



## CHEMOPROTECTIVE EFFECT OF *BUCHANANIA LANZAN* SEEDS EXTRACT ON TOXICITY OF CISPLATIN INDUCED NEPHROTOXICITY IN RATS

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### ABSTRACT

The clinical use of cisplatin is highly restricted, because of nephrotoxicity. The study objective was to investigate the protective effects of *Buchanania lanzan* on cisplatin induced nephrotoxicity. Wistar rats were assigned into 7 groups weighing 220–250 g, were included in the study. The control group received distilled water for 21 days. The second group of animals injected only with cisplatin (6mg/kg i.p) on day 17 to 21. Third and fourth group of animals administered with extract of *Buchanania lanzan* (200 and 400 mg/kg p.o.) and injected with Cisplatin on day 17 to 21. Fifth and sixth group of animals only injected with extract of *Buchanania lanzan* (200 and 400mg/kg p.o.) and seventh group of animals injected with standard Mesna for 21 days respectively and injected with cisplatin (6mg/kg i.p) on day 17 to 21. On day 22, the rats were decapitated, and blood and hepatic tissues were taken. The results indicated significant changes in serum urea and creatinine concentration in cisplatin group compared with control group. *Buchanania lanzan* was observed to have a protective effect on cisplatin induced Kidney toxicity.

**KEYWORDS:** *Buchanania lanzan* spreng seed, Cisplatin, Mesna, Nephroprotectivity.

### INTRODUCTION

Cisplatin is one of the important anti-neoplastic drugs that is useful in the treatment of many tumours including head, ovary, testis and lungs malignancies. Therapeutic effects of cisplatin are associated with severe side effect, mainly nephrotoxicity and neurotoxicity. The major restriction to use a high dose of cisplatin is its strong side effects in the kidney and gastrointestinal tract. Original pathways of cisplatin transport in renal cells are active transport, although cisplatin enters the cells through passive diffusion as well. The organic cation transporter 2 is the major transporter for cisplatin uptake in proximal tubular cells. Cisplatin also induces the production of free radicals and activates the mitogen-activated protein kinase (MAPK) intracellular signalling pathways. In the presence of cisplatin, reactive oxygen species (ROS) are generated in cells via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase.<sup>[1]</sup>

There is an increasing interest in herbal remedies because of their effectiveness, less side effects in clinical experiments and relatively low cost. *Buchanania lanzan* spreng. (*B. lanzan*) belongs to the Family Anacardiaceae, is commonly known as 'Chaar' in India.<sup>[2]</sup> In ayurvedic medicine various part this plant used as astringent, depurative, constipating, brain tonic, cardio tonic and for glandular swelling. The

Phytoconstituents reported in seeds of this plant are flavonoids, sterols, tannins, quercetin, glycosides, Triterpenoid, Saponins, carbohydrates, phenolic compounds. The reported pharmacological activity of *B. Lanzan* is Anti-inflammatory and analgesic activity, Antioxidant Activity, Antidiabetic and antihyperlipidemic, Antiulcer, Antidiarrheal, Wound healing activity, Memory booster, Antivenom and Diuretic activity.<sup>[3]</sup> Based on the reported findings for the presence of flavonoids and antioxidant property, the present study was undertaken to evaluate the effects of ethanolic extract of *Buchanania lanzan* seed in cisplatin induced nephrotoxicity.

### MATERIAL AND METHODS

#### Collection and Identification of plant Materials

*Buchanania lanzan* seed were collected from the surrounding area of rural Pune during August-September 2018. The plant was identified and authenticated by M/s. Shamantak Enterprises. Dr. Gautam, Botanist, Pune, India.

#### Preparation of Plant Extract

A weighed quantity (50g) of the air-dried powdered seeds of *B. lanzan* was drawn and then it was extracted with 90% ethanol in a Soxhlet extractor. The hydroalcoholic extract was concentrated in a rotary flash

evaporator at a temperature not exceeding 50<sup>0</sup> C to get a solid residue. The yield of *B.lanzan* was found to be 5% w/w.

### Experimental Animals

Male wistar rats weighing 220-250 g was used for the present studies. The rats were obtained from the animal house, AISSMSCOP, Pune. The rats were maintained at a room temperature of 22-24<sup>0</sup> C, with a 12 h light/dark cycle and humidity around 50±5%. During acclimatization, the rats were randomized into experimental and control groups and housed individually in sanitized cages housed with sterile paddy husk as bedding. The animals had free access to food and water ad libitum throughout study. All experimental procedures were in compliance with the Institutional Animal Ethical Committee approved protocol (CPCSEA/IAEC/PC-07/01/2K18).

### Phytochemical Analysis

The extract was subjected to preliminary phytochemical screening for phytochemical constituents such as Flavonoids, Tannins, Alkaloids, Glycosides, Triterpenoid, Saponins, Sterols, Carbohydrates, Phenolic compounds, Gum and Mucilage.

### Experimental Design

42 Male wistar rats were divided into seven groups, 6 in each. Group 1 was served as normal control which was given with distilled water only. Group 2 treated with Cisplatin (6mg/kg i.p) on 17-21 day of experiment. Group 3 treated with an oral dose of 200 mg of extract +6mg/kg of Cisplatin on 17-21 days. Group 4 treated with an oral dose of 400mg of extract + 6mg/kg of Cisplatin on 17-21 days. Group 5 treated with an oral dose of 200mg of extract for 21 days. Group 6 treated with an oral dose of 400mg of extract for 21 days. Group 7 treated with an oral dose of standard drug 200mg/kg of Mesna+ 6mg/kg of Cisplatin on 17-21 days.

At the end of study, i.e. on the day 22, the rats were anesthetized, blood sample were taken by retro orbital method and the animals were sacrificed. Blood samples were centrifuge at 1500rpm (Micro centrifuge) for 15 minutes within 1 hr of collection to obtain serum samples, which were immediately analysed for estimation of serum creatinine, blood urea nitrogen by using standard kit (Biolab, Diagnostics, Mumbai). A histopathological examination of Kidney was performed to analysed morphological analysis.<sup>[11]</sup>

### Histopathological Examination

Kidneys excised from each treatment group were subjected to histopathological examinations. After fixing the tissues in 10 % formalin, they were dehydrated and mounted in paraffin blocks. The sections of 3-5 µ thickness were cut and stained with haematoxylin-eosin stain.

### Statistical Analysis

Statistical analysis was carried out using the GraphPad Instat 3. All of the data are shown as the mean ±Standard error of the mean (S.E.M) and were analyzed using one-way analysis of variance (ANOVA). Significant differences between the control and experimental groups were determined using Tukey – Kramer's all comparison test, P<0.05 and P<0.001 was considered significant.

## RESULTS

### Effect of *B. lanzan* on renal function test

Administration of cisplatin has led to a significant impairment of renal function as indexed by remarkable decrease in levels of serum creatinine and blood urea nitrogen (BUN) when compared with control rats (p<0.05). Treatment with 200 mg/kg *Buchanania lanzan* daily for 21 days had no significant difference compared with cisplatin group. Treatment with 400 mg/kg *Buchanania lanzan* daily for 21 days showed a significant improvement in renal function as indicated by the significant (p<0.001) reduction in serum creatinine and BUN as compared with cisplatin group (Table 1).

**Table 1: Effect of *Buchanania lanzan* extract on Cisplatin induced Nephrotoxicity.**

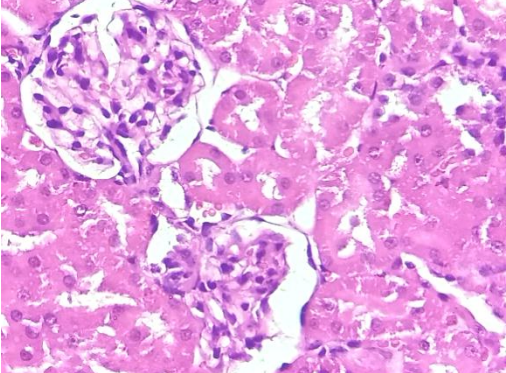
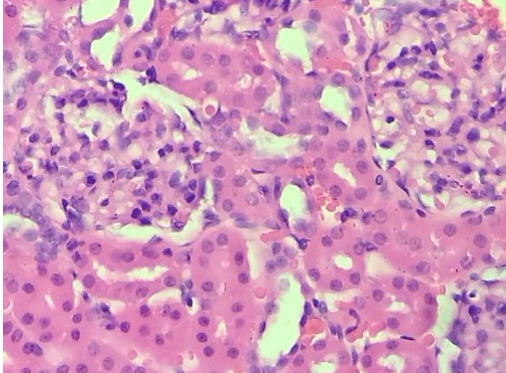
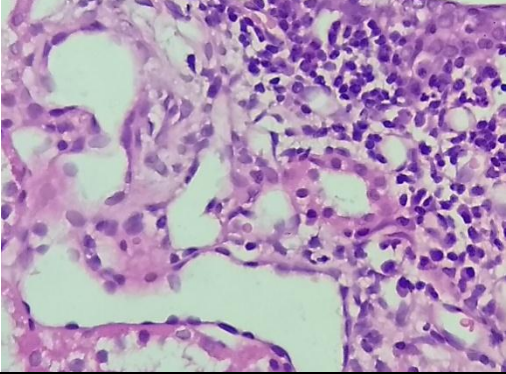
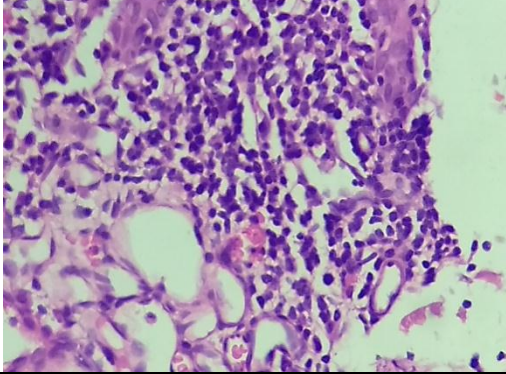
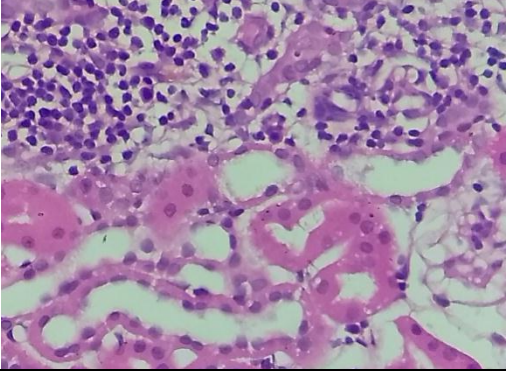
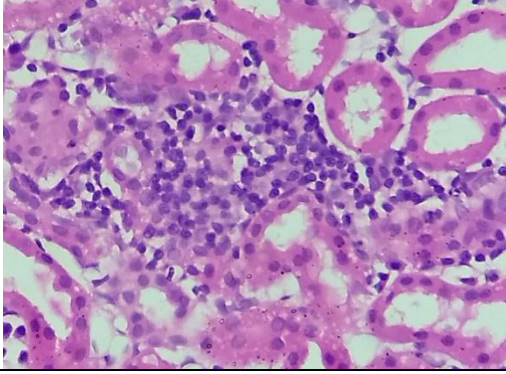
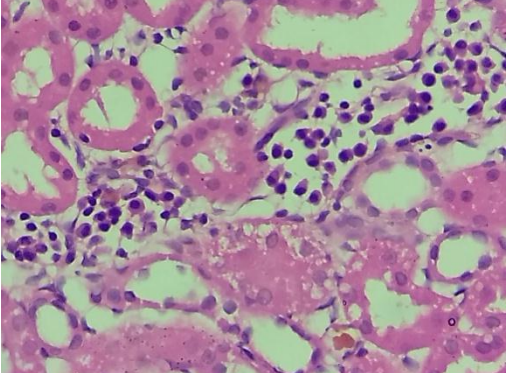
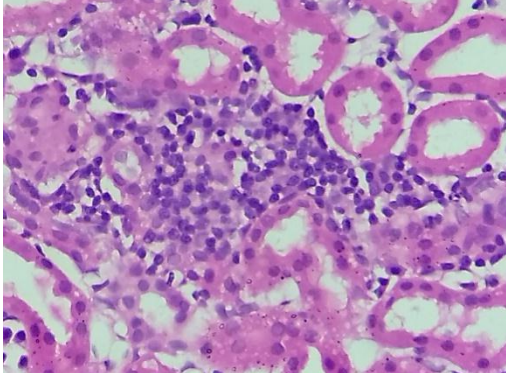
| Parameters                     | Creatinine (mg/dl) | BUN (mg/dl)    |
|--------------------------------|--------------------|----------------|
| Control                        | 16.64±0.362        | 51.88±0.768    |
| Cisplatin (6 mg/kg)            | 9.135±0.314        | 65.055±0.764   |
| <i>B. lanzan</i> 200mg+cis     | 9.085±0.302        | 50.48±0.410    |
| <i>B. lanzan</i> 400mg+cis     | 13.35±0.188*       | 52.50±0.478*   |
| <i>B. lanzan</i> 200mg         | 8.393±0.233**      | 49.958±0.512** |
| <i>B. lanzan</i> 400mg         | 9.908±0.322**      | 53.981±0.407** |
| Mesna (200mg/kg) +cis (6mg/kg) | 11.33±0.261**      | 90.45±0.602**  |

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. \*p<0.05.

### Histopathological Examination:

**Kidney:** Microscopic examination of kidneys treated with Cisplatin showed mild infiltration of inflammatory cells in renal tubules when compared with control group. Severity of infiltration was decreased in animals treated with mesna and *Buchanania lanzan* extract at 200 mg/kg

body weight and at 400 mg/kg of *Buchanania lanzan* extract did not revealed any renal damage when compared with cisplatin group. Animals treated with 200 and 400 mg/kg of extract did not show any lesion of pathological significance when compared with control group. (Figure 1).

|                                                                                                                  |                                                                                                                 |
|------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
|                                 |                               |
| Control; Kidneys; Right: Showing normal glomerulus and tubules. 10 X, H & E                                      | Control; Kidneys; Left: Showing normal glomerulus and tubules. 40 X, H & E                                      |
|                                |                              |
| Cisplatin; Kidneys; Right: Showing Infiltration of inflammatory cells (Mild). 40 X, H & E                        | Cisplatin; Kidneys; Left: Showing Infiltration of inflammatory cells (Mild). 40 X, H & E                        |
|                               |                             |
| Cisplatin + Standard; Kidneys; Right: Showing Infiltration of inflammatory cells (Minimal). 40 X, H & E          | Cisplatin + Standard; Kidneys; Left: Showing Infiltration of inflammatory cells (Minimal). 40 X, H & E          |
|                               |                             |
| Extract 200 mg/kg + Cisplatin; Kidneys; Right: Showing Infiltration of inflammatory cells (Minimal). 40 X, H & E | Extract 200 mg/kg + Cisplatin; Kidneys; Left: Showing Infiltration of inflammatory cells (Minimal). 40 X, H & E |

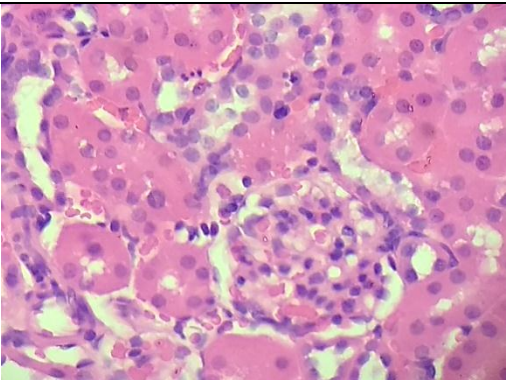
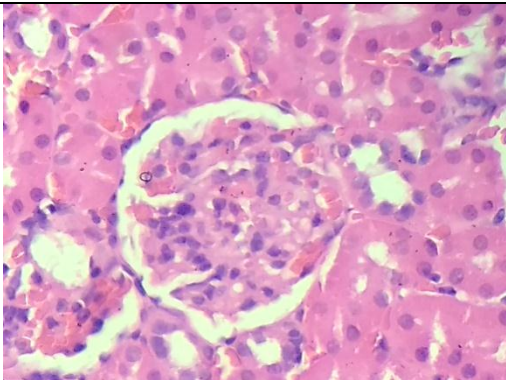
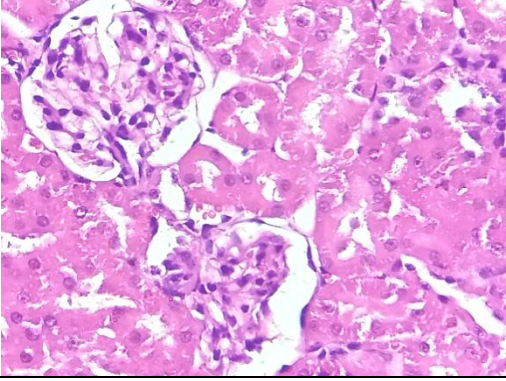
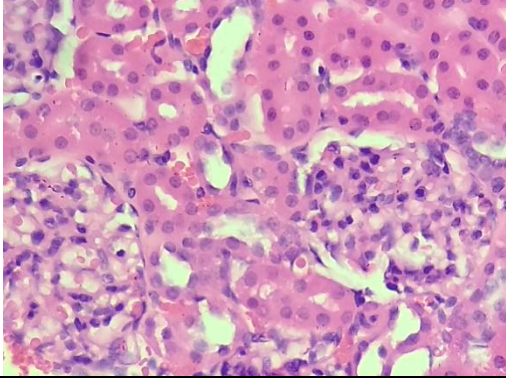
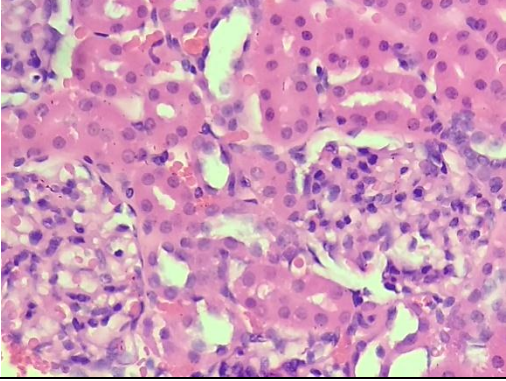
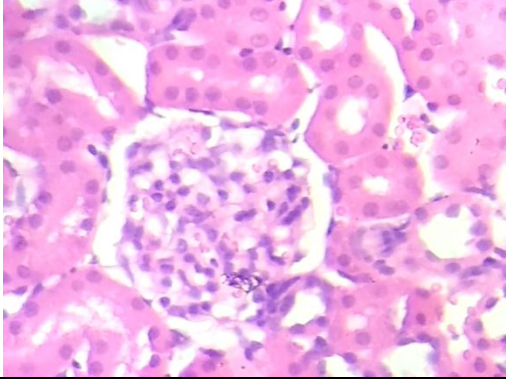
|                                                                                                             |                                                                                                            |
|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
|                            |                          |
| Extract 400 mg/kg + Cisplatin; Kidneys; Right: Showing normal glomerulus and tubules (Minimal). 40 X, H & E | Extract 400 mg/kg + Cisplatin; Kidneys; Left: Showing normal glomerulus and tubules (Minimal). 40 X, H & E |
|                           |                         |
| Extract 200mg/kg; Kidneys; Right: Showing normal glomerulus and tubules (Minimal). 40 X, H & E              | Extract 200mg/kg; Kidneys; Left: Showing normal glomerulus and tubules (Minimal). 40 X, H & E              |
|                          |                        |
| Extract 400 mg/kg; Kidneys; Right: Showing normal glomerulus and tubules (Minimal). 40 X, H & E             | Extract 400 mg/kg; Kidneys; Left: Showing normal glomerulus and tubules (Minimal). 40 X, H & E             |

**Fig. 1: Histopathological examination of Kidney of rats in Nephrotoxicity study.**

Therefore, On the basis of histopathology findings, it can be concluded that severity and incidence rate of infiltration of inflammatory cells in renal tubule was decreased in animals treated with mesna and *Buchanania lanzan* extract at 200 mg/kg suggestive of ameliorating effect of Extract. Animals treated with *Buchanania lanzan* extract at 200 and 400 mg/kg did not show any lesion of pathological significance.

## DISCUSSION

Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity. Nephrotoxicity can be

diagnosed through a simple blood test. Evaluation of nephrotoxicity through blood tests includes the measurements of blood urea nitrogen (BUN); concentration of serum creatinine, glomerular filtration rate and creatinine clearance. However, these assessments of nephrotoxicity are only possible when a majority of kidney function is damaged. Other mechanism of cisplatin-induced kidney injury involve inflammation, fibrogenesis, and oxidative stress. Cisplatin is an effective anticancer agent. However, its accumulation in renal cells causes nephrotoxicity. In recent years, use of natural remedies has developed to

reduce cisplatin nephrotoxic action. Among medicinal plants, *Buchanania lanzan* because of various pharmacologic properties and religious background.<sup>[1]</sup> The reported literature demonstrated to presence of flavonoids and other phenolic constituents in the plant extract. Cisplatin induced nephrotoxicity is associated with oxidative stress which contributes to cytotoxicity; hence, the use of antioxidants could counteract such cytotoxic effects.<sup>[4]</sup> *Buchanania lanzan* is reported to possess antioxidant and antiinflammatory properties. Therefore, in the present study we hypothesized that *B. Lanzan* extract may protect the kidney against cisplatin induced toxicity. Results of the present study demonstrated that cisplatin was significantly able to increase the urea and creatinine concentration in blood, which confirmed the accuracy of the experiment in induction of renal injury.<sup>[7,8]</sup> There are evidences indicating that cisplatin exerts its enhancing effects in serum urea and creatinine concentration by the reduction of glomerular filtration rate (GFR) that may be due to production of reactive oxygen species (ROS) in afferent and efferent arterioles and consequent increase in their resistance. ROS also increase the production of vasoconstrictor substances including endothelin, isoprostane, and thromboxane that in turn result in glomerular vasoconstriction and GFR reduction.<sup>[9,10]</sup>

The results of current study indicated that treatment with *B. lanzan* extract at 400mg/kg significantly increased the serum urea and creatinine concentrations in preventive+treatment group. This study indicates the nephroprotective effects of *B. lanzan* seed extract in cisplatin induced nephrotoxicity. The nephroprotective effect of *B. lanzan* extract may be due to presence of flavonoids and its associated antioxidant property.

## CONCLUSION

This study showed that the administration of the *B. lanzan* extract to reduce toxic effects of cisplatin in a time and dose-dependent manner. It was found in the current study that *B. lanzan* was protective against Cisplatin induced renal toxicity.

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