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# Ameliorative Effect of Cow Urine Against Cisplatin Induced Nephrotoxicity in Rats

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In this study, the nephroprotective activity of cow urine was undertaken. The effect of cow urine distillate was studied *in vivo* in rats intoxicated with cisplatin. The nephrotoxicity was induced with the administration of single i.p. dose of cisplatin 5 mg/kg body weight. The protective effect of cow urine distillate (in three dose levels) were studied in intoxicated rats. Parameters for the assessment of activity included kidney function tests and histological observations. The cow urine distillate produced dose dependent significant ($p < 0.05$) lowering of the elevated blood urea, serum creatinine and blood protein levels when compared with the toxic control. The presence of antioxidants in cow urine might cause the observed protective effects.

**Key Words: Nephrotoxic, Cow urine distillate, Cisplatin, Kidney function tests.**

## INTRODUCTION

The revered Indian cow, *Bos indicus* known as "Kamadhenu" in Indian scripts, is believed to be a "mobile hospital" for treatment of many diseases. A number of diseases can be cured by the use of medicines derived from the cow. Urine of cow is elaborately described in ancient Ayurvedic scriptures such as Charaka samhita, Shushruta samhita and Brahad-Wagbhatt as bitter, pungent, spicy and warm. It is used as an insecticide and as a regulator for various disorders like gas, acidity and cough. It promotes the power of wisdom in human beings, acts like a universal medicine and is easily digested by all[1].

The root cause of various diseases in human beings is believed to be due to shortage or accumulation of certain elements which are already in the body. The urine of the cow contains all such elements. Hence, according to Ayurveda it is considered as a natural and universal medicine to fulfill the shortage of element or to equalize and reduce the increased elements level in the body by restoring the excretion mechanisms of the body. Though Indian Ayurvedic literature cites the medicinal properties of cow urine, there is very little scientific evidence that supports the literature. Recently scientific attempts have been made to support the view[2].

## EXPERIMENTAL

**Cow urine distillate:** The early morning first voided urine of *Bos indicus* which were fed on open grass field was collected from the local cow sheds belonging to Sri Ramachandrapura math, Hosanagara, immediately distilled at 100 ºC using temperature controlled distillation apparatus and stored below 10 ºC for further use.

**Selection of dose:** For the evaluation of nephroprotecive activity of cow urine distillate, three dose levels were selected. The rat dose was calculated from human dose (60 mL per day), multiplying by a factor 0.018 × 5 which is equal to 5.4 mL/ kg body weight (first dose)[3]. The second dose was selected which was twice that of first dose *i.e.* 10.8 mL/kg body weight. The third dose was selected which was 50 % of the first dose *i.e.* 2.7 mL/ kg body weight.

**Animal treatment:** Albino Wistar rats of either sex (180-260 g) were obtained time to time from the laboratory of K.S Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore, India and were maintained on 12 h light/dark cycle and allowed food and water *ad libitum*. The institutional Animal Ethics committee of K.S. Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore, India, approved the experimental protocol in accordance with the guidelines provided by committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) with registration number KSHEMA/AEC/049/2007.

Animals were randomly assigned to five groups of six animals each and their body weight was noted. Group I received saline (10 mL/kg, p.o.) as normal control for ten days. Group II received saline (10 mL/kg, p.o.) for 10 days, followed by a single dose of cisplatin (5 mg/kg, i.p.) on the day eleven as treated control group. Groups III, IV, V, received cow urine distillate at the dose of 2.7 mL, 5.4 mL and 10.8 mL per kg body weight orally once daily respectively for 10 d followed by a single dose of cisplatin (5 mg/kg, i.p.) on the day eleven. The body weight of the animals was noted and percentage change in the body weight was calculated. From all the above groups blood was withdrawn through the retro orbital vein on the 16th day. The blood sample of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation and subjected for assessment of blood urea concentration, Serum creatinine level and blood protein level as renal function tests. Two animals from each group were sacrificed on the day of blood withdrawal. Kidneys were isolated and small pieces of the cortex of kidney of each animal were fixed in 10 % neutral buffered formalin, dehydrated in graded alcohol and embedded in paraffin wax. Sections (5 µm thick) were stained with haematoxyline and eosin (H&E) and subjected to microscopic examination for the presence of glomerular congestion, tubular casts, peritubular congestion, epithelial desquamation, blood vessel conjestion, interstitial edema and inflammatory cells[4].

**Statistical analysis:** The data were expressed as Mean ± SEM and analyzed using one way analysis of variance (ANOVA), followed by Dunnet's t-test. A probability value of $p < 0.05$ was considered as statistically significant.

## RESULTS AND DISCUSSION

The present study was undertaken to establish nephroprotective activity of cow urine. The results of nephroprotective effects of cow urine distillate on cisplatin intoxicated rats are shown in Table-1. Intoxication of rats with cisplatin significantly ($p < 0.05$) altered the biochemical parameters when compared with normal control rats. In the cisplatin treated group, blood urea, serum creatinine and protein levels were significantly elevated followed by significant loss in body weight of the experiment animals. Groups treated with cow urine distillate showed significant dose dependent decrease in blood urea, serum creatinine and protein levels ($p < 0.05$) when compared with cisplatin intoxicated rats. The animals showed the signs of recovery and an increase in the body weight was observed on the final day of observation. The histological observations basically support the results obtained from serum biochemical assays. When cisplatin intoxicated rat kidney sections (Fig. 2) are compared to normal sections (Fig. 1) showed marked congestion of the glomeruli with numerous tubular casts associated with epithelial desquamation. Marked peritubular congestion and edema were also observed. The interstitium showed infiltration with inflammatory cells and congestion. Groups treated with cow urine distillate (Fig. 3) showed almost normalization of kidney section. However mild glomerular, peritubular congestion and inflammatory cells were observed.

TABLE-1
EFFECT OF COW URINE DISTILLATE ON VARIOUS BIOCHEMICAL PARAMETERS IN CISPLATIN INDUCED RENAL DAMAGE

| Groups | % Change in body weight | Blood urea nitrogen (mg/dL) | Serum creatinine (mg/dL) | Blood protein (mg/dL) |
|---|---|---|---|---|
| I | 13.21 ± 2.13 [a] | 42.56 ± 1.76 [a] | 1.18 ± 0.31 [a] | 6.20 ± 0.1 [a] |
| II | -12.40 ± 3.01 | 127.25 ± 2.17 | 3.98 ± 0.63 | 8.02 ± 1.27 |
| III | 5.23 ± 1.18 [a] | 54.97 ± 3.98 [a] | 2.87 ± 0.3 [a] | 7.03 ± 0.23 [a] |
| IV | 6.19 ± 2.32 [a] | 52.10 ± 4.23 [a] | 2.61 ± 0.27 [a] | 6.91 ± 0.21 [a] |
| V | 6.29 ± 2.14 [a] | 47.27 ± 4.29 [a] | 1.28 ± 0.42 [a] | 6.61 ± 0.12 [a] |

Values are given as mean ± SEM for groups of six animals each; values are statistically significant at $p < 0.05$; [a]$p < 0.05$ significant as compared to group II.

*In vivo* and *in vitro* studies have demonstrated that reactive oxygen metabolites *viz.*, free radical species, super oxide, hydroxyl radical anion and hydrogen peroxide are important mediators of tissue injury[5]. Oxygen free radicals have been implicated in several biological processes, potentially important in glomerular diseases[6]. Reports suggest that cisplatin induces nephrotoxicity by initiation of lipid peroxidation and depletion of cellular thiols. Cisplatin inhibits the activity of antioxidant enzymes (super oxide dismutase, catalase, glutathione peroxidase) in rat kidneys[7], there by suggesting that cisplatin cytotoxicity results from generation of reactive oxygen species. The results obtained in the present study correlate with previous reports that lipid peroxidation contributes to cisplatin induced nephrotoxicity. In the present study,

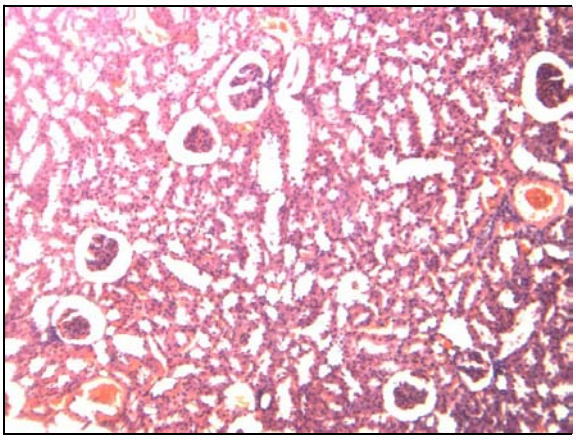

Fig. 1. Normal rat kidney showing normal glomeruli and tubules

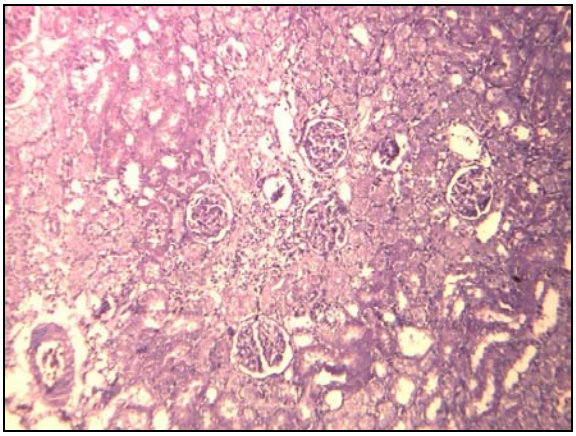

Fig. 2. Cisplatin intoxicated showing glomerular congestion, peritubular congestion, inflammatory cells and epithelial desquamation

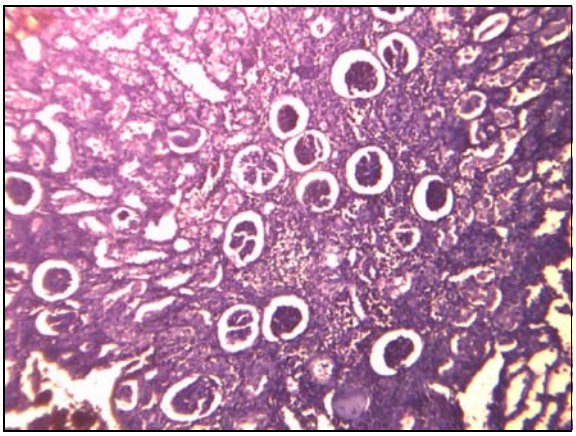

Fig. 3. Cisplatin + cow urine distillate showing mild inflammatory cell infiltration

cow urine was found to reverse the cisplatin induced nephrotoxicity. Hence the possible mechanism of nephroprotection by cow urine may be attributed to its anti-oxidant and free radical scavenging properties.

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