*Research Article*

# *In vivo* study of Nephroprotective potential of Shilajit by using Cisplatin induced nephrotoxicity model

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**Abstract**

**Background**: Cisplatin is a potent Antineoplastic agent and it is widely administered for tumours like neuroblastoma, lymphoma, neck carcinoma, endometrial neoplasm and bladder carcinoma but now days cisplatin having limited used due to renal toxicity, GI disturbances, like nausea, vomiting and myelosuppression. **Objective:** In this experiment, we have used cisplatin induced nephrotoxicity model and Shilajit Aqueous Extract as a test drug, it is potent Antioxidant and Herbomineral Drug. **Materials and Methods:** The healthy wistar rats of either sex weighing between 180-250gms we have prepared five groups for study of Nephroprotective Potential of Shilajit. This groups are follows Group I - Vehicle control, Group II- Negative control (cisplatin) Group III-Positive control (standard cystone), Group IV -Test1 (Treated Group),Group V-Test2 (Treated Group) and Group VI -Test3 (Treated Group). **Results and Conclusion**: On the comparisons between these groups with normal rats of group I, it was found that there is a significant decrease in the body weight and increase in the kidney weight. And when the comparisons between these groups with of cisplatin + cystone induced nephrotoxic rats showed significant increase in the body weight but decreased in the kidney weight. the cisplatin+shilajit200mg/kg Per Oral administered rats showed significant increased body weight, but not showed significant decrease kidney weight and cisplatin+shilajit 400mg/kg Per Oral and cisplatin+shilajit 800mg/kg Per Oral showed increased in the body weight and decreased in the kidney weight when compared cisplatin induced nephrotoxic rats. The histopathological analysis also showed nephroprotective potential of aqueous extract of Shilajit in cisplatin induced Nephrotoxicity.

***Keywords:*** Nephroprotecive, cystone, shilajit, cisplatin

## Introduction

Kidney is a vital organ of human body which purify the blood by excreting nitrogenous waste and toxic components from the urine. It helps maintaining electrolyte balance, homeostasis, blood pressure cisplatin induced nephrotoxicity by causing renal phosphor lipidosis through inhibition of lysosomal hydrolases such as sphingomyelinase and phospholipases in addition to causing oxidative stress etc. (Tran et al., 2013).

Nephrotoxicity occurs when toxins are damages to the kidneys. Whenever the kidney damages occure, kidneys unable to excrete nitrogenous waste from the body, like magnesium and potassium ions the number of drug concerned with development of cisplatin ,cisplatin is a Antineoplastic agent, widely used in the Lymphoma, neck carcarcinoama, Endometrial neoplasm. Cisplatin having major side effects like renal toxicity, mylosupression, Nausea and vomiting (Mohana and Reddy, 2012).

According to WHO, traditional medicines incorporate health practice, approaches knowledge and plant minerals and animal based medicines, applied singularly and combination to treat and prevent illness. Shilajit is the one of the major Herbomineral drug containing fulvic acid and humic acid. this components having Antioxidants properties which may exhibit Nephroprotection against cisplatin induced Nephrotoxicity (Mohana and Reddy, 2012).

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Upon literature review, it was found that the native practitioners claimed that Shilajit are traditionally used in a Nephroprotective activity. The Shilajit consist major organic constituents included benzoic acid, hippuric acid, fatty acids, resin and waxy materials, gums, albuminoids and vegetable matter with benzoic acid being the active ingredient. Shilajit also contains Shilajityl acetate, shilajitol, shilaceatatechol, shilaxanthone, shilanthranil (Ali, 2004).The various reported pharmacological activities include aphrodisiac activity and spermatogenic effect (Gupta, 2013). Parasympathomimetic effect (Sarabjeet, 2012), Cancer treatment (Akhilesh, 2015), Testosterone Induced Benign Prostrate hyperplasia (Sakhare and Bhagat, 2014), Anti-inflammatory and anti-arthritic (Lawley et al., 2013) for prevention and treatment of micro and macrovascular complication of type-ii DM (Gopa et al., 2016), effect of Shilajit on lipid profile of hyperlipidemic albino rats (Mudassara et al., 2012) etc. However, there are no reports on nephroprotective activity of Shilajit, hence, the present study was designed to verify the claim of the native practitioners.

## Materials and Methods

### Animals

The healthy wistar rats of either sex weighing between 180-250g were taken for the experiment. They were housed under the condition of temperature (23±2°C),relative humidity(55±5%) and 12 hour light and dark cycle. the animals were fed with standard pellet diet and reverse osmosis water .the experimental protocol approved by institutional animal ethical committee as per as CPCSEA guidelines (1670/PO/ReBiBt/S/12/CPCSEA) .

### Acute toxicity study

The Shilajit Aqueous Extract has been subjected to toxicity study as per the guidelines set by CPCSEA. The study was approved by Institutional Animal Ethics Committee (IAEC) no mortality and no signs of toxicity found after administration of dose at 4000mg/kg to female mice's. Hence 200mg and 400mg and 800mg of this extract selected for further study. To predict the mortality and b.w, animals were kept under observation up to 14 days after extract administration (Adsul and Pagar, 2017).

### Drugs and chemicals

Cisplatin was purchased from Alkem laboratories Ltd, Shilajit was purchased from Pantanjali and Other reagent were purchased from local market, all this chemicals used in this studied were analytical grade.

### Preparation and scheduled

The selected doses of Shilajit 200,400,800mg/kg b.w for the rats (Vivek et al., 2011; Bhargavi et al., 2013). They are given oral route using oral gavage standard cisplatin 7mg/kg b.w. was used.

### Experimental Protocol

Animals were randomized and divided into six groups (1 to 6) of six animals (n=6) in each groups (Table 1). Group I served as untreated control and was fed orally with normal saline daily for 14 days. Group II rats were treated with single dose of cisplatin (7 mg/kg body weight; i.p.) on day 1 treated as disease control. Group III served as standard received cystone (500 mg/kg; p.o.) for 14 days after single dose of cisplatin on day 1. Group IV, V and VI received Shilajit200, 400 and 800 mg/kg b.w; p.o. For 14 days after single dose of cisplatin on day 1respectively. On days 1st and 14th of the experiment, the rat weights were measured and average body weights were calculated (Nabila et al., 2015).

**Table 1.** Treatment & Dose of Shilajit to the various groups

| Sr. No. | Groups | Treatment and dose/ day |
|---|---|---|
| 1 | Group I<br>Vehicle control | Vehicle control (1 ml distilled water p.o.) 0 to 14$^{th}$ day |
| 2 | Group II<br>Negative control | Negative control cisplatin (7 mg/kg i.p.) 1$^{st}$ day |
| 3 | Group III<br>Positive Control | Cisplatin (7 mg/kg i.p.)1$^{st}$ day + Standard Cystone (500 mg/kg p.o.) 1$^{st}$ to 14$^{th}$ day |
| 4 | Group IV<br>Test 1 | Cisplatin (7 mg/kg i.p.)1$^{st}$ day + Shilajit dose(200 mg/kg p.o.)1$^{st}$ to 14$^{th}$ day |
| 5 | Group V<br>Test 2 | Cisplatin (7 mg/kg i.p.)1$^{st}$ day + Shilajitdose(400 mg/kg p.o.)1$^{st}$ to 14$^{th}$ day |
| 6 | Group VI<br>Test 3 | Cisplatin (7 mg/kg i.p.) 1$^{st}$ day + Shilajit dose (800 mg/kg p.o.) 1$^{st}$ to 14$^{th}$ day |

## Computation parameter of Nephroprotective activity

### Morphological parameter

These parameters were studied by recording the body weight and kidney weight.

### Biochemical parameter

Biochemical parameter were analyzed according to reported method methods as Creatinine, Uric acid, Urea, Sodium and Potassium to determine the functional state of the kidney (Bhargavi et al., 2013). The estimation of the serum enzymes individually are helpful in the differential diagnosis of the kidney diseases. The concentration of these enzymes increase in serum whenever the kidney tissue damaged, it is due to release of enzymes from the damaged cells. These elevations are the indicators of cellular leakage and loss of functional integrity of cell membrane.

### Urine analysis

Metabolic cages were cleaned to prevent contamination. The experimental animals were transferred to the separate metabolic cages after the last day administration. Twenty-four hour urines were collected. The collected urine samples were transferred to clean containers and mixed with suitable quantity of purified water.

A drop of concentrated HCl was added to the collected urine. This prevents the growth of microbes and also prevents metal hydrolysis. The collected urine was measured and transferred to a cleaned airtight container and used for the urine analysis**.** From the collected urine samples of rats, urine sodium, potassium, urinary creatinine, uric acid and urinary urea were estimated using auto analyzer and diagnostic kits.

**Kidney histopathology**

After withdrawing the blood from the animals they were sacrificed and the kidney was removed, fixed in 10% formalin embedded in paraffin section and stain with hematoxylin and eosin, the studies were read by pathologist who was not aware of the treatment.

**Statistical analysis**

Arithmetic means of the values of readings were calculated for each experiment the result obtained was used for statistical analysis using Prism pad software. The data obtained from various models of nephrotoxicity in rats experiments were subjected to analysis of variance (ANOVA) followed by Dunnett's test using Prism pad software. Value of $p<0.01$ was considered statistically significant.

**Results and discussion**

**Morphological study**

There is a significant decrease in the body weight of the Cisplatin induced nephrotoxic rats, when compared to normal rats. The Cisplatin+Cystone administered rats showed significant increase in the body weight, when compared to the cisplatin induced nephrotoxic rats. There is a significant increase in the body weight of Cisplatin+Shilajit 200mg/kg p.o., Cisplatin+Shilajit 400 mg/kg p.o. and Cisplatin+Shilajit 800mg/kg p.o. when compared to the cisplatin induced nephrotoxic rats. There is a significant increase in the kidney weight of the Cisplatin induced nephrotoxic rats when compared to normal rats. The Cisplatin+Cystone administered rats also showed significant decrease in the kidney weight, when compared to the cisplatininduced nephrotoxic rats. There is non-significant difference in the kidney weight of Cisplatin+Shilajit 200mg/kg p.o. administered rat when compared to the Cisplatin induced nephrotoxic rats and there is significant decrease in the kidney weight of Cisplatin+Shilajit 400 mg/kg p.o. and Cisplatin +Shilajit 800mg/kg p.o. when compared to the cisplatin induced nephrotoxic rats (Table 2).

**Table 2**. Effect of Shilajit on Body weight and Kidney Weight in Cisplatin Induced Nephrotoxicity

| Group No. | Treatment | Body Weight (gm) | Kidney Weight (gm) |
|---|---|---|---|
| 1 | Vehicle | 210.00±2.887 | 1.307±0.0135 |
| 2 | Cisplatin | 198.50±0.8466$^{\#\#}$ | 1.463±0.0120$^{\#\#}$ |
| 3 | Cisplatin + Cystone | 209.67±1.520$^{**}$ | 1.332±0.0079$^{**}$ |
| 4 | Cisplatin +Shilajit 200 | 205.50±0.4282$^{*}$ | 1.415±0.0156 |
| 5 | Cisplatin + Shilajit 400 | 205.67±1.116$^{*}$ | 1.383±0.0095$^{**}$ |
| 6 | Cisplatin + Shilajit 800 | 208.33±1.453$^{**}$ | 1.345±0.01668$^{**}$ |

N=6, values are expressed as Mean ± SEM. Comparisons were made as follows, *p < 0.05, **p < 0.01 when compared with normal control. *p <0.05, **p < 0.01 when compared with negative control (values are compared on 15$^{th}$ day by one way ANOVA Dunnett t test) N.S. – non significant

**Biochemical parameters**

***Urine Analysis***

A significant decrease in the urine volume in the Cisplatin induced nephrotoxic rats was observed when compared to the normal rats (Table 3). The Cisplatin+Cystone administered rats showed a significant increase in the urine volume when compared to Cisplatin induced nephrotoxic rats. There is non-significant difference in the urine volume of Cisplatin+Shilajit 200mg/kg p.o. administered rat when compared to the Cisplatin induced nephrotoxic rats and there is a significant increase in the urine volume of Cisplatin+Shilajit 400 mg/kg p.o. and Cisplatin+Shilajit 800mg/kg p.o. when compared to the Cisplatin induced nephrotoxic rats.

**Table 3.** Effect of Shilajit on Urine Volume, Urine Urea, Urine Creatinine and urine Uric acid in Cisplatin Induced Nephrotoxicity

| Group No. | Treatment Groups | Urine Volume (ml/day) | Urine urea (mg/dl) | Urine creatinine (mg/dl) | Urine uric acid (mg/dl) |
|---|---|---|---|---|---|
| 1 | Vehicle | 2.358±0.043 | 28.667±1.429 | 0.931±0.141 | 3.514±0.2060 |
| 2 | Cisplatin | 1.900±0.074$^{**}$ | 51.035±1.175$^{**}$ | 2.213±0.113$^{**}$ | 10.609±0.1763$^{**}$ |
| 3 | Cisplatin +Cystone | 2.230±0.0057$^{**}$ | 33.750±2.082$^{**}$ | 1.059±0.060$^{**}$ | 4.684±0.1215$^{**}$ |
| 4 | Cisplatin+Shilajit200 | 1.992±0.0300 | 49.553±1.826 | 1.937±0.088 | 9.942±0.1089 |
| 5 | Cisplatin+Shilajit400 | 2.133±0.0499$^{**}$ | 43.589±1.307$^{*}$ | 1.719±0.124$^{*}$ | 8.461±0.5525$^{**}$ |
| 6 | Cisplatin+Shilajit800 | 2.236±0.0053$^{**}$ | 32.517±0.784$^{**}$ | 1.184±0.105$^{**}$ | 4.815±0.2123$^{**}$ |

There is a significant increase in the urine urea, urine creatinine and urine uric acid in the Cisplatin induced nephrotoxic rats was observed when compared to the normal rats. The Cisplatin+cystone administered rats have showed significant decrease in the urine urea, urine creatinine and urine uric acid when compared to the Cisplatin induced nephrotoxic rats. There is a non-significant difference in the urine urea, urine creatinine and urine uric acid of Cisplatin+Shilajit 200mg/kg p.o. administered rat when compared to the Cisplatin induced nephrotoxic rats and there is significant decrease in the urine urea, urine creatinine and urine uric acid of Cisplatin+Shilajit 400 mg/kg p.o. and Cisplatin +Shilajit 800mg/kg p.o. when compared to the Cisplatin induced nephrotoxic rats.

### *$Na^+$ Ion & $K^+$ Ion Analysis*

There is a significant decrease in the urine sodium levels in the Cisplatin induced nephrotoxic rats was observed when compared to the normal rats. The Cisplatin+cystone administered rats have showed significant increase in the urine sodium levels when compared to the Cisplatin induced nephrotoxic rats. There is non-significant difference in the urine sodium levels of Cisplatin+Shilajit 200mg/kg p.o. administered rat when compared to the Cisplatin induced nephrotoxic rats and there is significant increase in the urine sodium levels of Cisplatin+Shilajit 400 mg/kg p.o. and Cisplatin+Shilajit 800 mg/kg p.o. when compared to the Cisplatin induced nephrotoxic rats (Table 4).

**Table 4.** Effect of Shilajit on Urinary $Na^+$ and Urinary $K^+$ in Cisplatin induced Nephrotoxicity.

| Group No. | Treatment Groups | $Na^+$ (meq/L) | $K^+$ (meq/L) |
|---|---|---|---|
| 1 | Vehicle | 148.32±2.143 | 4.499±0.260 |
| 2 | Cisplatin | 129.13±2.533$^{\#\#}$ | 9.570±0.193$^{\#\#}$ |
| 3 | Cisplatin +Cystone | 143.27±1.905$^{**}$ | 5.597±0.199$^{**}$ |
| 4 | Cisplatin +Shilajit200 | 131.16±2.497ns | 8.743±0.163$^{*}$ |
| 5 | Cisplatin +Shilajit400 | 138.88±1.582$^{*}$ | 7.642±0.222$^{**}$ |
| 6 | Cisplatin +Shilajit800 | 142.60±1.961$^{**}$ | 5.908±0.218$^{**}$ |

N=6, values are expressed as Mean ± SEM. Comparision were made as follows, *p < 0.05, **p < 0.01 when compared with normal control. *p <0.05, **p < 0.01 when compared with negative control (values are compared on 15th day by one way ANOVA Dunnett's t test) N.S. – non-significant

There is a significant increase in the urine potassium levels in the Cisplatin induced nephrotoxic rats was observed when compared to the normal rats. The Cisplatin+cystone administered rats have shown significant decrease in the urine potassium levels when compared to the Cisplatin induced nephrotoxic rats. There is a significant decrease in the urine potassium levels of Cisplatin+Shilajit 200mg/kg p.o., Cisplatin+Shilajit 400 mg/kg p.o. and Cisplatin+Shilajit 800mg/kg p.o. administered rat when compared to the Cisplatin induced nephrotoxic rats.

### *Serum Analysis*

There is a significant increase in the serum urea, serum creatinine and serum uric acid in the Cisplatin induced nephrotoxic rats were observed when compared to the normal rats. The Cisplatin +cystone administered rats have showed significant decrease in the serum urea, serum creatinine and serum uric acid when compared to the Cisplatin induced nephrotoxic rats. There is a non-significant difference in the serum urea, serum creatinine and serum uric acid of Cisplatin+Shilajit 200mg/kg p.o. when compared to the Cisplatin induced nephrotoxic rats. There is significant decrease in the serum urea, serum creatinine and serum uric acid of Cisplatin+Shilajit 400 mg/kg p.o and Cisplatin+Shilajit 800mg/kg p.o. administered rats when compared to the Cisplatin induced nephrotoxic rats (Nabila et al., 2015).

**Table 5**. Effect of Shilajit on serum urea, serum creatinine and serum uric acid in Cisplatin induced Nephrotoxicity

| Group No. | Treatment Groups | Serum Urea (mg/dl) | Serum Creatinine(mg/dl) | Serum uric acid (mg/dl) |
|---|---|---|---|---|
| 1 | Vehicle | 30.473±1.452 | 0.8557±0.1097 | 3.043±0.015 |
| 2 | Cisplatin | 53.870±1.371$^{**}$ | 1.730±0.05944$^{**}$ | 7.128±0.017$^{**}$ |
| 3 | Cisplatin +Cystone | 33.537±1.246$^{**}$ | 1.243±0.1163$^{**}$ | 4.015±0.009$^{**}$ |
| 4 | Cisplatin +Shilajit200 | 50.586±1.590 | 1.612±0.02522 | 6.803±0.028 |
| 5 | Cisplatin +Shilajit400 | 46.685±1.202$^{**}$ | 1.357± 0.01230$^{*}$ | 5.872±0.027$^{**}$ |
| 6 | Cisplatin +Shilajit800 | 35.531±1.257$^{**}$ | 1.257±0.1156$^{**}$ | 4.648±0.013$^{**}$ |

N=6, values are expressed as Mean ± SEM. Comparisons were made as follows, *p < 0.05, **p < 0.01 when compared with normal control. *p <0.05, **p < 0.01 when compared with negative control (values are compared on 15th day by one way ANOVA Dunnett's t test) N.S. – no significant

### Histopathological observations

The histopathological changes observed in the cisplatin induced nephrotoxic rats when compared to the kidneys of the normal rats with respect to glomerular congestion, glomerular hemorrhage, tubular dilatation, necrosis, cast in tubules, interstitial inflammation and changes in the blood vessels. Pathological changes were observed in the cisplatin induced nephrotoxic rats when compared to the normal rats. The rats administered with cisplatin+Shilajit have shown protection against tubular dilatation, necrosis, and casts in the tubules when compared to the cisplatin induced nephrotoxic rats.

**Table 6.** Results of the histopathological examination in Cisplatin induced Nephrotoxicity

| Group No. | Treatment Groups | Interstitial Inflammation | Blood vessels | Necrosis |
|---|---|---|---|---|
| 1 | Vehicle | Absent | Normal | Absent |
| 2 | Cisplatin | present | Congested | present |
| 3 | Cisplatin+ Cystone | Absent | Congested | Absent |
| 4 | Cisplatin+ Shilajit200 | present | Congested | present |
| 5 | Cisplatin+ Shilajit400 | present | Normal | Absent |
| 6 | Cisplatin+ Shilajit800 | present | Normal | Absent |

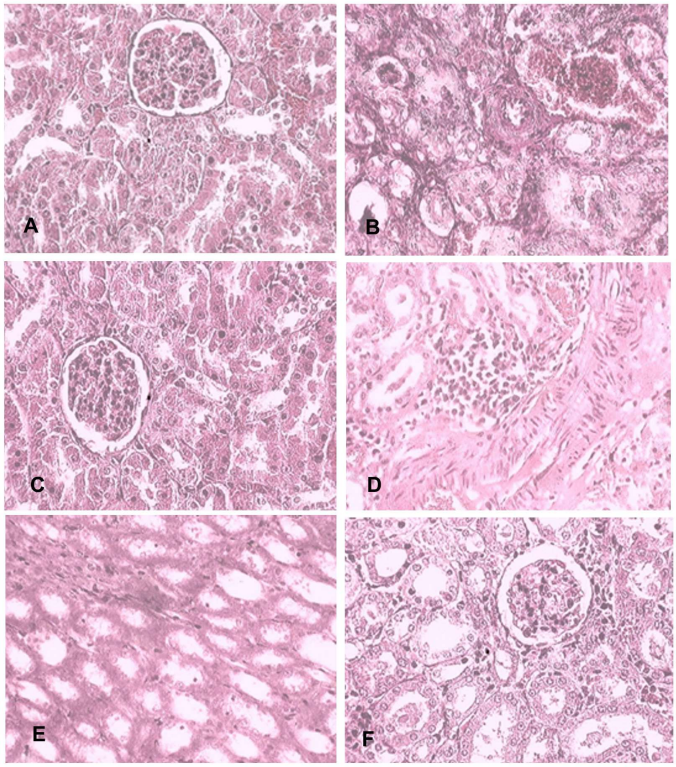


**Figure 1.** The histopatholgical observations of different groups in cisplatin induced nephrotxic Rats: Group I: Normal (X 40) showing normal glomeruli,(B) Group II: Negative control (cipaltin) ( X40),showing necrosis cast in tubules,glomerular Haemorrhage in congested blood vessels, (C) Group III : positive contains normal glomeruli with congested blood vessels, (D)Group IV: showing inflammation, cast in tubules,glomerular Haemmorrhage,(E) Group V: showing glomeruli with normal mild inflammation, (F) Group 6: Normal glomeruli.

## Conclusion

From the above data, Shilajit protects the cisplatin mediated nephrotoxicity .the was close relation between antioxidant capacity amount of chemical constituents presents in the herbominerals drug. Fulvic and humic acid having vital role in anti-oxidation as well as biological functions as well as biological functions. The anti-oxidants properties due to active ingredient such as fulvic acid and humic acid, etc.

**Conflicts of interest:** None

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**Conflicts of interest:** None

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