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Nephroprotective Effects of *Dioscoreaalata* and *Moriengaolifera* on Cisplatin Induced Nephrotoxicity in Rats

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Abstract

Nephrotoxicity, which is caused by mostly due to medication, is one of most important global health problem. Drugs are a common source of acute kidney injury. Drugs shown to cause nephrotoxicity exert their toxic effects by one or more common pathogenic mechanisms. . Most drugs found to cause nephrotoxicity exert toxic effects by one or more common pathogenic mechanisms. These include altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. Cisplatin, one of the many drugs, is responsible for severe nephrotoxicity. General preventive measures to avoid nephrotoxicity include using alternative non-nephrotoxic drugs; correcting risk factors; assessing baseline renal function before initiation of therapy, followed by adjusting the dosage; monitoring renal function and vital signs during therapy; and avoiding nephrotoxic drug combinations, using of several nephroprotective agents including medicinal plant extracts. Promising results showed that the use of some medicinal plant extracts (*Dioscoreaalata* and *Moriengaolifera*) gave rise to moderate restoration of normal physiology of kidney and liver of mice.

Key words: Nephroprotective, *Dioscoreaalata*, *Moriengaolifera,* cisplatin, rat.

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Introduction

The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs.[1] Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs.[2] Approximately 20% of nephrotoxicity is induced by drugs, but medication of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity.[3,4] However, these assessments of nephrotoxicity are only possible when a majority of kidney function is damaged.[5]

Mechanism of Drug-induced nephrotoxicity

Cisplatin is cleared by the kidney by both glomerular filtration and tubular secretion.[8] Cisplatin concentrations within the kidney exceed those in blood suggesting an active accumulation of drug by renal parenchymal cells. Previous studies using kidney slices,[9] cultured renal

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epithelial cells[10] and isolated perfused proximal tubule segments[11] have provided evidence for basolateral-to-apical transport of cisplatin. Studies in recent years have identified two different membrane transporters capable of transporting cisplatin into cells: Ctr1 and OCT2. Ctr1 is a copper transporter which was also shown to mediate cisplatin uptake into mammalian cells[12], including ovarian cancer cells.[13] Ctr1 is highly expressed in adult kidney and the protein localizes to the basolateral membrane of the proximal tubule.[14] Downregulation of Ctr1 expression in kidney cells *in vitro* decreased both cisplatin uptake and cytotoxicity, suggesting that Ctr1 is an important cisplatin uptake mechanism in these cells.[14] The role of Ctr1 in cisplatinnephrotoxicity *in vivo* has not been examined. In addition, the organic cation transporter OCT2 (SLC22A2) transports cisplatin.[15-18]

Cisplatin was shown to inhibit the uptake of other OCT2 substrates, consistent with the view that these substrates share a common transport pathway. Likewise, cimetidine, an OCT2 substrate, reduced cisplatin uptake and cytotoxicity *in vitro*[15-17] and cisplatin nephrotoxicity *in vivo.*[7]

Prevention of cisplatin induced nephrotoxicity, several approach

Many strategies have attempted to prevent or reduce its nephrotoxicity.[19,20] Early experience suggested that the administration of cisplatin by prolonged continuous infusion and saline hyperhydration, with or without frusemide or mannitol osmotic diuresis, reduces nephrotoxicity.[19] Amongst many others, the drugs studied have included a variety of sulfur-containing compounds, such as sodium thiosulfate, WR-2721 (amifostine), DDTC (sodium diethyldithiocarbamate), mesna, biotin, cephalexin and sulfathiazole, all of which probably react with nephrotoxic cisplatin metabolites to form less toxic products.[20,21]

Protective effects of plants materials in drug-induced nephrotoxicites

Several medicinal plants extract were used to prevent cisplatin induced nephrotoxicity. One study suggested that protective effect of terpenes isolated from the fruiting bodies of *Ganodermalucidum* (Fr) P. Karst found promising reduction of nephrotoxicity caused by the cisplatin, in mice. Intraperitoneal administration of cisplatin (16 mg/kg body wt) resulted in significant nephrotoxicity in mice. The results suggest the potential therapeutic use of *Ganoderma terpenes* to prevent nephrotoxicity caused during chemotherapy using cisplatin.[22]

One study suggested that after treatment with MNLE (methanolicneem leaves extract), the histological damage and apoptosis induction caused by cisplatin were improved.[23]

One study suggested that, *Taxilli Ramulus* could protect against cisplatin induced acute renal failure in mice.[24-26]

Many other herbal medicines such as *Dioscorea species* may reduce cisplatin induced nephrotoxicitieshas been reported. One study revealed that *Morienga. oleifera* seed powder at the dose rate of 600 mg/kg is as efficacious as silymarin in exerting nephroprotective and antioxidant effects.[27] We reviewed clinical and experimental literature on cisplatin nephrotoxicity to identify new information on the mechanism of injury and further study will be emphasis the potential approaches on some medicinal plants *Dioscoreaalata* and *Moringaolifera.*[27-31]

Place and duration of the research work: This research work was carried out in a) Indoor patient Department, Department of Oncology, Khwaja Yunus Ali Medical College**,** Hospital, Enayetpur, Sirajgonj. b) Centre for bioequivalence study, Khwaja Yunus Ali Medical College Hospital, Enayetpur, Sirajgonj c) Pharmacology Research Lab., Department of Pharmacology and Histology Laboratory of Anatomy of BSMMU, Shahbag, Dhaka from January 2012 to December 2014.

Materials and Methods

a) Methanolic extract of tuber part of the *Dioscoreaalata* and seed pods of *Moringaoleifera.*

b) Cisplatin induced liver and kidney of rat

1. Phytochemical investigation

Collection and identification of plant materials (tuber parts of *Dioscoreaalata* and seed pods of *Moringaoleifera*), Drying, Grinding and weighting of plant material, Extraction of plant materials with MeOH, Collection of powdered material from plant extracts-After evaporation gummy part of tuber parts of *Dioscoreaalata* and seed pods of *Moringaoleifera* were collected from the wall of volume flask weighted to 56 g and 34 g respectively. Two extracts were preserved in two separate air tight bottle for future biological investigations.

2. Histopathology of liver and kidney of Swiss albino rat

The most common procedure used in histologic research is the preparation of tissue sections or slices that can be studied with the light microscope. Under the light microscope, tissues are examined visually in a beam of transmitted light. Because most tissues and organs are too thick to pass through them, they must be sliced to obtained thin, translucent sections that are attached to glass slides for microscopic examination.[37]

Collection of rats

Animal House, ICDDR'B, Mohakhhali, Dhaka. (vide memo no. 0461). Total number of test animal (Swiss albino rats)[32]: 40. Average wt of rat: 130 g. Total number of groups =4. Group A- Cisplatin, Group B- Cisplatin + *D. alata*,[33,34] Group C- Cisplatin + *Moringaoleife*,[35,36] Group D- Placebo control.

Animal Dose & Duration: A. Injectable :(a) Cisplatin :16 mg / kg body wt, given every weeks for 2 weeks.(b) Metabolite2 :16 mg / kg body wt, given every weeks for 2 weeks.

(c) Metabolite 4 :16 mg / kg body wt, given every weeks for 2 weeks. B. Powdered extract: 250mg/kg body-wt.[39]

Steps in preparation of Slides

I. Sectioning of Tissue- Paraffin sectioning: The steps in paraffin sectioning are-a. Fixation of the Tissue: Formol-saline (Formalin 10 ml and normal saline 90 ml) is commonly used as a used as a fixative b. Dehydration of the tissue in ethyl alcohol and xylol c. Preparation of Block-The tissue is casted in wax. d. Section cutting- Sections are cut with microtome and taken on slide.

II. Staining of the sections by- Haematoxylin and Eosin (H & E) Stains. Characteristics of Haematoxylin and Eosin (H & E) Stains[37,38] are-

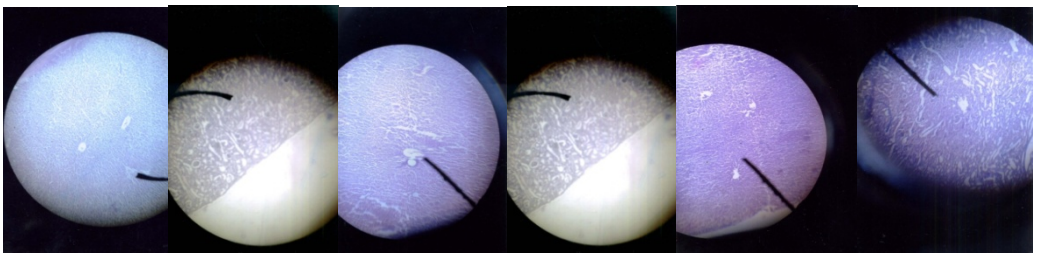
1. Nuclei stain blue. 2. Cytoplasm stain pink or red3. Collagen fibers stain pink4. Muscle stain pink.

Steps of preparing histological slide- 1. Treat with xylol to remove wax2. Treat with alcohol to remove xylol3. Rinse in water4. Stain in haematoxylin for 15-30 minutes.

5. Rinse in water for 15-30 minutes6. Differentiate in acid-alcohol till nuclei are only stained 7. Rinse in water for 20-30 minutes8. Stain in 1% eosin for 5-15 minutes 9. Wash in running water till eosin is differentiated10. Blot, dehydrate in alcohol, clear in xylol11. Mount the section in Canada balsam on a slide.

Results:

Photograph 1: Histopathology of liver and kidney of mouse after cisplatin administration.



Liver Kidney Liver Kidney Liver Kidney

Group A Group B Group C

Results of Histopathological changes in cisplatin induced liver and kidney of rat, group A showed that normal findings of liver architecture and in kidney proximal tubular necrosis, while

Table 1: Histopathology of liver and kidney of rat after cisplatin administration

Drug	Liver	Kidney
Cisplatin	Normal findings of liver architecture.	Proximal tubular necrosis –some proximal tubular cells became swollen and some of the cell's nucleus became shrinkaged (pyknotic cell)

in group B and group C that was treated with extract of *D. alata* and extract of *Maringa olifera* has showed restoration of normal proximal tubular cells though moderate tubular necrosis were seen (Table 1-4).

Table 2: Histopathology of cisplatin treated liver and kidney of rat after *D. alata* extract administration

Drug	Liver	Kidney
Cisplatin and *D. alata* extact	No changes were found in liver architecture.	Moderate amount of tubular necrosis were found. Rest of the tubular proximal cells became normal. Some of the proximal tubules show pyknotic cells and some are enucleated cells, some were swollen.

Table 3: Histopathology of cisplatin treated liver and kidney of rat after *M. olifera* extract administration

Drug	Liver	Kidney
Cisplatin and *M. olifera* extract	Normal hepatocytes was seen	Moderate amount of tubular necrosis were found. Rest of the tubular proximal cells became normal. Some of the proximal tubules show pyknotic cells and some are enucleated cells, some were swollen.

Discussion

Massive necrosis of the proximal convoluted tubules with distorted cellular architecture along with other distal tubular abnormalities were seen the rat groups that was treated with cisplatin. A promising results showed that the use of some medicinal plant extracts (*D. alata and Moriengaolifera*) gave rise to moderate restoration of normal physiology of kidney and liver of rat (Photograph 1 & Figure 1-4).

The experimental results reveal that, the methanolic extract of *D. alata* and *M. olifera* could help prevent nephrotoxicity manifested consequent to cisplatin chemotherapy. The effect is mainly due to the capacity of the extract to restore renal antioxidant defense system. Our earlier investigations have shown that of methanol extract of *D. alata*and *M. olifera* occurring in South India possessed significant antioxidant and antitumour activities. Further study concerning the structure of suspected metabolites will be elucidated by using HPLC-mass spectrometry.

Conclusion

The extracts of *D. alata and Moriengaolifera* had some effects on renal histology in injured mice kidney was very promising and significant. Results suggest that *D. alata and Moriengaolifera* extract may be used to cure or prevent not only cisplatin-induced renal toxicity but also other drug induced nephrotoxicities without any adverse effect; hence it can serve as a novel combination agent with cisplatin and other drugs to limit renal injury.

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