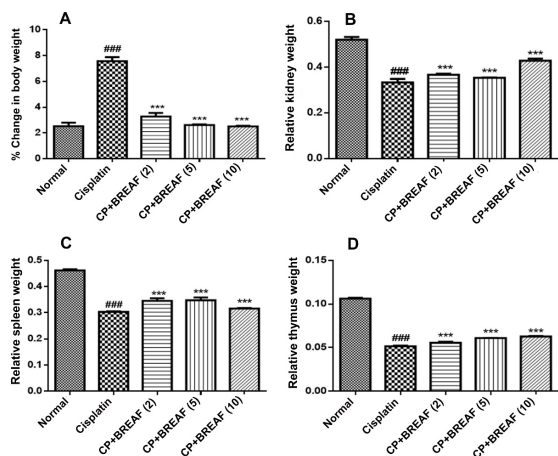
with normal-control group, suggesting severe kidney damage following cisplatin injection (Figs 2A to D). Administration of BREAF (2, 5, and 10 mg/kg p.o.) to animals exerted significant ( $p < 0.001$ ) nephroprotective effect, as evidenced by decreased levels of creatinine and BUN, as compared to cisplatin control group (Figs 2A and B). The four-fold increase in alkaline phosphate was observed in cisplatin-control group, as compared to normal-control group. In the present study, BREAF showed significant ( $p < 0.001$ ) and dose-dependent decrease in serum alkaline phosphate levels and non dose-dependent increase in albumin level as compared to cisplatin-control group (Figs 2C and D).

### BREAF diminished Cisplatin-Induced Oxidative Stress

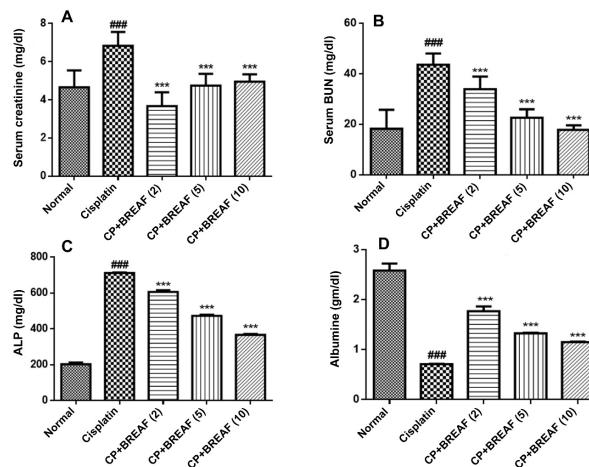
Single-dose administration of cisplatin resulted in the notable increase of MDA content along with significant ( $p < 0.001$ ) decrease in GHS, SOD, and catalase content as compared with normal-control group (Figs 3A to D). Alternatively, treatment of animals with BREAF at the dosage of 2, 5, and 10 mg/kg p.o. for 5 days resulted in significant ( $p < 0.001$ ) increase in GHS, SOD, and catalase level along with significant ( $p < 0.001$ ) decrease in MDA level. The effect of BREAF on SOD level was in dose-dependent manner; however, the effect of BREAF on GHS, catalase, and MDA was found to be non-dose dependent (Figs 3A to D).

### BREAF attenuated Cisplatin-Induced Renal Histopathological Changes in Rats

Histopathological study of kidney tissues in normal rats demonstrated the normal appearance of renal tubules. The kidney tissues in cisplatin exposed group showed necrosis

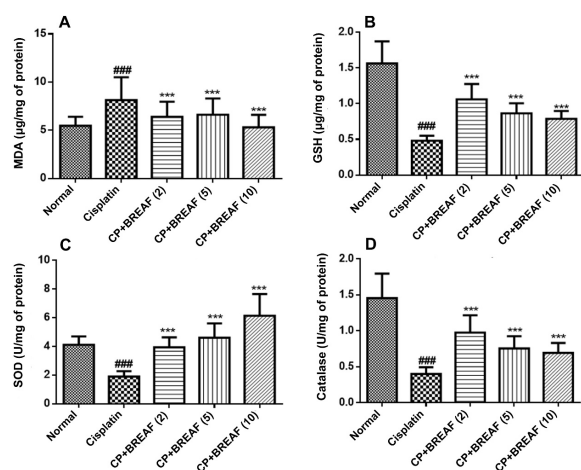


**Fig. 1:** Effect of BREAF on body weight changes and relative organ weight in cisplatin-induced nephrotoxicity in rats; **A)** % change in body weight; **B)** Relative kidney weight; **C)** Relative spleen weight; **D)** Relative thymus weight; values represent mean  $\pm$  SEM ( $n = 6$ ) animals per group; ### $p < 0.001$  as compared with normal group; \*\*\* $p < 0.001$  as compared with control group (one-way ANOVA followed by Dunnett's multiple comparison post hoc test)



**Fig. 2:** Effect of BREAF on biomarkers of kidney function in cisplatin-induced nephrotoxicity in rats; **A)** Serum creatinine (mg/dL); **B)** Serum BUN (mg/dL); **C)** ALP (mg/dL); **D)** Albumin (gm/dL); values represent mean  $\pm$  SEM ( $n = 6$ ) animals per group; ### $p < 0.001$  as compared with normal group; \*\*\* $p < 0.001$  as compared with control group (one-way ANOVA followed by Dunnett's multiple comparison post hoc test)



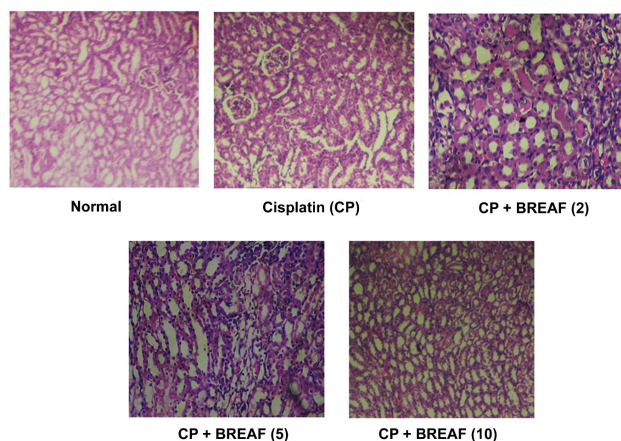


**Fig. 3:** Effect of BREAF on oxidative stress parameters in cisplatin-induced nephrotoxicity in rats; **A)** MDA ( $\mu\text{g}/\text{mg}$  of protein); **B)** GSH ( $\mu\text{g}/\text{mg}$  of protein); **C)** SOD ( $\text{U}/\text{mg}$  of protein); **D)** Catalase ( $\text{U}/\text{mg}$  of protein); values represent mean  $\pm$  SEM ( $n = 6$ ) animals per group; ###  $p < 0.001$  as compared with normal group; \*\*\*  $p < 0.001$  as compared with control group (one-way ANOVA followed by Dunnett's multiple comparison post hoc test)

of renal tubules, cast formation, interstitial leukocyte infiltration, and congestion of inter-tubular blood vessels indicating the kidney damage (Fig. 4). Group treated with low and middle (2 and 5 mg/kg p.o.) dose of BREAF showed evidence for the necrosis of renal tubules, presence of cast, and leucocyte infiltration. But, the group treated with high dose of BREAF (10 mg/kg p.o.) normalized the cisplatin-induced histopathological changes, and decreased the infiltration of inflammatory cells. These observations reinforce the earlier results of the anti-inflammatory action of BREAF.<sup>[20]</sup>

## DISCUSSION

Cisplatin is effective chemotherapeutic agent used for the treatment of several cancers. Despite its anti-cancer potential, the use of cisplatin is associated with severe side effects, especially nephrotoxicity.<sup>[3,29]</sup> There is a lack of drugs that can offer protection to the kidneys against cisplatin-induced toxicity, and search for the nephroprotective drugs is continuing. Several studies have been conducted to enhance our understanding of the pathogenesis and treatment of cisplatin-induced nephrotoxicity.<sup>[36]</sup> Pathophysiology of cisplatin-induced tubular damage is complex, which shows the involvement of many interconnected factors. The mechanisms of cisplatin-induced nephrotoxicity may involve accumulation of cisplatin in renal tubules, conversion of cisplatin into nephrotoxins, mitochondrial dysfunction, and DNA damage.<sup>[3,12]</sup> Additional mechanisms include, oxidative stress, inflammation, and fibrogenesis along with activation of the apoptotic pathway through the activation of cell cycle arrest, disturbance of renal tubular cell transport systems, and alterations in ATPase activity.<sup>[37-39]</sup>



**Fig. 4:** Effect of BREAF on histopathological changes in kidney tissues in cisplatin-induced nephrotoxicity in rats; Kidney tissue specimens were cut into slices of 5  $\mu\text{m}$  thickness, followed by staining with hematoxylin and eosin, according to routine staining protocols; Microscopic examination was carried out under light microscopy

The various parts of *B. racemosa* are reported to possess biological activities, like anti-bacterial, anti-fungal, anti-oxidant, analgesic, anti-inflammatory, anti-arthritis, and anti-diarrhoeal activity.<sup>[17,21,23,25,40,41]</sup> The present study was conducted to evaluate the protective effect of BREAF in cisplatin-induced nephrotoxicity in rats. We observed that BREAF decreased cisplatin-induced nephrotoxicity through the minimization of renal tubular damage, body weight changes, oxidative stress, and inflammation. Intraperitoneal administration of cisplatin resulted in augmented oxidative stress through increase in MDA and decrease in anti-oxidant enzymes, as well as, increased creatinine and BUN levels, suggesting the induction of nephrotoxicity in cisplatin exposed rats. Results demonstrated that oral treatment of animals with BREAF at the dosage of 2, 5, and 10 mg/kg had nephroprotective effect as evident through observational, biochemical, and histopathological investigations in cisplatin and BREAF treated animals.

In the present study, the rats treated with cisplatin showed a decrease in total body weight along with a decrease in relative kidney, thymus, and spleen weights. The oral treatment of rats with BREAF (2, 5, and 10 mg/kg) showed significant recovery from cisplatin-induced total body weight and organ weight loss. The cisplatin-induced decrease in weight loss could be due to reduced food intake by the animals and gastrointestinal toxicity.<sup>[42,43]</sup> Creatinine and urea are the major biomarkers of nephrotoxicity, and their level significantly increases in nephrotoxicity. Increase in serum creatinine and BUN levels in cisplatin-treated rats, which occurred due to glomerular and renal tubal damage, is well established through several studies.<sup>[13,26,44,45]</sup> Serum creatinine is easily measurable and important indicator of renal function. Creatinine is mainly filtered through the kidneys and any injury to kidney that leads to an increase in serum creatinine

level.<sup>[10]</sup> Administration of cisplatin at single-dose causes acute renal failure.<sup>[10]</sup> The peak creatinine level following cisplatin administration is reported on the 5th day of cisplatin injection. Increased serum creatinine represents irreversible renal tubular injury, and it is the indirect measure of glomerular filtration rate.<sup>[10,46]</sup> Serum urea level, usually referred to as BUN, is also used to determine renal function. Ammonia formed during the protein metabolism is transformed into urea, which is excreted through the kidney. Renal dysfunction leads to decrease in the elimination of urea that causes accumulation of urea in the bloodstream.<sup>[10,46]</sup> BREAF administration offered protection through the improvement of renal function as revealed by a notable decrease in serum creatinine and BUN in comparison with the cisplatin-control group. ALP is an important biomarker of kidney damage. The ALP level is increase in pathological conditions, especially liver and kidney diseases. In the present study, treatment of animals with BREAF resulted in a decreased level of ALP.

Oxidative stress is a major factor contributing to acute renal failure.<sup>[47]</sup> Usually, the endogenous anti-oxidants, like SOD, GSH, and catalase detoxifies the ROS produced during the course of normal cellular processes. However, the administration of cisplatin causes excessive accumulation of ROS, leading to increased lipid peroxidation and endogenous anti-oxidant depletion.<sup>[48,49]</sup> Increased MDA level following cisplatin administration indicate the enhanced LPO due to deleterious effect of ROS on cell membrane lipids. Excessive accumulation of MDA in kidney tissues leads to overutilization and subsequent depletion of anti-oxidant enzymes, including GSH.<sup>[50,51]</sup> The notable decrease in the activity of anti-oxidant enzymes in the present study may occur due to increased activity of NADPH oxidase and xanthine oxidase, which causes subsequent depletion of anti-oxidant enzymes (catalase and SOD), GSH, and enhancement of MDA in kidney tissues.<sup>[48,52]</sup>

GSH is essential anti-oxidant tripeptide for the maintenance of cellular integrity and cell metabolism.<sup>[53]</sup> Oxidative stress caused by cisplatin treatment in rats has been reported to deplete the GSH levels in kidney tissues.<sup>[54,55]</sup> A decrease in GSH levels following treatment of animals with cisplatin can be attributed to its utilization in the detoxification of toxicants and regulation of cellular homeostasis.<sup>[56]</sup> GSH offers protection against potential nephrotoxicity through the formation of GSH adducts.<sup>[57]</sup> ROS scavenging by GSH may involve various enzymatic and non-enzymatic mechanisms. Lipid peroxidation after cisplatin administration is attributed to the depletion of GSH and impaired anti-oxidant enzyme activity. Treatment of animals with BREAF (2, 5, and 10 mg/kg p.o.) resulted in an increased level of GSH as compared with cisplatin-control group. This effect of BREAF on GSH levels may involve its direct anti-oxidant effects, increased biosynthesis of GSH, or enhanced levels of other anti-oxidants.<sup>[43,58]</sup>

Superoxide is highly reactive, and cytotoxic ROS produced during oxygen metabolism. The SOD has an important role in the conversion of toxic superoxide into the less toxic hydrogen peroxide. SOD is the first line of defense against ROS-mediated tissue injuries.<sup>[47]</sup> In the present study, level of SOD was decreased in cisplatin-control group, as compared to the normal-control group, which may occur due to exhaustion of zinc and copper that are essential for the activity of anti-oxidant enzymes.<sup>[10,59]</sup> Whereas, the treatment of animals with BREAF at 2, 5, and 10 mg/kg p.o., resulted in an increased level of SOD in renal tissues. Catalase is an enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen.<sup>[60]</sup> Catalase offers protection against several ROS-mediated toxicities, including cisplatin-induced nephrotoxicity.<sup>[10,61]</sup> Treatment of animals with BREAF at all the tested dosages showed significant increase in catalase level. It signifies the anti-oxidant activity of BREAF during cisplatin-induced kidney toxicity. Overall results of this study demonstrate that BREAF caused a decrease in oxidative stress and an increase in anti-oxidant enzymes. These results of BREAF are in agreement with previous studies reporting the anti-oxidant potential of *B. racemosa*.<sup>[14,15,19]</sup>

Histopathological observation also showed that BREAF decreased the tissue necrosis, interstitial edema, and inflammatory cell infiltration caused by cisplatin. This study substantiates that intraperitoneal injection of cisplatin at the dose of 5 mg/kg in single-dose induced severe nephrotoxicity in rats. BREAF ameliorated cisplatin-induced nephrotoxicity at the minute dosage of 2, 5, and 10 mg/kg p.o. The nephroprotective effect of BREAF involves the anti-oxidant mechanisms, and this activity of BREAF is attributed to its bartogenic acid content.

In conclusion, bartogenic acid is a pentacyclic triterpenoid present in the fruits of *B. racemosa*. It was reported to possess the anti-cancer activity in DMBA-croton oil-induced two-stage carcinogenesis model in mice. The present study highlights the nephroprotective activity of BREAF against cisplatin-induced nephrotoxicity in rats. The anti-cancer and nephroprotective activities of BREAF can facilitate the activities of various chemotherapeutic drugs and offer protection against cisplatin-induced nephrotoxicity. Therefore, BREAF could be a suitable adjuvant to anti-cancer chemotherapy.

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