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Full Length Research Paper

Comparative study of the protective effects of ethanol extract of neem leaves and vitamin E on cisplatin-induced kidney damage in wistar rats

Terkula Kpela[1]*, Elizabeth B. Umoren[2] and Abue D. Andrew[3]

[1]Department of Anatomy, Benue State University College of Medicine, Makurdi, Nigeria
[2]Department of Physiology, University of Calabar, Calabar, Nigeria
[3]Department of Anatomical Science, University of Abuja, Abuja, Nigeria.

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The protective effects of neem leaf extract and vitamin E on Cisplatin-induced kidney damage in wistar rats were compared. Twenty rats weighing 180-200g were divided into four groups, each containing five animals. Group I served as control. Group II received a single intraperitoneal dose of Cisplatin (10 mg/kg) on day one. Group III received a single intraperitoneal dose of cisplatin (10 mg/kg), followed by oral administration of neem leaf extract at a dose of 500 mg/kg/day for 14 days. Group IV received a single intraperitoneal dose of cisplatin (10 mg/kg), followed by daily oral dose of vitamin E (6 mg/kg) for 14 days. A day after drug and extract administration, the animals were sacrificed under chloroform anaesthesia and 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 (H and E) as well as deoxyribonucleic acid (DNA) staining. Results showed that cisplatin-induced necrosis of tubule cells and raised serum electrolytes, urea and creatinine levels were partially normalized by treatment with neem leaf extract but relatively unaffected by treatment with vitamin E. We conclude, therefore, that neem leaf extract, unlike vitamin E, 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). It is a well-known and relatively common cause of death. Cisplatin is one of the most widely used cytotoxic therapeutic 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 (Katzung, 2004; Kamanyire, 2008). 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; Maliakel et al, 2008; Nisar and Feinfeld, 2002; Pfeifle et al, 1985; Tebekeme and

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

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 extracts (Bhanwra et al, 2000; Chattopadhyay, 2003; Dorubabu et al, 2006; Gupta et al, 2004; Mbah et al, 2007; Kpela et al, 2012).

Vitamin E has numerous functions in the body such as protecting molecular structures from oxidative damage. Because of its chemical structure, vitamin E acts as a naturally occurring antioxidant and inhibits the oxidation of highly unsaturated substances. It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Murray et al, 2006; Herrera and Barbas, 2001; Traber and Atkinson, 2007). Vitamin E is considered as the 'standard antioxidant' to which other compounds with antioxidant activities are compared, especially in terms of its biological activity and clinical relevance. It is against this background that the present study was conceived to compare the possible protective effects of ethanolic extract of Neem leaves and vitamin E 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. 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 obtained 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. Vitamin E (Nutriheal vitamin E, RHovid) was also purchased from Kamel Pharmacy LTD, Calabar. Each capsule contained 1000 IU (667mg) of d-alpha tocopherol in a 1ml oily medium.

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 a single dose of Cisplatin 10mg/kg intraperitoneally, followed by Neem extract 500mg/kg/day orally for 14 days.

Group IV received a single dose of Cisplatin 10mg/kg intraperitoneally, followed by Vitamin E 6 mg/kg orally for 14 days. Animals were sacrificed one day after administration and blood collected through cardiac puncture into labeled specimen bottles. Serum was obtained by centrifugation and used for estimation of potassium, sodium, bicarbonate, urea and creatinine. The kidneys were removed and washed with cold saline, blotted dry and weighed. The kidneys were processed through paraffin sections for Hematoxylin and Eosin staining using Drury and Wailington method of 1967 and Deoxyribonucleic acid staining using Feulgen and Rossenbeck method of 1924.

Data generated by was subjected to analysis of variance (ANOVA). The level of statistical significance was taken as $P<0.05$.

RESULTS

Morphological analysis

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

Animals in group II which received a single dose of Cisplatin on the first day of the 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, kidney sections in this group showed

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(Cisplatin injection 10 mg stat on day one followed by neem extract 500 mg/kg daily for 14 days)	0.49±0.015*
IV(Cisplatin injection 10 mg stat on day one followed by vit.E 6 mg/kg daily for 14 days)	0.50±0.030*

p<0.05.

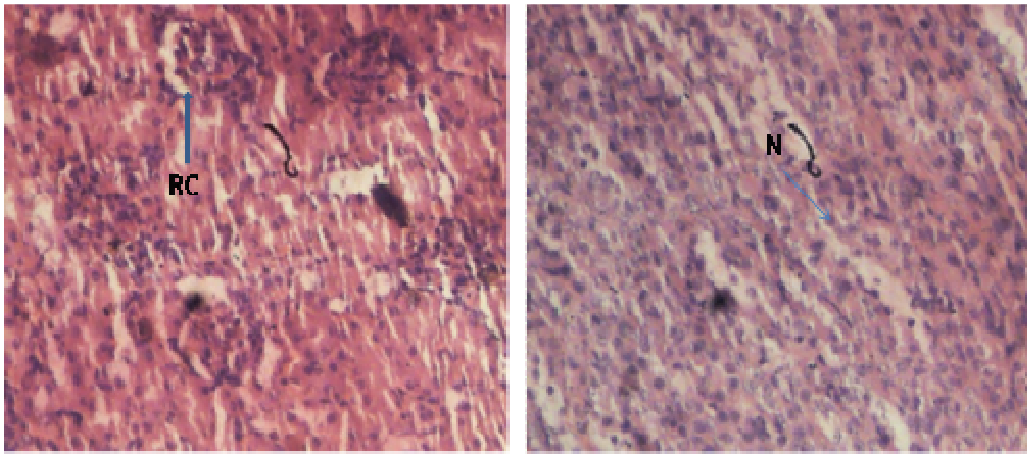

Plate 1. photomicrograph of kidney of animals from control group. Left – H and E section showing normal renal corpuscles RC and tubular cells. Right – histochemical section showing normal staining intensity and tubular cells with prominent nuclei N within the section.
Magnification: ×400

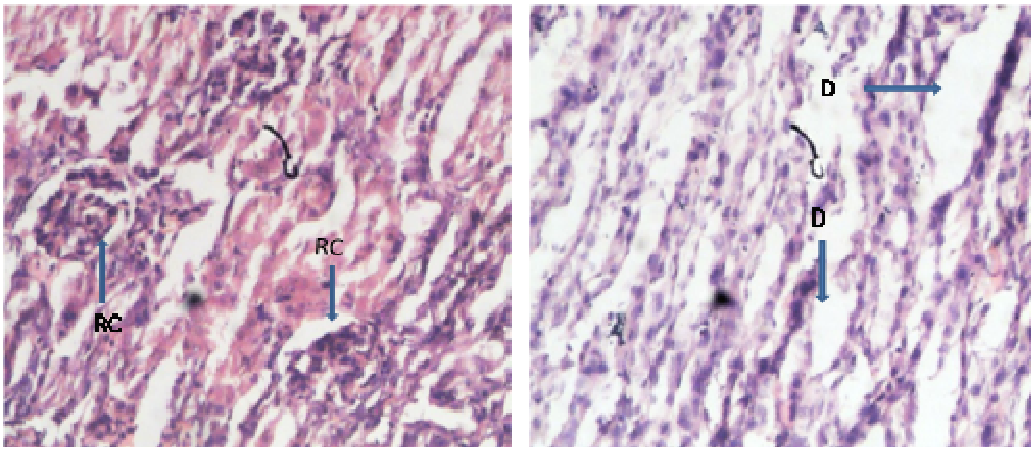

Plate 2. photomicrograph of the kidney of animals in group II treated with cisplatin only. Right - H and E section showing renal corpuscles RC, distortions, and dilatations D of the renal tubules. Left - histochemical section showing very reduced staining intensity and abnormal dilatations of the renal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

abnormal proximal tubular cell sizes, gaps and cystic dilatation of tubules suggestive of necrosis (Plate 2).

Animals in group III which received Cisplatin injection followed by oral neem extract administration for 14 days, showed significantly increased kidney weight relative to control (p<0.05) (Table 1). Light microscopy showed

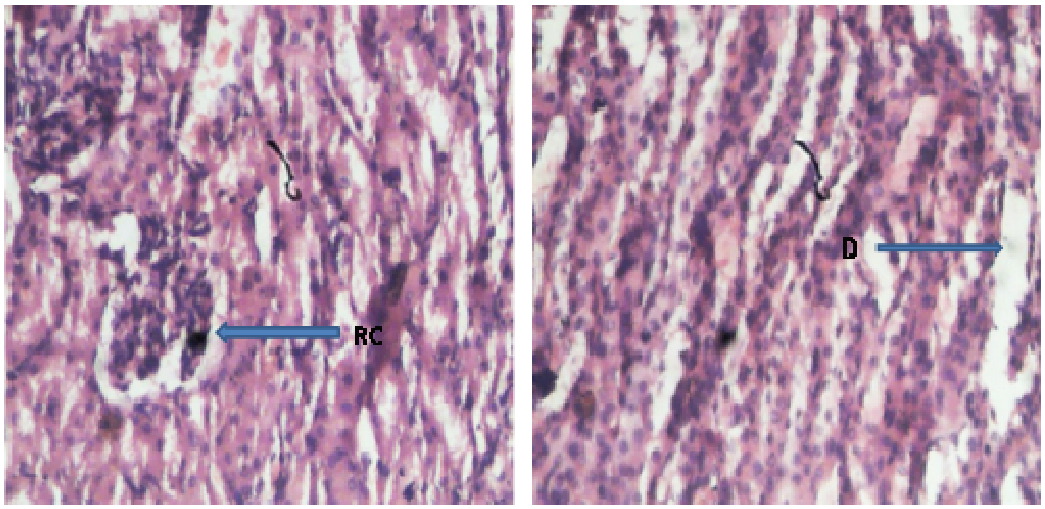

Plate 3. photomicrograph of the kidney of animals in group III treated with cisplatin injection followed by neem oral extract. Right - H and E section showing a renal corpuscle and tubular cells in the cortex. Left - histochemical section showing normal staining intensity and only mild tubular dilatation in the section.
Magnification: ×400.

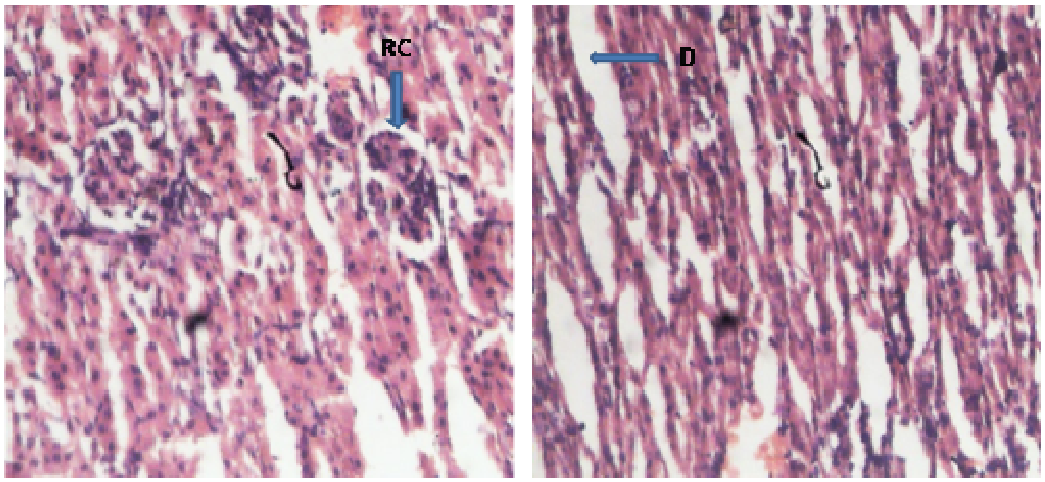

Plate 4. photomicrograph of the kidney of animals treated with cisplatin followed by vitamin E. Right - H and E section showing renal corpuscle and abnormal spaces depicting cell loss. Left - histochemical section showing reduced staining intensity and abnormal dilatations of the renal tubules D.
Magnification: ×400.

slight dilatations of renal tubules and leucocytic infiltration (Plate 3).

Animals in group IV which received Cisplatin injection followed by oral vitamin E administration for 14 days, had significantly higher kidney weight relative to control ($P<0.05$) (Table 1). Light microscopy showed tubular dilations similar to those observed in group animals (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 had significantly raised mean serum levels of urea, creatinine and potassium and significantly reduced mean serum level of bicarbonate relative to control ($p<0.05$) (Table 2).

Table 2. Serum biochemical parameters among the various groups

Groups	Biochemical parameters				
	Na+ (mmol/L)	K+ (mmol/L)	HCO3- (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(Cisplatin injection 10 mg 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*
IV(Cisplatin injection 10 mg stat on day one followed by vit.E 6 mg/kg daily for 14 days)	141.8±3.4	4.7±0.8*	22.8±1.5*	13.6±3.6*	126±5.7*

$p<0.05$.

Animals in group III had higher mean serum 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).

Animals in group IV had significantly raised mean serum levels of urea, creatinine and potassium but significantly reduced mean serum level of bicarbonate relative to control ($p<0.05$).

There was no significant difference in the mean serum level of sodium 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 have 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).

Although animals that received a single intraperitoneal dose of cisplatin (10 mg/kg), followed by oral administration of neem leaf extract at a dose of 500 mg/kg/day for 14 days showed significantly increased kidney weight and serum urea and creatinine levels relative to control ($p>0.05$), there was no associated hyperkalemia and metabolic acidosis. Renal histology showed only mild tubular dilatations. It can be stated, therefore, that, the morphological and biochemical findings in this group represent an amelioration of some of the effects of Cisplatin on the kidneys by neem leaf extract. 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. It is, therefore, conceivable that neem extract could ameliorate cisplatin toxicity.

In this study, the administration of vitamin E to animals had not improved cisplatin toxicity. Essentially, all the study parameters were similar to those of the group that received cisplatin alone. One possible explanation to this lies in the biochemical properties of vitamin E. The stability of the tocopheroxyl free radical means that it can penetrate further into cells and, potentially, propagate a chain reaction. Therefore, vitamin E may, like other antioxidants, also have pro-oxidant actions, especially at high concentrations. This may also explain why, although studies have shown an association between high blood concentrations of vitamin E and lower incidence of atherosclerosis, trials of high doses of vitamin E have been disappointing (Murray et al., 2006). Again, during exposure to toxic substances, the delicate balance between free radicals and cell injury is shifted in favour of pro-oxidants resulting in oxidative stress (Sies, 1996).

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. The mechanism by which neem leaf extract reduces cisplatin-induced kidney damage probably involves neutralizing or inactivating oxygen-derived free radicals that are released during tissue inflammation. Vitamin E does not demonstrate a

clear ability to reduce biochemical and morphological evidence of cisplatin-induced renal damage probably because it also has pro-oxidant properties.

REFERENCES

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): 64-8.

Chattopadhyay RR (2003). Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: part II. J. Ethanopharmacol. 89 (2-3): 217-219.

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

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.

Herrera E, Barbas C (2001). Vitamin E: action, metabolism and perspectives. J. Physiol. Biochem. 57 (2):43-56.

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.

Kpela T, Ekanem TB, Akpantah AO (2012). Protective effect of ethanol extract of neem leaves on cisplatin-induced kidney damage in wistar rats. Glo. Adv.Res. J. Med. and Med. Sci. 1 (9): 249-254.

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: 315-327.

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, et al (2007). Fractionated neem leaf extract is safe and increases CD4+ cell levels in HIV/AIDS patients. Am. J. Ther. 14 (4): 369-374.

Murray RK, Granner DK, Rodwell VW (2006). Harper's illustrated biochemistry, 27th edition, Mc Graw Hill, p.495.

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, et al (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.

Sies H (ed) (1996). Antioxidants in disease, mechanisms and therapy. Academy press, New York.

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

Traber MG, Atkinson J (2007). Vitamin E,and nothing more. Free Radic. Biol. Med. 43 (1): 4-15.

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.

Wang X,Quinn P (1999). Vitamin E and itsfunctions in membranes. Prog. Lipid. Res. 38 (4): 309-336.

Yamate J, Ishida A, Tsujino K, et al (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.