ORIGINAL ARTICLE

Protective properties of dietary inclusion of *Ocimum sanctum* on cisplatin-induced nephrotoxicity in rats

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Abstract

Background: Nephrotoxicity is a frequent severe side effect of cisplatin chemotherapy, limiting its clinical use despite being one of the most potent chemotherapy drugs. In this study, we investigated the nephroprotective potential of dietary containing *Ocimum sanctum* against cisplatin-induced nephrotoxicity in rats. Materials and **Methods:** Adult male rats were randomly divided into four groups with six animals in each group. Groups 1 and 2 were fed basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil, and 4% mineral and vitamin premix) for 6 weeks. Groups 3 and 4 were fed basal diet supplemented with 2% and 4% *O. sanctum* leaves, respectively, for 6 weeks. Groups 2-4 received a single intraperitoneal dose of cisplatin (7.5 mg/kg BW) after the 5th week of the experiment. Histopathological study of kidney was performed using light microscopy. **Results:** The blood urea, creatinine, uric acid, total protein, blood urea nitrogen (BUN), urine volume, and urine pH were analyzed. The administration of basal diet supplemented with 2% and 4% *O. sanctum* to rats significantly reduced the creatinine, urea, uric acid, urine pH, BUN, and total protein compared to cisplatin control group, while a significant increase in urine output was observed. Pretreatment with a basal diet supplemented with 2% and 4% *O. sanctum* were significantly prevented histopathological changes in kidney toward normal. **Conclusion:** These results suggest that dietary inclusion of *O. sanctum* could protect against cisplatin-induced nephrotoxicity.

Key words: Creatinine, nephrotoxicity, *Ocimum sanctum*, urea, uric acid

INTRODUCTION

Synthetic drugs are a common source of acute injury of kidney. Compared with 30 years ago, the average patient today is older, has more comorbidity and is exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function. Drugs shown to be caused nephrotoxicity exert their toxic effects by one or more common pathogenic mechanisms. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. Therefore, successful prevention requires knowledge of pathogenic mechanisms of renal injury, patient-related risk factors, drug-related risk factors and preemptive measures, and coupled with vigilance and early intervention. General preventive measures include using alternative non-nephrotoxic drugs whenever possible; correcting risk factors, if possible; by adjusting the dosage; monitoring renal function; vital signs during therapy; and avoiding nephrotoxic drug combinations.[1,2]

The use of chemotherapeutic agents for the treatment of cancer has opened new prospective for improvement of the quality of life of cancer patients. However, besides its success, many anticancer drugs have been shown to be teratogenic and carcinogenic in experimental systems.[3,4] Cisplatin (cis-diamminedichloroplatinum II) is a major antineoplastic chemotherapy drug for the treatment of various forms of cancers such as ovarian, testicular, bladder, head and neck, and uterine cervix carcinomas. However, the clinical use of cisplatin is limited because of its unwanted side effects such as nephrotoxicity and hepatotoxicity. Thus, there is a continuous search for agents that provide nephroprotection against cisplatin drugs.[5,6]

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Ocimum sanctum is a well-known medicinal plant, which grows wild as well as in households and temples in India. It has been traditionally regarded as possessing rejuvenating, tonic and vitalizing properties that contribute to longevity and a healthy life. Leaves of *O. sanctum* possess expectorant, diaphoretic, antiseptic, spasmolytic, nephroprotective, stimulant, and anticatarrhal properties and are used as cold and cough remedies, for fever, pain, gastrointestinal disorders (such as dyspepsia and vomiting), worm infestations, skin diseases, snakebite, and scorpion sting.[7-10] Significant nephroprotective activity of *O. sanctum* was reported earlier, and in view of its nephroprotective properties, it is worthwhile to investigate and establish the nephroprotective potential of dietary containing *O. sanctum* against cisplatin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Collection and Identification of Plant Material

O. sanctum of vouchered herbarium specimens was prepared and preserved along with crude drug sample at the herbarium (BOT/541) of Department of Botany, Govt. P.G. College, BHEL, Bhopal, Madhya Pradesh, India. The plant materials were shade dried, reduced to coarse powder and stored in an airtight container until further use.

Preparation of Dietary Inclusion

The basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil, and 4% mineral and vitamin premix) was prepared and fed to normal and control group animals. The basal diet supplemented with 2% and 4% preparation of dietary containing *O. sanctum*.

Selection of Animals

Male Wistar rats (150-200 g) were used, and kept in quarantine for 10 days under standard husbandry conditions (27.3°C, relative humidity 65% ± 10%) for 12 h in dark and light cycle, respectively, and were given standard food and water *ad libitum*. All experiments were approved by the Institutional Ethical Committee and were carried out according to the Animal Ethics Committee guidelines.

Chemoprotective Activity of Dietary Inclusion Against Cisplatin-Induced Nephrotoxicity

The basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil, and 4% mineral and vitamin premix) was prepared and fed to normal and control group animals. The basal diet supplemented with 2% and 4% *O. sanctum* and fed to cisplatin-induced nephrotoxicity animals. After acclimatization, the rats were randomly divided into four groups of six animals each. Groups 1 and 2 were fed basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil, and 4% mineral and vitamin premix) for 6 weeks. Groups 3 and 4 were fed basal diet supplemented with 2% and 4% *O. sanctum* leaves, respectively, for 6 weeks. Groups 2-4 received a single intraperitoneal dose of cisplatin (7.5 mg/kg BW) after the 5th week of the experiment. All the animals were sacrificed after 1 week of cisplatin administration.

At the end of experimental period, all the animals were sacrificed under diethyl ether anesthesia. Blood samples were collected from the rat using retro-orbital puncture method, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Biochemical Parameters

On the respective day of completion of studies, blood was collected from rats by retro-orbital puncture method and subjected to biochemical parameters, i.e., estimation of blood urea, creatinine, uric acid, total protein, and blood urea nitrogen (BUN) were analyzed estimations by using prietest biochemical kits by ROBONIK biochemical analyzer.

The kidney weight, body weight of animals, urine volume, and urine pH were measured.[11-15]

Histopathology of Kidney

Removed kidney was fixed in 10% neutral buffered formalin for 24 h at room temperature. After the fixation, the samples were dehydrated by bathing them in a graded series of mixtures of ethanol and water. This was followed by a hydrophobic clearing agent (xylene) to remove the alcohol, and finally the infiltration agent (paraffin wax), which replaces the xylene. Finally, tissue samples are embedded in paraffin. Samples from each specimen are sectioned at 50 microns in thickness using a rotary microtome.

The sections are stained with hematoxylin (hematoxylin solution) and eosin (eosin Y solution 0.5% alcoholic). Sections were observed under a microscope (Olympus CH20*i*) at ×40 and ×100 magnification.[16,17]

Statistical Analysis

Results were analyzed using one-way analysis of variance followed by the Tukey's test using a statistical software package, GraphPad Prism; version 5.03. Values were expressed as mean ± SEM and the $P < 0.05$ was considered as statistically significant.

RESULTS

Chemoprotective Activity of Dietary Inclusion Against Cisplatin-Induced Nephrotoxicity

The rats treated with cisplatin leads to significant increase in serum creatinine, urea, uric acid, BUN, and total protein compared to normal rats group [Table 1]. The administration of basal diet supplemented with 2% and 4% *O. sanctum* to rats significantly reduced the creatinine, urea, uric acid, BUN, and total protein compared to cisplatin control group.

The body weight of cisplatin control rats decreased significantly compared to normal groups. The rats treated with basal diet supplemented with 2% and 4% *O. sanctum* enhanced the body weight indicating recovering of nephrotoxicity. The urine volume of control group decreased significantly while the pH of urine increased. However, administration of basal diet supplemented with 2% and 4% *O. sanctum* increased the urine output and decreased the pH of the urine compared to control group. Cisplatin-treated group also showed that kidney weight was significantly increased compared to normal control but downregulated by basal diet supplemented with 2% and 4% *O. sanctum* [Table 2].

Histopathological Studies

Histopathological study was performed using light microscopy. Microscopic examination of normal kidney showing tubular brush borders and intact glomeruli without any structural alterations in renal tissues [Figure 1a]. In cisplatin treated renal tissues (Group 2) showed swelling and massive and diffuse cell necrosis in proximal tubules of kidneys indicates cell injuries [Figure 1b]. Pretreatment with basal diet supplemented with 2% and 4% *O. sanctum* was significantly prevented histopathological changes toward normal [Figure 1c and d]. Cisplatin-induced nephrotoxicity was evidenced by biochemical measurements and histopathological changes that coincide with the observations of other investigators.

This inhibitory action of basal diet supplemented with 2% and 4% *O. sanctum* against nephrotoxin was confirmed through biochemical and histopathological studies. This

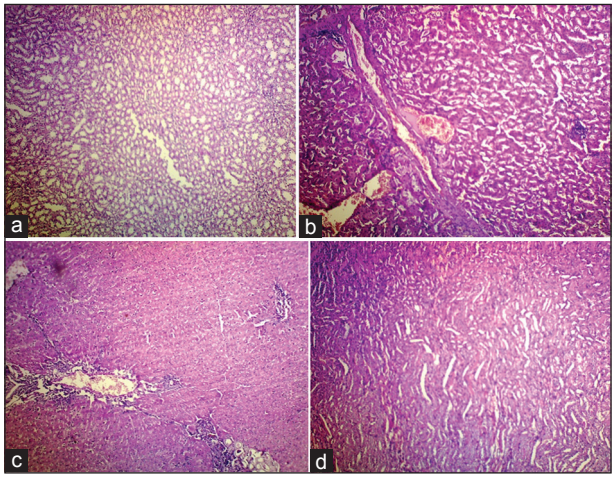

Figure 1: (a) Microscopically photograph of liver section of normal rats (Group-1), (b) microscopically photograph of liver section of cisplatin control rats (Group-2), (c) microscopically photograph of liver section of diet supplemented with 2% *O. sanctum* (Group-3), and (d) microscopically photograph of liver section of diet supplemented with 4% *O. sanctum* (Group-4)

Table 1: Effect of diets supplemented with *O. sanctum* on kidney function test in animals treated with cisplatin

Treatment	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	BUN (mg/dL)	Total protein (g/dL)
Normal rats (basal diets)	38.24±0.11	1.53±0.42	0.48±0.82	19.46±2.17	4.92±0.46
Control rats (cisplatin + basal diets)	163.15±0.63*	6.13±0.35*	3.21±1.53*	112.42±3.25*	21.73±1.59*
O. sanctum (2%) + cisplatin (6 mg/kg)	89.62±0.35[a]	2.36±0.61[a]	1.58±1.68[a]	72.59±1.78[a]	8.68±2.05[a]
O. sanctum (4%) + cisplatin (6 mg/kg)	37.83±0.58[a]	1.72±0.78[a]	0.53±0.61[a]	26.17±2.92[a]	4.52±2.38[a]

Values are expressed as mean±SEM, *n*=6 in each group. *P<0.05 when compared with normal group, [a]P<0.05 when compared with cisplatin treated group. *O. sanctum: Ocimum sanctum*

Table 2: Effect of diets supplemented with *O. sanctum* on body weight, urine volume, urine pH, and kidney weight treated in rats

Treatment	Body weight (g)	Urine volume (ml)	Urine pH	Kidney weight (g)
Normal rats (basal diets)	185.43±2.56	31.86±1.21	5.24±0.59	1.24±1.43
Control rats (cisplatin + basal diets)	128.36±3.17*	11.54±1.41[a]	9.73±01.18*	3.61±0.91*
O. sanctum (2%) + cisplatin (6 mg/kg)	171.62±2.91	23.94±0.86	6.24±0.84	1.82±1.29
O. sanctum (4%) + cisplatin (6 mg/kg)	192.72±1.48[a]	33.15±1.36*	5.17±1.51[a]	1.19±1.05[a]

Values are expressed as mean±SEM, *n*=6 in each group. *P<0.05 when compared with normal group, [a]P<0.05 when compared with cisplatin treated group. *O. sanctum: Ocimum sanctum*

activity may due to the presence of secondary metabolites such as flavonoid and polyphenolic compounds which may be responsible for the kidney protective activity.

DISCUSSIONS

In this study, the observed nephrotoxicity due to cisplatin treatment was manifested by marked increases in serum creatinine, urea and uric acid accompanied by a decrease in urine creatinine, urea, uric acid, and creatinine clearance. This may be due to the decrease in the glomerular filtration rate or may be secondary due to the increase of the reactive oxygen species which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that thereby decrease the glomerular filtration rate.

Furthermore, the destruction of proximal and distal tubules observed in the cisplatin treated rats preceded the renal homodynamics, suppressed the reabsorption, increased vascular resistance and caused the elevation in BUN and creatinine levels.

For over a decade, oxidative stress has been regarded as one of the most widely accepted factors that contribute to cisplatin nephrotoxicity. Cisplatin treatment can lead to accumulation of endogenous ROS and oxidative stress within the renal tubular cells and kidney slices, as well as *in vivo* in whole animals. Meanwhile, during cisplatin treatment, a robust inflammatory reaction occurs and inflammasomes are also stimulated, further exacerbating renal tissue damage.[18-21] In our present study, we found that the pretreatment with basal diet supplemented with 2% and 4% *O. sanctum* significantly attenuated cisplatin-induced nephrotoxicity not only by its antioxidation effect but also by decreasing the expressions of several proinflammatory cytokines. The above findings support the observation of histopathological studies.

CONCLUSION

The study demonstrated that administration of cisplatin induces nephron damage in rats. However, *O. sanctum* supplemented diets ameliorate this cisplatin-induced nephrotoxicity through improvement in the rats' antioxidant status and modulating oxidative stress. Consequently, dietary inclusion of *O. sanctum* may be a cheap management strategy in the management of acute nephrotoxicity or cisplatin-induced renal damage.

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