Available online on 15.04.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Prophylactic Effect of *Butea monosperma* against Cisplatin-Induced Nephrotoxicity in Rats

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ABSTRACT

Cisplatin is an effective chemotherapeutic agent for solid tumors; however its use is limited by nephrotoxicity. The current study investigated the effect of *Butea monosperma* in cisplatin-induced nephrotoxicity and oxidative stress in rats. Kidneys have a vital role in the normal physiology of humans. Worldwide chronic kidney disease has become a major cause for disability and in worst circumstances leads to death. Major renal disorders occur due to diabetes and its complications termed as diabetic nephropathy (DN). The animals were divided into four groups of six rats each (n=6). The control group (Group 1) received 1%CMC, Group 2 received only cisplatin, a 7 days of extract of *Butea monosperma* (200 mg/kg bwt) pre-treatment was applied to the animals in Group 3 before administration of cisplatin; a 7 days of extract of *Butea monosperma* was performed following administration of cisplatin for the animals in Group 4. Cisplatin (6 mg/kg) was intraperitoneally injected as a single dose and extract of *Butea monosperma* (200 and 400 mg/kg bwt) was administered by gavage in 1%CMC. Biochemical and histopathological methods were utilized for evaluation of the nephrotoxicity. The concentrations of creatinine, uric acid and blood urine nitrogen, in plasma and levels of malondialdehyde and reduced glutathione as well as glutathione peroxidase and catalase activities were determined in kidney tissue. Administration of cisplatin to rats induced a marked renal failure, characterized with a significant increase in plasma creatinine and uric acid and Blood urea nitrogen. levels of rats received cisplatin alone were significantly different compared to control group but they had higher kidney malondialdehyde, and lower reduce glutathione concentrations, superoxide dismutase and catalase activities. Extract of *Butea monosperma* administration produced amelioration in biochemical indices of nephrotoxicity in both serum and kidney tissues when compared to toxic inducer group; pre-treatment with extract of *Butea monosperma* being more effective. Results from this study indicated that the novel natural antioxidant extract of *Butea monosperma* might have protective effect against cisplatin-induced nephrotoxicity and oxidative stress in rat.

Keywords: *Butea monosperma,* Cisplatin, Kidneys, Nephrotoxicity, Malondialdehyde

Article Info: Received 12 Feb 2019; Review Completed 20 March 2019; Accepted 22 March 2019; Available online 15 April 2019

Cite this article as:

Bajaj J, Jain S, Sharma S, Prophylactic Effect of *Butea monosperma* against Cisplatin-Induced Nephrotoxicity in Rats, Journal of Drug Delivery and Therapeutics. 2019; 9(2-s):62-66 **http://dx.doi.org/10.22270/jddt.v9i2-s.2456**

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INTRODUCTION

The important role of kidneys in normal physiology comprises plasma filtration of metabolic waste products, regulation of plasma volume, hormone secretion and acid-base balance. Any changes in the above indicators lead to a large number of diverse, life threatening renal diseases. Globally, the 12th cause of death in humans is due to chronic kidney disease (CKD) and leads to 17th cause of disability. People with CKD are more prone to cardiovascular disorders (CVD) rather than to reach end-stage renal disease (ESRD) [1]. Around 30% of diabetes mellitus patients (DM) fall ill with diabetic nephropathy (DN) and CKD incidence. According to the Diabetes Atlas 2006 (India), patient's population with DM is presumed to rise to 69.9 million by 2025 in the absence of preventive measures[2]. From the classical times the nature stands a golden mark and provided the armory of remedies to cure all ailments of mankind. Herbs have always been the predominant form of medicine in India and currently they are becoming popular globally. India has an age old system of medicine known as Ayurveda system along with some other traditional system such as Siddha and Unani. There is rampant change in the international interest of herbal medicines[3]. Medicinal plants play a major role in research and new drug development in the field of pharmaceutical science. Cisplatin is a standout amongst the most broadly utilized anticancer medications for the treatment of different tumors[4]. However, in spite of its wide therapeutic benefits as an anticancer drug, its clinical use is limited due to its dose dependent hepatonephrotoxicity[5]. Although intensive prophylactic measures, irremediable renal and hepatic damage occurs within days in nearly one-third of cisplatin-treated patients[6,7]. There are many evidences that cisplatin induces oxidative stress which plays a critical role in liver and kidney diseases[8,9]. Oxidative stress was attributed to the combination of multi-ways, such as the generation of reactive oxygen species (ROS), which could interact with the antioxidant defense system and cause oxidative damage in different tissues and reaction with glutathione and thiols in protein, which could lead to cell dysfunction in liver and kidney[10]. Reactive oxygen species (ROS) directly act on cellular units such as, proteins, lipids

and DNA to devastate their structure[11]. The free radicals devastate the lipid units of the cell membrane by denaturing proteins and peroxidation, causing enzymatic deactivation and lead to mitochondrial dysfunction[12]. Previous literature revealed that kidney toxicity and oxidative stress caused by cisplatin was decreased by the various methods. The method includes antioxidant including sodium selenite[13, 14], Vitamin C[15, 16], curcumin[17] and the carotenoid bixin[18, 19]. *Butea monosperma*, is a moderate sized deciduous tree, belonging to *fabaceae* family. It is known as The Flame of forest or Bastard teak in English and Palash or dhak in Hindi They consists one of the largest families of flowering plants with 630 genera and 18000 species. The genus *Butea* includes *Butea monosperma* parviflora, *Butea minor* and *Butea superba* widely distributed throughout India. It is elucidated in Upanishads, Vedas, Susrirta Samhita, Charaka Samhita, Astanga Sangraha, Ashtanga Hrdaya[20]. This is a moderate sized deciduous tree which is widely distributed throughout India, Burma and Ceylon extending in the North West Himalayas as far as Jhelum except in very acrid parts[21]. It is considered as a sacred tree. It grows well in the alkaline, marshy condition in sunny location. The number of different chemical constituents obtained from various parts of the plant are used as anti-inflammatory,anti-diabetic,anti-fungal,anti-asthamatic,astringent, aphrodisiac[22].The gum which is obtained from the slit made on the bark of the tree is known as 'kamarkas' or Bengal Kino which is rich source of tannins. The dyeing agent present in the flowers that imparts its color is used as insecticide and coloring agent. The flavonoids Butin, Butein, Butrin, Isobutrin, Palasitrin, Coreopsin, Isocoreopsin, Sulphuresin, Monospermoside, Isomonospermoside and 7,3,4- trihydroxyflavone have been isolated from the flower of this plant. From the stem and the pods of this plant species Euphane triterpenoid 3a-hydroxyeuph-25-ene and the alcohol 2, 14-dihydroxy-11, 12-dimethyl-8-oxo-octadec-11- enylcyclohexane and Imide palasimide has been isolated respectively. It acts as the host for lac insect and plays a role in the production of lac[23]. Bark fibers are utilized for making cordage. Wood pulp is useful for newsprint manufacturing[24]. The aim of the current study is to investigate the effect of *Butea monosperma* in cisplatin-induced nephrotoxicity in rats.

MATERIAL AND METHODS

Collection and authentication of plant material

Leaves of *Butea monosperma* were collected in August-October locally from Bhopal (M.P.). Special precautions were taken to collect healthy plants avoiding foreign materials. Herbarium of plant were prepared and submitted for authentication to Safia College of Science (Department of Botany), Bhopal, India. Authentication was done by Dr. Zia-Ul-Hasan, botanist (Head, Department of Botany). Authentication voucher number was 448/Bot/Saifia/13.

Chemicals

Cisplatin (Himedia) was purchased from Himedia, India, GSH, glutathione reductase, thiobarbituric acid, hydrogen peroxide, NADPH, ethanol, NBT, PMS and other reagents were supplied from Sigma (Mumbai, India).

Extraction

In the present study, extraction was performed using continuous hot Soxhalation[25]. Pulverised and weighed quantity of dried leaves of *B. monosperma* was placed in thimble of Soxhlet apparatus. Extraction was performed using petroleum ether, followed by methanol. For each solvent, soxhalation was continued till no colour was observed in siphon tube and completion of extraction was confirmed till solvent in the siphon tube does not left any residue after evaporation. Obtained extracts were concentrated under vacuum at 40°C-45°C. The extracts were further dried in shade. Dried extracts were weighed and percentage yield for extract was determined and packed in air tight container and labelled for further use.

Acute oral toxicity

The acute toxicity study was performed for *Butea monosperma* extract according to OECD (423) guidelines. Wistar rats were selected randomly. The test groups include four treatment groups with dosages at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. Individual doses were calculated on the basis of body weight of the animal on the day of treatment. The animals were observed almost constantly for behavioral changes, mortality and appearance during firstly for first 4 hours, periodically during the 24 hours and then every day for a period of two weeks.

Animals

In this investigation, 24 healthy adult *Albino wistar* rats either sex (8 weeks old weighing 150–200 g) were used. The animals were obtained from the Pinnacle Biomedical Research Institute, Bhopal (M.P.), India. The animals were kept under standard laboratory conditions (12-h light: 12-h dark and 24 ± 3 ◦C). Animals were provided with food and water *ad libitum*. Experiments were strictly conducted and approved by Animal ethics Committee, Pinnacle Biomedical Research Institute, Bhopal, M.P., INDIA (Reg: 1824/PO/c/09/CPCSEA and protocol reference no. PBRI/IAEC/PN-17069).

Experimental design

The rats were divided into four groups; each group containing six rats. Cisplatin was injected to animals' intra-peritoneally at the dose of 6 mg/kg, which is well documented to induce nephrotoxicity in rats[25, 27]. Methanolic leaf extract of *Butea monosperma* was administered to animals by gavage orally at the doses of 200 mg/kg and 400 mg/kg body weight once daily for 7 days. The dose *Butea monosperma* extract used in this study was selected on the basis of the acute oral toxicity[28]. Group 1 (control) received 0.1% CMC daily for 7 days, Group 2 received 0.1% CMC drug orally for 7 days and a single dose cisplatin on 7th day intra-peritoneally post dosing of CMC. Group 3 and Group 4 received extract of *Butea monosperma* at a dose of 200 and 400mg/kg bw orally once daily respectively and a single dose of Cisplatin (6 mg/kg bwt) in both group post dosing of extract.

Sample collection

At termination of experiments, animals of each group were sacrificed at the 8th day. Blood samples were collected and samples were centrifuged at 200 × g for 5 min at +4 ◦C to separate their plasma and one of the kidney tissue samples was kept it in 0.2M Phosphate buffer for further analysis.

In-vivo antioxidant assay

Kidney tissue were dissected out and homogenized. Briefly, tissues were weighed and homogenized with buffers (provided by the kits). The homogenates were then determined following the procedures to study the enzyme activity via catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and LPO spectrophoto metrically (UV spectrophotometer – Model Systronic 2202).

Superoxide dismutase

The Superoxide Dismutase Assay Kit utilizes a tetrazolium salt for detection of superoxide radicals generated by red formazan dye reduction produced. One unit (U) of SOD

activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Briefly, 0.1 ml sample was added with 1.2 ml of 0.052M sodium pyrophosphate buffer 0.1 ml of 186 µM phenazine methosulphate + 0.3 ml of 300 µM Nitroblutetrazolium + 0.2 ml of 750 µM NADH. Incubate at 30°C for 90 seconds Add 0.1 ml glacial acetic acid Stir with 4.0 ml n-butanol Allow to stand for 10 min Centrifuge and butanol layer were separate. The reduction was measured at 560 nm in UV spectrophotometer – Model Systronic 2202 and percentage of SOD inhibition as compared to the blank was determined. One unit of SOD was calculated by the amount of protein needed to achieve the 50% inhibition and hence expressed as unit SOD/mg protein[29].

Malondialdehyde (MDA)

Tissue peroxidation value was detected by measuring thiobarbituric acidreactive substance (TBARS). In brief, 0.2 ml of aliquot was added with 0.2 ml 8.1 % SDS, 1.5 ml 20 % acetic acid, 1.5 ml 8 % TBA (make up volume up to 4 ml with distilled water). Heat on water bath (95°C) for 60 min using glass ball as condenser, cool and make up volume up to 5 ml Add 5 ml of butanol : pyridine (15:1). The mixture was vortex for 2 minutes then centrifuge at 3000 rpm for 10 min. take out the upeer layer form the mixture and then absorbance of pink-colored product was taken at 532 and 600 nm wavelength using UV-Vis Spectrophotometer (Systronic-2202). The difference between absorbance was measured and compared to that of the standard malonaldehyde tetramethyl acetal solutions of different concentrations. MDA activity was expressed as nmol MDA/g protein[30].

Glutathione peroxidase

The Glutathione Peroxidase Assay Kit measures GPx activity indirectly by a coupled reaction With GR. Oxidized glutathione, produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to NADP+ is accompanied by adecrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A340 is directly proportional to the GPx activity. One unit (U) of GPx activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP+ per minute at 25^{o}C [31].

Catalase

CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm and was expressed as k/g protein, where k is the first-order rate constant. Protein concentrations were measured[32].

Biochemical assays

Plasma creatinine, Blood urea nitrogen, uric acid were measured by using test kit (Span Diagnostics Ltd.) in biochemical auto analyzer (Star 21 plus-E119147).

Histopathological examinations

The other kidney was excised after sacrifice. The tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 5 mm and were stained with hematoxylin–eosin.

Statistical analysis

All data were calculated as Mean ± SD. Results were analyzed using One Way ANOVA followed by Bonferroni's test. P<0.05 was considered as level of significance.

RESULTS

In acute oral toxicity study, the methanolic extract of *Butea monosperma* were employed as per OECD guideline in wistar rats. Extract did not show any mortality and toxicity at any concentration not even at highest does of 2000 mg. kg^{-1}. Therefore, in present study 200mg (low) and 400mg (high) doses of methanolic drug extract were selected for determination of nephrotoxicity activity. In *invivo* oxidative stress study parameters, the production of MDA level in the renal tissue were assessed as a measure of LPO. A single dose of cisplatin increased the formation of lipid peroxides 24 h after i.p. cisplatin, compared to control groups (P < 0.05). This increase was inhibited by administration of methanolic extract of *Butea monosperma* in rats sacrificed on 8th day after cisplatin administration (P > 0.05). Kidney tissues in cisplatin-treated group showed a statistically significant reduction in the MDA production in animals as compared to pretreated with methanolic extract of *Butea monosperma* (P < 0.05). The SOD level were decrease in Cisplatin injected group whereas pretreated with extract group shows significant increment in the SOD level but did not reach the level of control group. In the cisplatin treated group, the cisplatin dose resulted in decrease in renal glutathione, after treatment as compared to the control group (P < 0.05). The levels of renal glutathione were restored to normal level in group 4 as compared to the control group (P < 0.05). Likewise in catalase assay decomposition of hydrogen peroxide is decreased in ciplatin induced group whereas extract pretreated group shows increment in the catalase level Table 1. In the cisplatin induced nephrotoxicity study using cisplatin as inducer, the protective effects of methanolic extract of *Butea monosperma* reach those of the control group. Table 2 showed the effects of oral extract of *Butea monosperma* on cisplatin mediated increases in serum creatinine and decreases in creatinine clearance 24h after the i.p. treatment. The cisplatin alone led to augment the value of serum creatinine compared to the control group (P < 0.05). A significant decline in the level of creatinine (P < 0.05) was observed in rats treated with cisplatin and *Butea monosperma* extract when compared to the control group. Blood urea nitrogen also increased in cisplatin induced group but pre treated with methanolic extract of *Butea monosperma* decreases the level of BUN similarly the Uric acid level also increased in cisplatin induced group whereas pretreated with extract significantly decreased the level.

Table 1: Chemoprotective activity against cisplatin induced nephrotoxicity

Group No.	Treatment Group	Enzymes involved in oxidative stress in Kidney (Mean ± SD)			
		LPO (nM MDA/gm wet tissue)	SOD (U/gm wet tissue)	GSH (nmol/gm wet tissue)	Catalase (U/gm wet tissue)
1	Control	8.17±0.753	14.50±1.871	65.50±7.036	21.17±2.229
2	Control + Cisplatin	75.17±5.037*	2.07±0.574*	19.33±4.502*	4.89±1.812*
3	Extract (200mg/kg bwt) + Cisplatin	54.33±4.761#	3.41±0.369#	23.83±2.483#	6.50±1.265#
4	Extract (400mg/kg bwt) + Cisplatin	45.50±3.728#	4.89±0.630#	29.50±2.258#	7.35±0.477#

(* – p<0.05 compared to Group I (vehicle treated group); #- p<0.05 as compared to group II [Cisplatin (6mg/kg, i.p)], n=3)

Table 2: Effect of methanolic extract of *Butea monosperma* on kidney function test in rats treated with cisplatin

Group No.	Group No.	Kidney function test (Mean ± SD)		
		Creatinine	BUN	Uric Acid
1	Control	0.08±0.009	10.17±2.317	1.16±0.169
2	Control + Cisplatin	3.87±0.389*	57.31±5.981*	61.78±5.212*
3	Extract (200mg/kg bwt) + Cisplatin	2.77±0.328#	48.79±2.728#	43.28±4.618#
4	Extract (400mg/kg bwt) + Cisplatin	2.17±0.228#	42.99±3.136#	34.37±3.701#

(* – p<0.05 compared to Group I (vehicle treated group); #- p<0.05 as compared to group II [Cisplatin (6mg/kg, i.p)], n=3)

Histopathological Studies

Histopathological study was performed using light microscopy. In control group, kidney showing tubular brush borders and intact glomeruli without any structural alterations in renal tissues. In cisplatin treated group showed swelling and massive and diffuse cell necrosis in proximal tubules of kidneys indicating injury. Pretreatment with Methanolic extract of *Butea monosperma* was significantly prevented histopathological changes. This inhibitory action Methanolic extract of *Butea monosperma* against cisplatin was confirmed through histopathological studies Figure 1.

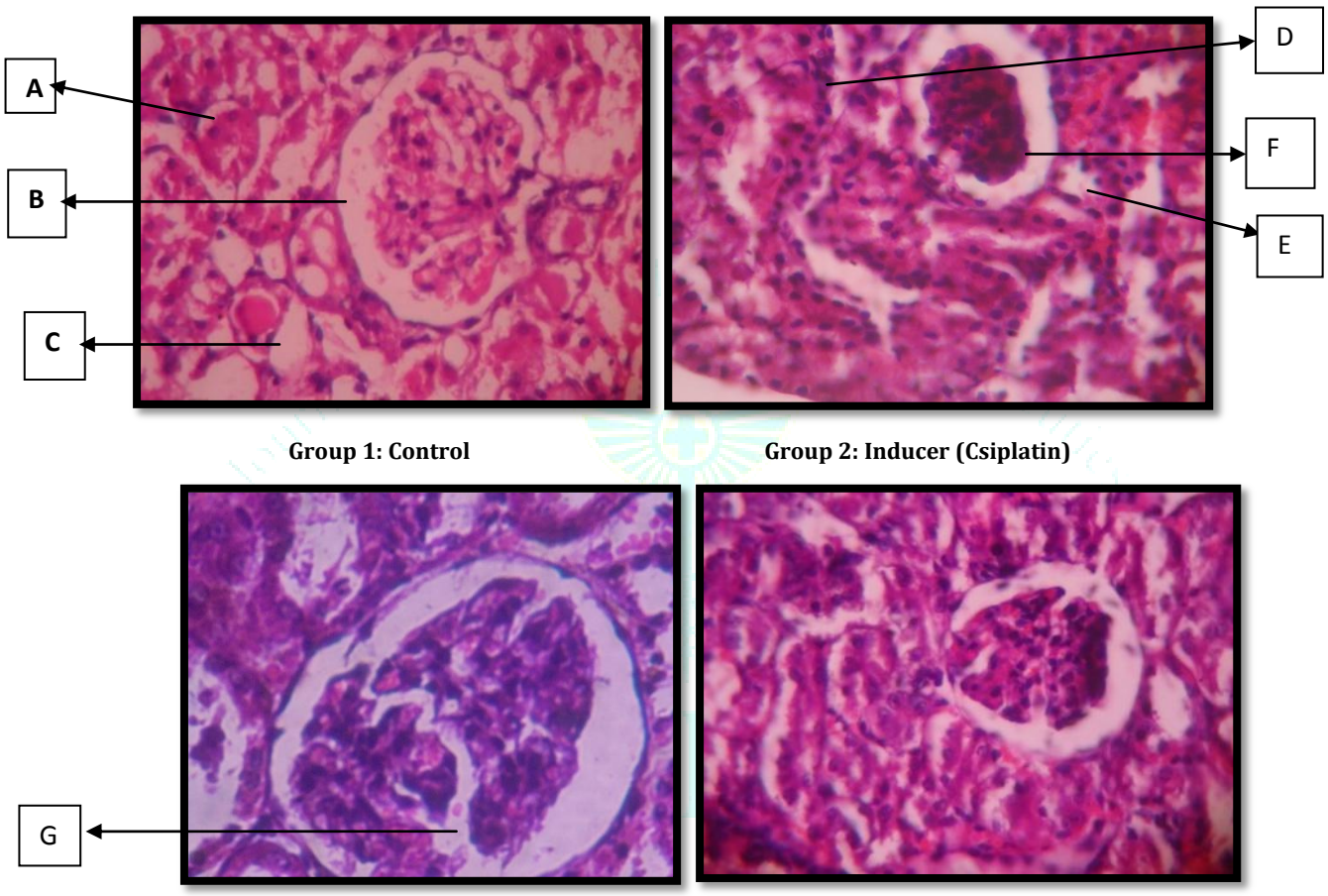

Figure 1: Photomicrograph of renal sections showing a protective effect of methanolic extract of *Butea monosperma* cisplatin-induced tubular damage. Group 1: control group, Group 2: Toxic control group, Group 3 Treatment group with 200 mg/kg, orally of methanolic extract of *Butea monosperma*, Group 4: Treatment group with 400 mg/kg, orally of methanolic extract of *Butea monosperma*. The details of histology pictures were as A: PCT- proximal convoluted tubule, B: Normal Glomerulli, C: DCT- distal convoluted tubule, D: Vacuolated cytoplasm, peritubular capillary dilation and congestion E: pyknosis, F: Dehydrated Bowman's Capsule, G: Bowman's space reached to its normal position

DISCUSSION

The exact mechanism of cisplatin-induced nephrotoxicity is unknown while studies supporting the involvement of lipid peroxidation and free radicals. Cisplatin generates ROS such as superoxide anion and hydroxyl radicals, and stimulates renal lipid peroxidation[33, 34]. The study regarding the role of lipid peroxidation and its position in the chain of events that leads to nephrotoxicity still controversial. The study in which free radical generation is not the direct cause of cisplatin-induced renal injury was supported by Kruidering [35]. In present study, the administration of cisplatin resulted in damage, evaluated by the increase in the MDA content when compared to control group. These data are supported by previous investigations where this antitumoral induced lipid peroxidation and damage in the renal tissues of rats. The increase in renal MDA content 24h after cisplatin injection was significantly prevented by pretreatment with extract, but it was not observed in animals sacrificed at 24 h. It was evidenced that cisplatin nephrotoxicity occurs as a result of oxidative stress and increased generation of superoxide anion, hydrogen peroxide, and hydroxyl radicals due to the increased activity of NADPH oxidase and adenosine deaminase [36]. These ROS damage the lipid gears of the cell membrane via proteins denaturation, which subsequently lead to enzymatic inactivation [37]. Moreover, cisplatin induced

kidney damage could be explained by the fact that, as fast as cisplatin is in the interior of the cells, the hydrolysis product reacts with GSH in the cytoplasm and DNA in the nucleus [38]. The produced cisplatin-DNA intrastrand cross-links result in cytotoxicity (apoptosis/ necrosis) [39].

CONCLUSION

Finally, on the basis of these investigations, we may partially conclude that the methanol extract of *Butea monosperma* with antioxidant properties could be a potent nephroprotective and curative agent for the next generation. The nephroprotective effects of the doses of *Butea monosperma* were accompanied with attenuation of cisplatin-induced alteration in oxidative stress.

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