International Scholars Journals

International Journal of Anatomy and Physiology ISSN 2326-7275 Vol. 12 (4), pp. 001-006, April, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Protective effect of ethanol extract of neem leaves on cisplatin-induced kidney damage in wistar rats

Terkula Kpela[1]*, Theresa B. Ekanem[2] and Amaber O. Akpantah[2]

[1]Department of Anatomy, Benue State University College of Health Sciences, Makurdi, Nigeria.
[2]Department of Anatomy, University of Calabar, Calabar, Nigeria.

Accepted 27 January, 2023

The protective effect of neem leaf extract on cisplatin-induced kidney damage in wistar rats was investigated. Twenty rats weighing 180-200g were divided into four treatment groups, each containing five animals. Group I served as control. Group II received a single intraperitoneal injection of cisplatin (10 mg/kg) on day one. Group III received oral administration of neem leaf extract at a dose of 500 mg/kg/day for 14 days followed by intraperitoneal injection of cisplatin at a dose of 10 mg/kg. Group IV received a single intraperitoneal injection of cisplatin at a dose of 10 mg/kg on day one followed by oral doses of neem leaf extract (500 mg/kg/day) for 14 days. All animals were sacrificed under chloroform anaesthesia two days after the last treatment day of the experiment. Blood was collected by cardiac puncture for biochemical analysis of serum electrolytes, urea and creatinine. The kidneys were removed and processed through paraffin sections for hematoxylin and eosin as well as deoxyribonucleic acid staining. Results showed that cisplatin-induced apoptosis, necrosis, and raised serum electrolytes, urea and creatinine levels were normalized by pretreatment with neem leaf extract. We conclude, therefore, that neem leaf extract can attenuate cisplatin-induced nephrotoxicity in wistar rats.

Keywords: Nephrotoxicity, cisplatin, neem leaf extract, wistar rat.

INTRODUCTION

Cancer is basically a disease of the cells characterized by a shift in the control mechanisms that govern cell proliferation and differentiation (Cotran et al, 1999). The incidence, geographic distribution and behavior of specific types of cancers are related to multiple factors such as sex, age, race, genetic predisposition and exposure to environmental carcinogens (Cotran et al, 1999). Cancer is a well-known and relatively common cause of death. With present methods of treatment, one third of patients are cured with local modalities, such as surgery or radiotherapy, which are quite effective when the tumor has not metastasized by the time of treatment. However, in the remaining cases, early micro metastasis is a characteristic of the neoplasm, indicating that a systemic approach such as chemotherapy is required, often along with surgery or radiotherapy, for effective cancer management (Katzung, 2004). An ideal anticancer drug would eradicate cancer cells without harming normal tissues. Unfortunately, no currently available agents meet this criterion, and clinical use of these drugs involves a weighing of benefits against toxicity in a search for a favorable therapeutic index (Katzung, 2004). cisplatin is one of the most widely used cytotoxic therapeutic

*Corresponding Author E-mail: dookpelu@yahoo.com; Tel: +234 806 783 8483

agents for the treatment of different cancers including testicular, germ cell, head and neck, bladder and lung cancers. It is an alkylating agent which at effective higher doses causes many adverse effects such as nephrotoxicity and genotoxicity (Kamanyire, 2008). Several strategies have been explored to reduce the side effects of cisplatin therapy but most of these measures have shown limited success. The question, therefore, is, 'How can the kidneys be effectively protected against cisplatin-induced damage'? Many studies have been directed towards reducing the cytotoxic impact of cisplatin on the kidneys (Gamel el-Din and Al-Bekairi, 2006; Howle and Gale, 1970; Maliakel et al 2008; Nisar and Feinfeld, 2002; Pfeifle et al, 1985; Tebekeme and Prosper, 2007; Umeki et al, 1988).

Of recent, beneficial effects of medicinal plants against some pathologies have gained considerable interest, neem being one of such well-known medicinal plants. Several studies have been undertaken on the protective effects of neem (Arivazhagan et al, 2004; Bhanwra et al, 2000; Biswas et al, 2002; Chattopadhyay, 2003; Dorubabu et al, 2006; Gupta et al, 2004; Mbah et al, 2007). It is against this background that the present study was conceived to evaluate the possible protective effect of ethanolic extract of neem leaves on cisplatin- induced nephrotoxicity in wistar rats.

MATERIALS AND METHOD

This experimental study was carried out in the department of Anatomy, College of Basic Medical Sciences, university of Calabar, Nigeria.

Extract preparation

Fresh neem leaves were harvested from the Botanical Garden of the university, duly identified and authenticated by the chief Herbarium. They were washed with water to remove debris and sand and spread under shed to remove excess water. The leaves were oven-dried and ground into powder using a table grinder. 1.5kg of the powder was mixed with 2.8 liters of Ethanol and the mixture was allowed to stand overnight in the refrigerator. The following morning, this mixture was blended and filtered. The filtrate was evaporated using a desiccator and the resultant yield was 60g of the extract which was about 4% yield. This was preserved in the refrigerator at 40c until ready for use. Before administration, a sensitive balance was used, each day, to weigh out 3g of the extract which was dissolved in 20 ml of distilled water. The resultant solution had a concentration of 150mg/ml (or 75mg/0.5ml) and this was given at a dose of 500mg/kg/day for 14 days.

Drug procurement

Cisplatin (kemoplant, RDurbar Pharma LTD) was purchased from Kamel Pharmacy LTD, Calabar. It is a sterile solution of cisplatin USP 1.0mg/ml (50ml pack) and sodium chloride USP 9mg/ml in water for injection USP. It was stored at room temperature but protected from light until ready for use.

Animal preparation

Twenty adult male wistar rats weighing 180-200g were used. They were purchased from the Animal House in the Department of Agriculture and kept in the Animal House of Anatomy Department, university of Calabar under standard laboratory conditions (12h light and 12h dark). The rats were fed on grower's mash produced by Bead Feed and flour mills limited, Calabar. Food and water were provided ad libitum. The animals were randomized into four groups with five animals per group.

Treatments

Group I (control) received water and food only.

Group II received a single dose of cisplatin (10mg/kg) intraperitoneally on first day of the experiment.

Group III received neem extract 500mg/kg/day orally for 14 days, followed by a single dose of cisplatin 10mg/kg intraperitoneally on day 14.

Group IV received a single dose of cisplatin 10mg/kg intraperitoneally on first day, followed by neem extract 500mg/kg/day orally for 14 days.

A day after drug and extract administration, the animals in all the groups were sacrificed under chloroform anesthesia. Blood was collected directly, through cardiac puncture for measurement of serum potassium, sodium, bicarbonate, urea and creatinine levels. The kidneys of each animal were removed and washed with cold saline, blotted dry on filter paper and weighed. They were then processed through paraffin sections for hematoxylin and eosin staining using Drury and Wailington method, 1967 and deoxyribonucleic acid staining using Fuelgen and Rossenbeck method, 1924.

Data generated by weighing the kidneys and animals as well as biochemical estimation of serum creatinine, electrolytes and urea was subjected to analysis of variance (ANOVA). The level of statistical significance was taken as $P<0.05$.

Table 1. Ratio of kidney weight to total body weight among the various groups

Groups	Ratio of kidney weight to total body weight
I(Control)	0.43±0.013
II(Cisplatin injection 10 mg stat on day one)	0.58±0.019**
III(Neem extract 500 mg/kg daily for 14 days followed by cisplatin injection 10 mg stat on day 14)	0.45±0.021*
IV(Cisplatin injection10 mg stat on day one followed by neem extract 500 mg/kg daily for 14 days)	0.49±0.015*

p<0.05.

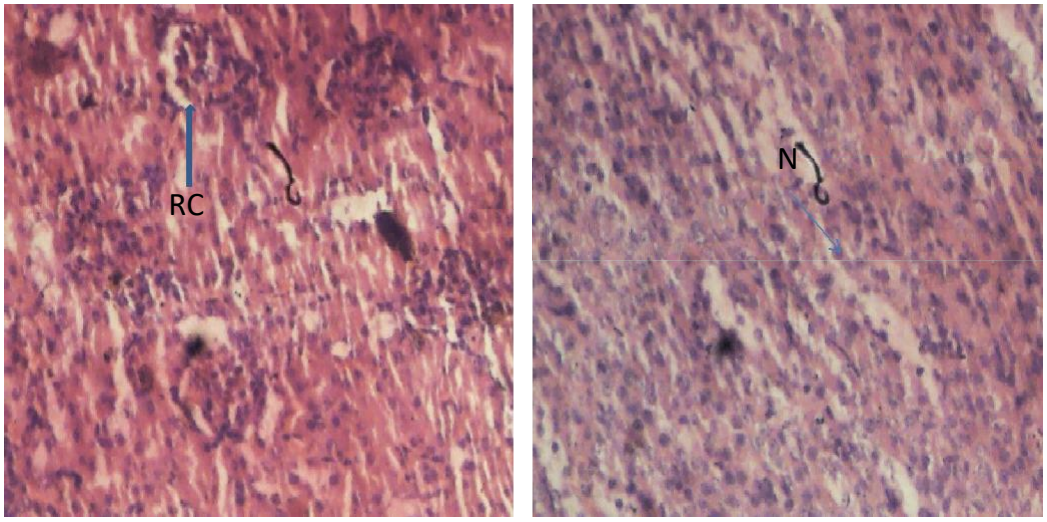

Plate 1. photomicrograph of kidney of animals from control group. Left – H and E section showing normal renal corpuscles RC and tubular cells. Right – histochemical section showing normal staining intensity and tubular cells with prominent nuclei N within the section.
Magnification: ×400.

RESULTS

Morphological analysis

The kidneys of animals in group I which served as control had no morphological defects or histological abnormalities (Table 1 and Plate 1).

Animals in group II which received only cisplatin injection on the first day of treatment developed nephrotoxicity manifested by significant increase in mean kidney weight as a percentage of total body weight relative to the control group (p<0.05) (Table 1). Microscopically, the sections of kidneys in this group showed abnormal cell sizes, gaps, and cystic dilatation of tubules suggestive of necrosis (Plate 2).

Animals in group III which received oral neem extract before cisplatin injection had a significantly increased kidney weight relative to control (p<0.05) (Table 1). Kidney microscopy showed no abnormalities (Plate 3).

Animals in group IV which received cisplatin injection followed by oral neem extract showed significantly increased kidney weight relative to control (p<0.05) (Table 1). Light microscopy revealed cystic dilatations of renal tubules (Plate 4).

Biochemical analysis

Animals in group I (control) showed normal mean levels of serum electrolytes, urea and creatinine (Table 2).

Animals in group II which received only cisplatin injection had significantly raised mean serum levels of urea, creatinine and potassium but significantly reduced mean serum level of bicarbonate relative to control (p<0.05) (Table 2).

Animals in group III which received oral neem extract followed by cisplatin injection had higher mean serum level of urea but lower mean serum levels of potassium and bicarbonate relative to control but these differences were not statistically significant (p>0.05). The mean serum creatinine level in this group was significantly higher than that of the control group (p<0.05) (Table 2).

Animals in group IV which received cisplatin injection followed by oral neem extract had higher mean serum

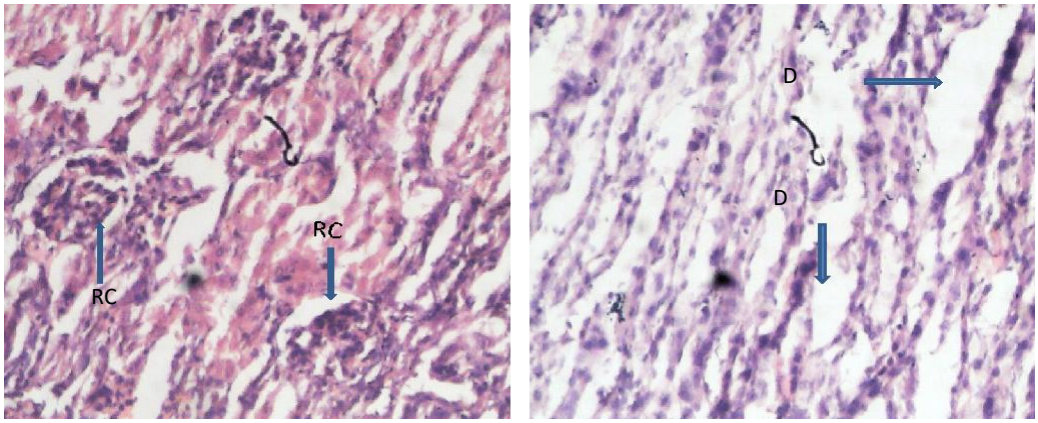

Plate 2. photomicrograph of the kidney of animals in group II treated with cisplatin only. Right - H and E section showing renal corpuscles RC, distortions, and dilations D of the renal tubules. Left - histochemical section showing very reduced staining intensity and abnormal dilatations of the renal tubules D.
Magnification: ×400.

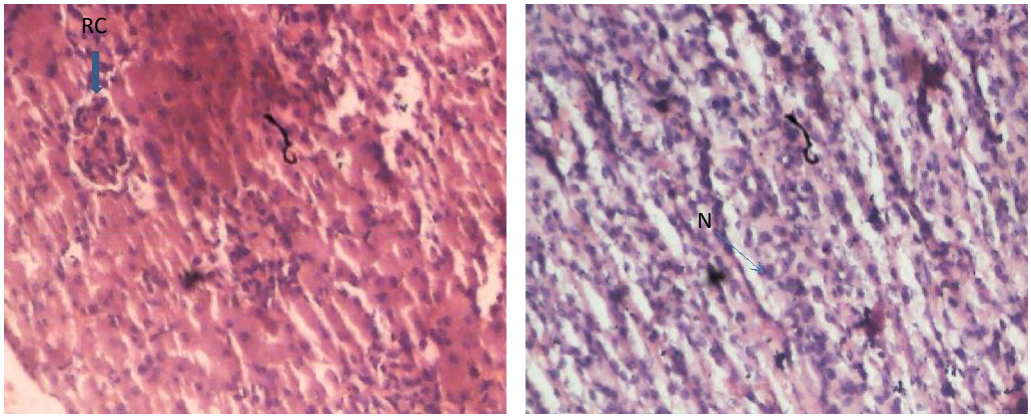

Plate 3. photomicrograph of the kidney of animals in group III treated with neem extract for 14 days followed by cisplantin injection on day 14. Right – H & E section showing a renal corpuscle and normal tubular cells. Left – histochemical section showing normal staining intensity.
Magnification: ×400.

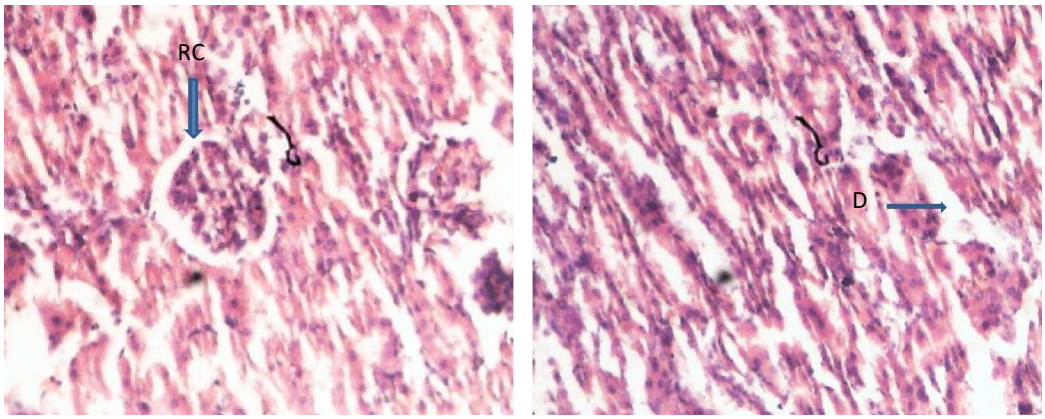

Plate 4. photomicrograph of the kidney of animals in group IV treated with cisplatin injection on day one followed by oral neem extract for 14 days. Right – H and E section showing a normal renal corpuscle and abnormal spaces in the section depicting cell loss. Left – histochemical section showing reduced staining intensity and spaces in the section.
Magnification: ×400.

Table 2. Serum biochemical parameters among the various groups

Groups	Biochemical parameters				
	Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)	U (mmol/L)	Cr (µmol/L)
I(Control)	141.1±1.3	4.1±0.5	27.4±1.3	4.5±1.4	85.3±9.2
II(Cisplatin injection 10 mg stat on day one)	141.2±1.6	5.5±0.3*	20.4±2.4*	16.5±4.2*	133.7±13.1*
III(Neem extract 500 mg/kg daily for 14 days followed by cisplatin injection 10 mg stat on day 14)	140.8±0.9	4.0±0.2	25.1±3.3	6.4±1.8	108.9±11.2*
IV(Cisplatin injection 10mg stat on day one followed by neem extract 500 mg/kg daily for 14 days)	142.4±2.5	4.3±0.4	24.2±2.0	11.9±2.3*	117.6±2.6*

$p<0.05$.

level of potassium and lower mean serum level of bicarbonate relative to control but these differences were not statistically significant ($p>0.05$). The mean serum creatinine and urea levels in this group were significantly higher relative to control ($p<0.05$) (Table 2).

The mean serum sodium level was not significantly different among all the study groups ($p>0.05$) (Table 2).

DISCUSSION

The observed morphological changes in the kidneys of animals that received cisplatin only and the attendant biochemical derangements are indicative of cisplatin nephropathy. Other Researchers arrived at similar findings (Gamal el-Din and Al-Bekairi, 2006; Prasad et al, 2006; Howle and Gale, 1970). Despite the clinical effectiveness of cisplatin as an anti-tumour drug, nephrotoxic side effect has significantly restricted its use. Experimental studies have shown acute cytotoxic effects following cisplatin treatment, mostly affecting tubular epithelial cells (Yamate et al, 1996; Razzaque et al 1999). Once within renal cells, cisplatin could abnormally reduce ATPase activity, inflict mitochondrial damage, induce cell cycle arrest and impair cellular transport system. The combined effects of these events can induce apoptosis or necrotic cell death (Lau, 1999; Lieberthal et al, 1998; Santos et al, 2007).

Animals which received neem leaf extract before and those that received the extract after cisplatin injection had significantly increased kidney weight relative to control indicating that at the dose of 10mg/kg/day, cisplatin is nephrotoxic in wistar rats. However, only those that received neem extract after cisplatin injection developed microscopic evidence of nephrotoxicity in the form of tubular dilatation. Biochemically, it is also only this group of animals that showed significantly high levels of both serum urea and creatinine which indicates renal compromise. The implication of this observation is that when neem extract is administered for up to two weeks prior to cisplatin injection, it may ameliorate some of the toxic effects of cisplatin on the kidney in wistar rats. Phytochemical analysis reports that neem contains bioflavonoids which are among the most potent antioxidant substances. Many components of neem, such as nimbidin, reportedly have anti-inflammatory properties (Biswas et al, 2002). The probable mechanism of neem-mediated renoprotection, therefore, may involve neutralizing or inactivating oxygen-derived free radicals arising during tissue inflammation consequent upon cisplatin therapy. From the findings of this study, administration of neem extract prior to induction of tissue injury proved more beneficial than afterwards. Presumably, the extract, when administered prior to onset of kidney injury, had more time to permeate the tissues and establish contact with individual cells and, therefore, afforded better protection against tissue damage. Similar protective effects of neem leaf extract on other tissues have been reported by other workers (Bhanwra et al, 2000; Chattopadhyay, 2003; Mbah et al, 2007 and Dorubabu et al, 2006).

CONCLUSION

From the result of this experiment, it can be concluded that cisplatin induces nephrotoxicity in wistar rats as it does in humans and this can be ameliorated by administration of neem leaf extract. When administered prior to cisplatin mediated injury, neem leaf extract proves more useful and has greater protective ability than when applied after cisplatin nephrotoxicity.

REFERENCES

Arivazhagan S, Velmurugan B, Bhuvaneswari V, Nagini S (2004). Effects of aqueous extracts of garlic (Allium sativum) and Neem (Azadirachta indica) leaf on hepatic and blood oxidant-antioxidant status during experimental gastric carcinogenesis. J. Med. Food. 7 (3): 334-9.

Bhanwra S, Singh J, Khosla P (2000). Effect of Azadirachta indica (neem) leaf aqueous extract on paracetamol-induced liver damage in rats. Ind. J. Physiol. Pharcacol. 44 (1): 64-68.

Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U (2002). Biological activities and medicinal properties of neem (Azadirachta indica). Curr. Sci. 82 (11): 1336-1345.

Chattopadhyay RR (2003). Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: part II. J. Ethanopharmacol. 89 (2-3):217-9.

Cotran RS, Kumar V, Collins T (1999). Robbins Pathologic basis of disease, 6th edition, Saunders.

Dorubabu M, Joshi MC, Bhawani G, Kumar MM, Chaturvedi A, Goel RK (2006). The effect of aqueous extract of neem (Azadirachta indica) leaves on offensive and defensive gastric mucosal factors in rats. Ind. J. Physiol. Pharmacol. 50 (3): 241-249.

Gamel el-Din AM, Al-Bekairi AM (2006). Carvedilol, a β-adrenoceptor Blocker with Antioxidative Potential, Attenuates Cisplatin induced Nephrotoxicity in Rats. J. Appl.Sci. Res.2(6):331-335.

Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC (2004). Protective role of extracts of neem seeds in diabetes caused by streptozocin in rats. J. Ethnopharmacol. 90 (2-3): 185-189.

Howle JA, Gale GR (1970). Cis-dichlorodiammineplatinum (II). Persistent and selective inhibition of deoxyribonucleic acid synthesis in vivo. Biochem. Pharmacol. 19: 2757-2762.

Kamanyire R. Cisplatin: a monograph for UKPID.

Katzung BG (2004). Basic and Clinical Pharmacology, 9th edition, Mc Graw Hill.

Lau AH (1999). Apoptosis induced by Cisplatin nephrotoxic injury. Kidney Int. 56: 1295-1298.

Lieberthal W, Menza SA, Levine JS (1998). Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. Am. J. Physiol. 274: F315-327.

Maliakel DM, Kagiya TV, Nair CKK (2008). Prevention of Cisplatin induced nephrotoxicity by glucosides of ascorbic acid and α-tocopherol. Experimental and toxicol. pathol. 60 (6): 521-527.

Mbah AU, Udeinya IJ, Shu EN, Chijioke CP, Nubila T, provide names of other authors (2007). Fractionated neem leaf extract is safe and increases CD4+ cell levels in HIV/AIDS patients. Am. J. Ther. 14 (4): 369-74.

Nisar S, Feinfeld DA (2002). N-acetylcysteine as salvage therapy in Cisplatin nephrotoxicity. Ren Fail 24 (4):529-533.

Pfeifle CE, Howell SB, Felthouse RD, Woliver TBS, Markman M, Murphy MP (1985). High- dose Cisplatin with sodium thiosulfate protection. J. Clin. Oncol. 3: 237-244.

Prasad SB, Rosangkima G, Kmynriam D (2006). Cisplatin-induced Toxicological Effects in Relation to the Endogenous Tissue Glutathione Level in Tumour-Bearing Mice. Asian J. Exp. Sci. 20 (1): 55-68.

Razzaque MS, Ahsan N, Taguchi T (2002). Role of apoptosis in fibrogenesis. Nephron. 90: 365-372.

Santos NA, Catao CS, Martins NM, Curti C, provide names of other authors (2007). Cisplatin induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch. Toxicol. doi.10.1007/s00204-006-0173-2.

Tebekeme O, Prosper AE (2007). Garcinia kola extract reduced Cisplatin induced kidney dysfunction in rats. Afr.J. Biochem.Res.1 (6): 124-126.

Umeki S, Watanabe M, Yagi S, Soejima R (1988). Supplemental fosfomycin and/or steroids that reduce Cisplatin-induced nephrotoxicity. Am. J. Med. Sci.; 295 (1): 6-10.

Yamate J, Ishida A, Tsujino K, provide names of other authors (1996). Immunohistochemical study of rat renal interstitial fibrosis induced by repeated injection of Cisplatin, with special reference to the kinetics of macrophages and myofibroblasts. Toxicol. Pathol. 24:199-206.