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## Effects of butein against cisplatin-induced nephrotoxicity and oxidative stress in mice

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### Abstract

Nephrotoxicity is one of the life-threatening side effects of cisplatin when used in the treatment of a wide variety of both pediatric and adult malignancies. Accumulating evidence suggests that cisplatin treatment induces oxidative stress and reduces antioxidant status leading to apoptosis of renal cells. The purpose of this study was aimed to explore the effect of butein, a potent antioxidant, against cisplatin induced nephrotoxicity in mice. Single intraperitoneal administration of dose of cisplatin (20 mg/kg) caused marked renal damage, characterized by a significant increase in serum creatinine and blood urea nitrogen (BUN). Cisplatin also increased relative weight of kidney with higher kidney lipid peroxidation levels, and lowered kidney superoxide dismutase (SOD) and catalase (CAT) activities. Histological examinations revealed renal tubular degeneration, extensive epithelial vacuolization and a large number of infiltrated inflammatory cells in cisplatin alone treated mice. The results showed that treatment with butein attenuated cisplatin induced kidney injury and significantly inhibited serum creatinine and BUN levels and restored antioxidant enzyme activities in kidney tissue. Histological studies exhibited that butein treatment reduced tubular degeneration, and epithelial vacuolization with reduce infiltrated inflammatory cells in kidney. The present study demonstrates that butein can effectively suppress cisplatin induced nephrotoxicity by inhibiting oxidative stress.

**Keywords:** Nephrotoxicity; Cisplatin; Oxidative stress; Butein; Antioxidant

### 1. Introduction

Cis-Diamminedichloroplatinum (cisplatin) is a potent chemotherapeutic agent and is widely used as the frontline therapy for the treatment of tumors of head, neck, lungs, and genitourinary tract [1]. Although effective, cisplatin is associated with many adverse drug reactions, such as renal damage, gastrointestinal dysfunction, auditory toxicity, and peripheral nerve toxicity [2]. Nephrotoxicity, in particular, is a major complication and a dose-limiting factor for cisplatin therapy [3]. Administration of cisplatin is frequently associated with renal insufficiency and tubular dysfunction. The possible involvement of peroxidative damage caused by a reactive oxygen species (ROS) has been suggested in the pathogenesis of cisplatin-induced renal failure [4]. In particular, the hydroxyl radical is highly reactive among oxygen radicals. Several antioxidants and oxygen radical scavengers have been reported to be effective in protection against these injuries.

Ayurveda is one of the oldest medical systems in the world, providing innumerable leads to find active and therapeutically useful compounds for drug development from plants. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including diabetes, cancer, malaria, cardiovascular diseases, neurological disorders, and kidney disorders. *Butea monosperma* (Lam.) Taub belong to family Fabaceae, widely distributed in India and in all Asian hemispheres. *Butea monosperma* has been traditionally used for the various disease conditions in Asia for centuries [5]. It has been used for the treatment of different ailments such as cancer, diabetes, diarrhoea, dysentery, fever and jaundice. Recent *in vivo* and *in vitro* studies have indicates its anti-diabetic, anti-cancer, anti-inflammatory, anti-asthmatic, anti-oxidant, anti-convulsant, anti-microbial, anti-viral and hepatoprotective properties [6]. We have previously reported that the n-butanolic fraction of flowers of *Butea monosperma* (Lam.) Taub have protective role against doxorubicin induced nephrotoxicity [6]. Butein, butrin and isobutrin are main active constituents in -butanolic fraction of flowers of *Butea monosperma* (Lam.) Taub [7]. Butein (2', 3, 4, 4'-tetrahydroxychalcone) have potent antioxidant and anti-inflammatory activity [8-10]. In the present investigation, the effect of butein was assessed for its probable nephroprotective potential in cisplatin induced nephrotoxicity in mice.

## 2. Materials and Methods

### 2.1 Chemicals

5,5-dithio-dinitro bisbenzoic acids (DTNB), thiobarbituric acid were purchased from S.D. Fine Chem. Ltd, India. Cisplatin was obtained from Fresenius Kabi Oncology Ltd. All other chemicals and reagents were purchased from Sigma- Aldrich chemicals, USA, unless stated otherwise.

### 2.2 Animals

Swiss albino female mice (20-25 g) were obtained from the animal house of Bombay College of Pharmacy, Kalina, Santacruz (East), Mumbai. Animals were housed in standard polypropylene cages with wire mesh top and maintained at  $23 \pm 2$  °C and relative humidity  $60 \pm 5\%$  with 12-h light-dark cycle. Animals were fed with commercially available standard rodent pellet diet (Amrut mice feed manufactured by Nav Maharashtra Chakan Oil Mill Ltd). The animal feed contains crude protein, crude oil, crude fiber, ash and sand silica. Water supplied by Municipal Corporation of Greater Mumbai was provided to the animals *ad libitum*. For all animal experimentation protocols prior approval was obtained from 'Institutional Animal Ethics Committee', of Bombay College of Pharmacy, Mumbai and all studies were performed in accordance with 'Committee for the Purpose of Control and Supervision on Experiments on Animals' (CPCSEA) guidelines.

### 2.3 Experimental protocol

To evaluate the protective effect of the butein, mice were randomly divided into four experimental groups (n=6) as follows: Control group (no treatment), cisplatin group, and pre-treatment with butein at doses of 25–50 mg/kg orally for three consecutive days. One hour after the last dose of butein, cisplatin (20 mg/kg) was injected intraperitoneally. Seventy two hours after cisplatin injection, mice were sacrificed and kidneys were quickly removed for biochemical and enzymatic estimation. Blood was collected, and the serum was extracted after centrifugation and stored at -80 °C until analysis.

### 2.4 Assessment of renal function

Blood samples were centrifuged at 6000 g for 10 min and sera were collected. Serum creatinine and Blood Urea Nitrogen (BUN) were estimated using commercially available diagnostic kit (Erba Diagnostic Mannheim GmbH). In addition, relative weight of kidneys (kidney to body weight ratio) was also assessed to evaluate the renal injury.

### 2.5 Determination of lipid peroxidation

Lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) according to the method of [11]. This method is based on the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) to form TBARS, which is measured spectrophotometrically at 532 nm. Briefly, the reaction mixture contained 0.2 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid in 0.27M hydrochloric acid (pH 3.5), and 1.5 mL of 0.8% aqueous solution of TBA. The mixture was finally made up to 4.0 mL with ultrapure water, and heated at 95 °C for 1 hr. After cooling with tap water, 1.0 mL of ultrapure water and 5.0 mL of the mixture of n-butanol and pyridine (15: 1, v/v) were added, and the mixture was shaken vigorously. After centrifugation at 5000 rpm for 10 min, the absorbance of the organic layer (upper layer) was measured at 532 nm to measure the TBARS. The amount of MDA was calculated using molar extinction coefficient  $1.56 \times 10^5 \text{ M}^{-1} \text{ Cm}^{-1}$  and

reported as nmoles of MDA/mg protein

### 2.6 Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured spectrophotometrically as described previously by [12]. In brief, the reaction mixture contained 50  $\mu\text{L}$  of the kidney homogenate; 1.5 mL of 0.05 M carbonate buffer (pH 10.2) and 0.5 mL of 0.1 mM EDTA solution. The reaction was then initiated by adding 0.5 mL of 0.3 mM epinephrine in order to obtain a rate of increase in absorbance of per minute at 480 nm. One unit of SOD is defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50%.

### 2.7 Determination of catalase activity

Catalase (CAT) activity was assessed based on the ability of the enzyme to break down hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) as described previously by [13, 14]. In brief, one mL of  $\text{H}_2\text{O}_2$  (30 mM) diluted with phosphate buffer (50 mM, pH 7.0) was added in 2 mL of diluted supernatant to initiate the reaction. The blank was prepared by mixing 2 mL supernatant with 1 mL phosphate buffer (50 mM, pH 7.0). The decrease in the absorbance was read at 240 nm for 3 min. The molar extinction coefficient of  $\text{H}_2\text{O}_2$ ,  $43.6 \text{ M}^{-1} \text{ cm}^{-1}$  was used to determine the catalase activity. One unit of activity is equal to 1 mmol of  $\text{H}_2\text{O}_2$  degraded per minute and is expressed as U/mg protein.

### 2.8 Histological analysis of kidney

Kidney tissue was fixed in 10% buffered formalin for 48 h and embedded in paraffin. The samples were cut on a microtome into  $4\mu\text{m}$  sections and stained with hematoxylin-eosin (HE) for histological examination. Stained sections were examined at  $400\times$  magnification by microscope (Motic® Research Microscope BA410 Elite, China) and examined for tubular degeneration, epithelial vacuolization and necrosis and infiltration of inflammatory cells

### 2.9 Statistical analysis

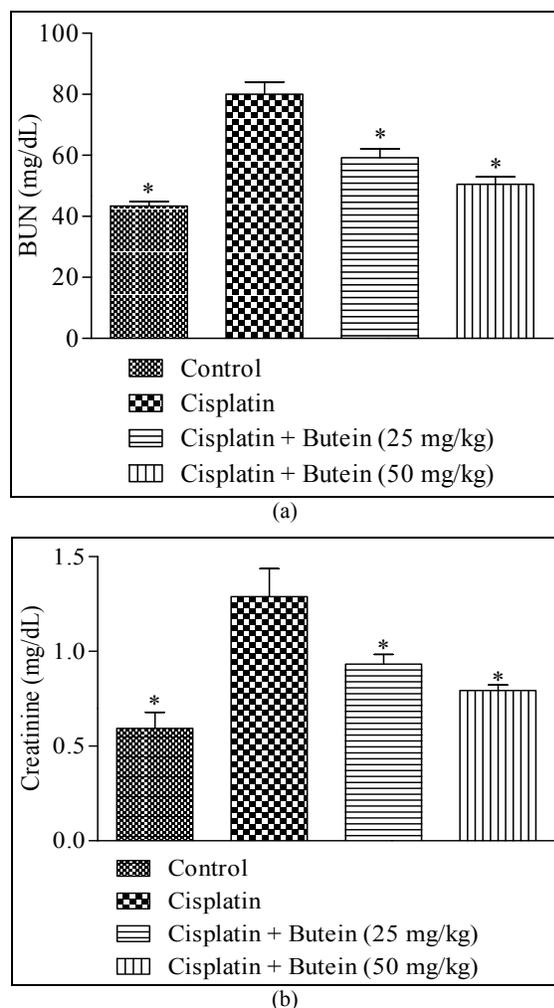
The experimental data were analyzed by one-way ANOVA and expressed as mean  $\pm$  SEM followed by Bonferroni Multiple Comparisons employing Graph Pad Prism 5 software. The significance was considered when  $p < 0.05$ .

## 3. Results and discussion

### 3.1 Butein treatment reduced nephrotoxicity markers

Chemotherapeutic agent like cisplatin continues to be an exceptional treatment against numerous types of cancers, despite of its side effects like nephrotoxicity. Various previous studies have identified the number of drugs or chemicals such as curcumin, resveratrol, melatonin, vitamin E and N-acetyl-cysteine have been used to prevent cisplatin-induced renal injury [15-17]. In this study, we have evaluated whether the nephrotoxic effects of cisplatin could be prevented or ameliorated by treatment with butein. As shown in Fig 1, the cisplatin alone treated animals confirmed a marked deterioration of their renal function 72h after the cisplatin injection. The serum levels of nephrotoxicity biomarkers, such as BUN and creatinine were significantly increased in cisplatin treated animals when compared with the vehicle control animals indicating the induction of severe nephrotoxicity. Nephrotoxic action of cisplatin is also considered to be via drug-induced reactive oxygen species (ROS) generation [18]. Treatment with butein at both the doses (25 and 50 mg/kg) followed by cisplatin significantly attenuated the elevation of BUN and serum creatinine levels.

Here, we provide evidence that treatment of butein with cisplatin decreases BUN and creatinine level, indicating improved renal function up to certain extent.



**Fig 1:** Effect of butein on a) BUN and b) creatinine levels in cisplatin treated mice

**Table 1:** Effect of butein on body weight, kidney weight and antioxidant status in kidney

Treatment	Control	Cisplatin	Cisplatin + Butein (25 mg/kg)	Cisplatin + Butein (50 mg/kg)
Body weight (g)	24.5 ± 2.58*	19.7 ± 1.98	21.8 ± 2.32*	23.1 ± 1.79*
Kidney weight (g)	0.34 ± 0.012*	0.47 ± 0.089	0.38 ± 0.017*	0.35 ± 0.014*
SOD (U/mg protein)	0.751 ± 0.22*	0.216 ± 0.11	0.627 ± 0.12*	0.674 ± 0.15*
CAT (U/mg protein)	26.78 ± 5.41*	5.93 ± 2.62	17.6 ± 3.74*	20.3 ± 4.81*
TBARS (nmole MDA)	5.28 ± 1.16*	19.2 ± 3.89	10.04 ± 2.81*	7.98 ± 1.61*

**Note:** Data are mean ± SEM (n = 6 for each group).

Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test. \*:  $p < 0.05$  when compared with Cisplatin alone treated mice. SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid reactive substances

### 3.4 Butein protects kidney morphology

As shown in Fig. 2, vehicle control treated mice showed apparently normal kidney architecture. However, the

Note: Data are mean ± SEM (n = 6 for each group). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test. \*:  $p < 0.05$  when compared with Cisplatin alone treated mice.

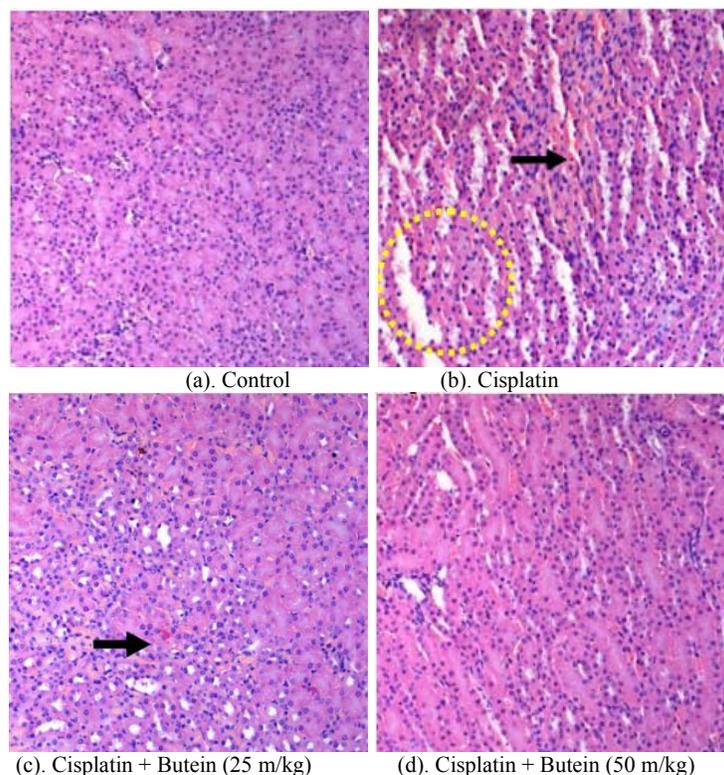
### 3.2 Butein treatment improve body weight and kidney weight

*In vivo*, animals treated with cisplatin showed significant decrease in body weight and increase in the relative weight of the kidneys. The reduction in body weight following cisplatin treatment may probably be attributed to gastrointestinal toxicity and subsequent decrease in appetite [19]. Moreover, the increase in relative kidney weight might be due to the edema of renal parenchyma since cisplatin could cause renal inflammation [19]. As shown in Table 1, the relative weight of kidneys was significantly increased and the body weight was significantly decreased in cisplatin alone treated group when compared with the vehicle control group. On other hand, treatment with butein prevented the increase in relative weight of kidneys and the body weight loss compared with the cisplatin alone treated mice.

### 3.3 Butein ameliorates kidney antioxidant status

Increased production of ROS and subsequent disturbance in antioxidant defence in kidneys has been extensively reported in cisplatin-induced animals. Various antioxidants and free radical scavengers including vitamin C, vitamin E, and flavonoids, tannins have been reported to protect renal tissues against cisplatin-induced nephrotoxicity [16, 20]. A number of experiments have suggested that renal antioxidant enzyme activities are decreased and MDA level is increased after cisplatin injection in rodents [21]. In our study, we reported that the activities of antioxidant enzymes (CAT, SOD) were significantly decreased and MDA levels increased in cisplatin treated mice, suggesting an increased oxidative stress. Treatment with the butein significantly elevated the SOD and CAT levels and decreased MDA level compared to cisplatin alone treated mice (Table 1). These data are in agreement with previous studies, which showed that antioxidants had protective activity against cisplatin-induced nephrotoxicity [22, 23].

histopathological changes of the kidney of cisplatin alone treated mice showed marked pathological lesions characterized by severe grade renal tubular degeneration, extensive epithelial vacuolization and necrosis and a large number of infiltrated inflammatory cells. However, pre-treatment with butein followed by cisplatin treatment markedly attenuated the histopathological changes and prevented the infiltration of inflammatory cells.



**Fig 2:** Effects of butein administration on renal tissue. Representative hematoxylin and eosin

(H&E) staining of kidney sections from the a) control, b) cisplatin alone, c) cisplatin + butein (25 mg/kg) and cisplatin + butein (50 mg/kg). Cisplatin alone treated mice kidneys were characterized by the presence of extensive vacuolization and necrosis (yellow colour outlined) and a large number of infiltrated inflammatory cells (black arrow)

#### 4. Conclusions

The mechanism by which butein has exerted its potential nephroprotective action is not certain. The nephroprotective effect of butein could be due to the reduction of oxidative stress and restoration of antioxidant enzyme activities. Taken together, butein could be a promising candidate for clinical use in patients undergoing treatment with cisplatin to ameliorate the nephrotoxicity.

#### 5. Acknowledgements

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