

## ***Cymbopogon citratus* aqueous extract alleviates cisplatin-induced renal oxidative stress and toxicity in albino rats**

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### **ABSTRACT**

Cisplatin is an effective chemotherapeutic agent for a wide variety of tumors, but is reported to cause renal toxicity. Therefore, the present study was aimed at evaluating the dose dependent (5% and 10%) and time course curative potential of aqueous leaf extract of *Cymbopogon citratus* (C.C.) on cisplatin induced renal oxidative damage in rats using biochemical and histopathological approaches. Male albino rats weighing between 150-200g were randomly separated into four different groups. Tissue damage was induced in rats of groups 2, 3 and 4 by a single intraperitoneal administration of cisplatin (5mg/kg b.w). Test rats in groups' 3 and 4 were treated 3 days after cisplatin injection intraperitoneally (i.p) with 5% and 10% C.C. accordingly for 3,6,9 and 12 days. Rats in group 2 were given sterile water in place of the extracts while rats in group I were the untreated controls. They were all allowed unlimited access to tap water and growers' mash. Cisplatin treatment caused increase ( $P \leq 0.05$ ) in malondialdehyde (MDA) concentration from  $394.17 \pm 0.25$  to  $1209.60 \pm 0.96$  nmol/g, reduction ( $P \leq 0.05$ ) in the activities of superoxide dismutase (SOD) from  $7.790 \pm 0.01$  to  $4.557 \pm 0.01$  U/mg, catalase (CAT) and glutathione peroxidase (GPx) in kidney homogenate. Parallel to these changes, cisplatin treatment enhances renal damage as evidenced by sharp increase in urea, uric acid, urine volume and serum creatinine ( $P \leq 0.05$ ). Additionally, the impairment of renal function corresponds to the histopathology. However most of these changes were alleviated by prophylactic treatment with aqueous extract of *Cymbopogon citratus* dose and time dependently ( $P \leq 0.05$ ). The results of this present study indicated that aqueous leaf extracts of *Cymbopogon citratus* has anti-nephrotoxic action against cisplatin induced renal toxicity in rats which might be ascribed to its antioxidant and free radical scavenging property.

**Key words:** *Cymbopogon citratus* renal toxicity cisplatin antioxidant anti-nephrotoxic

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## Introduction

Cisplatin is an effective chemotherapeutic agent for a wide variety of tumors (Park *et al.*, 2009). Nevertheless, it has several side effects including hepatotoxicity (Mansour *et al.*, 2006; Pratibha *et al.*, 2006) and nephrotoxicity (Park *et al.*, 2009). Mora *et al.* (2003) showed that a decrease in antioxidant enzymes resulted from cisplatin induced tissue toxicity. They added also that the development of therapies to prevent the appearance of cisplatin-induced tissue toxicities has focused on administration of antioxidants along with cisplatin treatment thus many studies dealing with protective effects using extracts of natural products and dietary antioxidant against cisplatin induced tissue toxicities have been reported (Behling *et al.*, 2006; Mansour *et al.*, 2006). Although, the precise mechanism for the cisplatin-induced toxicity is not well understood, Cisplatin is preferentially taken up and accumulated in the liver and kidney cells (Stewart *et al.*, 1982; Ravi *et al.*, 1995). Therefore, the enhanced production of Reactive Oxygen Species (ROS) (Saad *et al.*, 2004), oxidative stress, (Saad *et al.*, 2004) and the decrease in antioxidant enzymes (Mora *et al.*, 2003) in kidneys have been implicated in the pathogenesis of cisplatin induced renal injury (Yilmaz *et al.*, 2004). However, the involvement of oxidative stress in cisplatin induced toxicity is further supported by the fact that many antioxidants prevent cisplatin induced nephrotoxicity ( Lee *et al.*, 2007).

The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth. Herbal medicine was practiced by people in Africa, Asia, Europe and the Americas (Wargovich *et al.*, 2001). *Cymbopogon citratus* of the Poaceae family is a tall aromatic coarse grass of 1.5 m high. It is a monocotyledonous hypogeal perennial plant with slender sharp edged green leaves that has a pointed apex. The stem is reddish brown in colour and it is attached to the bulb by stalk. The entire plant is attached to the soil by fibrous root (Burkill, 1996).

In folk medicine *C. citratus* of Brazil is believed to have anxiolytic, hypnotic and anticonvulsant properties (Rodrigues *et al.*, 2006; Blanco *et al.*, 2009) and also cytoprotective, antioxidant, anti-inflammatory properties (Lee *et al.*, 2008; Figueirinha *et al.*, 2010; Tiwari *et al.*, 2010). However, Leite *et al.*, (1986), reported that this same herb had no effect on humans. The present study was aimed at evaluating the dose dependent and time course curative potential of aqueous leaf extract of *Cymbopogon citratus* (C.C.) on cisplatin induced renal toxicity and oxidative stress.

## MATERIALS AND METHODS

### Animals

Seventy two (72) adult healthy male albino rats, weighing between 150 and 200 g were used in this study. The rats were obtained from the animal house of the Niger Delta University, College of Health Sciences, Bayelsa State and housed in standard cages. They were then allowed free access to standard feed (growers mash) and water for a period of two weeks to acclimatize to the cage environment prior to the commencement of the experiment. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

### Drugs and chemicals

Cisplatin was a product of Korea United Pharm INC, KOREA. Kits from Teco diagnostics Ltd. USA, HUMAN diagnostics Ltd. Germany, Fortress diagnostics Ltd. United Kingdom, Sigma-Aldrich Ltd., U.S.A. were used. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

### Preparation of extracts

The leaves of *Cymbopogon citratus* were collected from Sagbama in Bayelsa State of Nigeria and were identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

Air dried leaves of *Cymbopogon citratus* was grounded and later milled into powder form. 50g portion of the milled leaf was weighed and soaked in 500ml of distilled water in a beaker. The mixture was shaken and kept on the laboratory bench for 24hrs before filtering. The filtrate was evaporated to dryness at room temperature in a rotary evaporator to obtain a paste which was further dried in a dessicator with constant changing of the self- indicating silica gel. Appropriate

weights of the residue were prepared in distilled water to obtain concentrations of 5% and 10% (w/v) of *Cymbopogon citratus* that were administered orally to each of the rats.

### **Experimental design and procedures:**

#### **Cisplatin model for evaluation of anti-nephrotoxic activity**

Cisplatin BP (50mg/50ml) was administered to the test rats intraperitoneally at a dose of 5mg/Kg body weight (Mansour *et al.*, 2006; Okoko and Oruambo, 2008).

#### **Evaluation of curative potential**

The rats were divided into four equal groups of eighteen (18) rats per group. In group 1 the rats received no cisplatin. Normal saline was administered i.p. The second group was injected with a single dose of cisplatin (5 mg/kg, i.p) at the beginning of the experiment (Mansour *et al.*, 2006).

Tissue damage was also induced in rats in groups 3 and 4 by a single intraperitoneal-administration of cisplatin (5 mg/kg body weight).

Three days later, 2ml/kg body weight of 5% and 10% aqueous extract of *Cymbopogon citratus* were administered to rats in groups 3 and 4 respectively through the oral route using the gavage once daily for 3, 6, 9 and 12 days.

Rats in group 2 were given sterile water in place of the extracts.

Rats in group I were untreated controls. They were all allowed unlimited access to tap water and growers' mash. During the experimental period, animal behavior and body weights were recorded daily. Randomly selected animals of different groups were anaesthetized with urethane.

Blood samples were collected by cardiac puncture after 0, 3, 6, 9, 12 and 15 days for biochemical analyses.

Parts of the kidney tissues were immediately taken and fixed in 10% neutral buffered formalin for histopathological examination.

### **Preparation of kidney Homogenate**

The livers and kidneys were also excised and washed in cold saline. Ten percent tissue homogenates were prepared in 0.1M Tris -HCl buffer (pH 7.4). Perinuclear fractions were obtained after centrifuging homogenates at 1500 rpm for 20 minutes using a centrifuge.

### **Biochemical Analysis**

After the experimental period, animals in different groups were sacrificed. Blood was collected in tubes without anticoagulant to separate serum for various biochemical estimations.

### **Renal markers**

Serum urea was assayed by the modified Berthelot method according to Tobacco *et al* (1979). Creatinine was determined by the colorimetric kinetic method by Bartels *et al.* (1971). Serum uric acid was estimated using the enzymatic colorimetric method employing uricase according to Duncan *et al.* (1982).

### **Markers of oxidative disturbances**

Catalase activity was determined by the method of Cohen *et al.* (1970). Super oxide dismutase (SOD) activity was by the methods of Misra and Fridovich (1972). The determination of glutathione peroxidase (Gpx) activity was by the method of Chance and Maehly (1955) as provided by Sigma-Aldrich Ltd., U.S.A. The assay method of Hunter *etal.* (1963) as modified by Gutteridge and Wilkins (1980) was adopted for the assay of Malondialdehyde (MDA) concentration.

### **Histopathological study**

Small pieces of kidney tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 $\mu$ m in thickness were cut and stained with hematoxylin and eosin.

## Statistical analysis

Data was expressed as Mean  $\pm$  SD of three estimations. The statistical significance was evaluated by ANOVA using SPSS Version 16 and the individual comparison were obtained by LSD and Tukey method. Values were considered statistically significant when  $P < 0.05$ . In order to discern the possible Interaction between cisplatin and *Cymbopogon citratus*, two-way analysis of variance was used.

## RESULTS

Intraperitoneal administration of cisplatin (5mg/kg i.p.) caused abnormal renal function in all rats. Serum urea, creatinine and uric acid increased ( $P \leq 0.05$ ) in the group treated with cisplatin only, after 6 days when compared with the normal (control). There were, however, slight decreases ( $P \geq 0.05$ ) on the 9th and 15th day (Tables 1, 2 and 3).

The serum urea, creatinine and uric acid of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of *Cymbopogon citratus* in groups 3 and 4 respectively increased significantly on the 3<sup>rd</sup> day but decreased on the 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day when compared to the cisplatin treated group. ( $P \leq 0.05$ ) (Tables 1, 2 and 3).

Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on serum urea, creatinine and uric acid ( $P \leq 0.05$ ).

**Table 1: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON SERUM UREA (mmol/l) ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1.Control ( normal saline)	6.67 ± 0.06 <sup>a</sup>	5.52 ± 0.01 <sup>a</sup>	5.57 ± 0.15 <sup>a</sup>	6.00 ± 0.10 <sup>a</sup>	5.83 ± 0.06 <sup>a</sup>	5.20 ± 0.10 <sup>a</sup>
2.Cis (5mg/kg i.p) +2ml water	5.73 ± 0.06 <sup>b</sup>	14.23 ± 0.11 <sup>b</sup>	13.90 ± 0.10 <sup>b</sup>	13.73 ± 0.20 <sup>b</sup>	14.03 ± 0.12 <sup>b</sup>	13.00 ± 0.10 <sup>b</sup>
3.Cis (5mg/kg i.p) )+2ml 5% C.C	6.97 ± 0.11 <sup>d</sup>	13.20 ± 0.48 <sup>c</sup>	10.83 ± 0.03 <sup>e</sup>	9.53 ± 0.10 <sup>e</sup>	8.13 ± 0.06 <sup>d</sup>	7.13 ± 0.15 <sup>c</sup>
4.Cis (5mg/kg i.p) )+2ml 10% C.C	5.20 ± 0.01 <sup>c</sup>	13.28 ± 0.29 <sup>c</sup>	10.97 ± 0.06 <sup>e</sup>	8.97 ± 0.58 <sup>c</sup>	8.20 ± 0.10 <sup>d</sup>	7.05 ± 0.13 <sup>c</sup>
Results of one-way ANOVA						
F- value	145.97	462.08	1785	1217	3245	1323
P- value	p < 0.05	p < 0.05	p < 0.05	p < 0.05	p < 0.05	p < 0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 1489, p<0.05. Treatment effect, p<0.05, F = 3415; time effect, p < 0.05, F = 5779

**Table 2: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON SERUM CREATININE (umol/l) ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1.Control ( normal saline)	69.73 ± 0.25 <sup>a</sup>	74.30 ± 0.30 <sup>a</sup>	69.87 ± 0.25 <sup>a</sup>	73.13 ± 0.32 <sup>a</sup>	73.07 ± 0.25 <sup>a</sup>	74.33 ± 0.67 <sup>a</sup>
2.Cis (5mg/kg i.p) +2ml water	72.17 ± 0.42 <sup>b</sup>	159.53 ± 0.85 <sup>b</sup>	155.67 ± 0.49 <sup>b</sup>	150.30 ± 0.80 <sup>b</sup>	147.00 ± 0.26 <sup>b</sup>	149.70 ± 0.46 <sup>b</sup>
3.Cis (5mg/kg i.p) )+2ml 5% C.C	68.97 ± 0.21 <sup>a</sup>	156.63 ± 0.35 <sup>e</sup>	133.10 ± 0.30 <sup>d</sup>	115.53 ± 0.80 <sup>e</sup>	113.80 ± 0.26 <sup>e</sup>	102.47 ± 0.90 <sup>e</sup>
4.Cis (5mg/kg i.p) )+2ml 10% C.C	70.23 ± 0.15 <sup>a</sup>	163.77 ± 0.31 <sup>c</sup>	130.60 ± 0.61 <sup>c</sup>	114.33 ± 1.10 <sup>e</sup>	108.73 ± 0.45 <sup>f</sup>	79.83 ± 0.32 <sup>f</sup>
Results of one-way ANOVA						
F- value	166.93	15190	3404	2252	5211	6874
P- value	p< 0.05	p<0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 8828, p<0.05. Treatment effect, p<0.05, F = 18830; time effect, p < 0.05, F = 38280



**Table 3: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON SERUM URIC ACID (umol/l) ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1.Control ( normal saline)	127.90 ± 2.42 <sup>a</sup>	133.63 ± 0.67 <sup>a</sup>	127.00 ± 1.25 <sup>a</sup>	124.53 ± 0.60 <sup>a</sup>	132.43 ± 0.50 <sup>a</sup>	129.63 ± 0.95 <sup>a</sup>
2.Cis (5mg/kg i.p) +2ml water	133.30 ± 2.75 <sup>a</sup>	208.33 ± 2.43 <sup>b</sup>	246.20 ± 1.35 <sup>b</sup>	240.47 ± 1.35 <sup>b</sup>	236.80 ± 1.05 <sup>b</sup>	219.80 ± 1.10 <sup>b</sup>
3.Cis (5mg/kg i.p) +2ml 5% C.C	127.03 ± 2.79 <sup>a</sup>	208.50 ± 0.96 <sup>b</sup>	247.43 ± 1.68 <sup>b</sup>	200.07 ± 1.32 <sup>e</sup>	168.67 ± 0.35 <sup>e</sup>	166.10 ± 0.30 <sup>e</sup>
4.Cis (5mg/kg i.p) +2ml 10% C.C	130.43 ± 2.05 <sup>a</sup>	206.80 ± 1.49 <sup>b</sup>	239.73 ± 0.85 <sup>c</sup>	186.50 ± 0.72 <sup>f</sup>	161.63 ± 1.88 <sup>f</sup>	162.60 ± 1.11 <sup>f</sup>
Results of one-way ANOVA						
F- value	8.59	953.08	2459	3427	3124	2846
P- value	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 2375, p<0.05. Treatment effect, p<0.05, F = 6010; time effect, p < 0.05, F = 9173

Intraperitoneal administration of cisplatin (5mg/kg i.p.) caused renal oxidative damage in all rats. Kidney superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) decreased ( $P \leq 0.05$ ) in the group treated with cisplatin only, after 15 days when compared with the normal (control) (Tables 4, 5, and 6 respectively).

The kidney SOD, catalase and GPx of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of *Cymbopogon citratus*, in groups 3 and 4 decreased significantly on the 3<sup>rd</sup> day but increased on the 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day when compared to the cisplatin treated group ( $P \leq 0.05$ ) (Tables 4, 5 and 6 respectively). The increase in kidney SOD, catalase and GPx by both extracts though not statistically significant ( $P \geq 0.05$ ) were dose dependent. The effect of time of administration of aqueous extract of *C.citratus* on kidney SOD, catalase and GPx were statistically significant ( $P \leq 0.05$ ).

Two way analysis of variance indicated that cisplatin and the extract showed significant interaction between time and doses on kidney SOD, catalase and GPx ( $P \leq 0.05$ ) (Tables 4, 5 and 6).

In addition, there was significant ( $P \leq 0.05$ ) increase in kidney MDA concentration in the cisplatin treated rats after 15 days when compared with the normal (control) rats (Table 7). The kidney MDA concentration of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of *Cymbopogon citratus* in groups 3 and 4 respectively, increased significantly on the 3<sup>rd</sup> day but decreased on the 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day when compared to the cisplatin treated group ( $P \leq 0.05$ ) (Table 7).

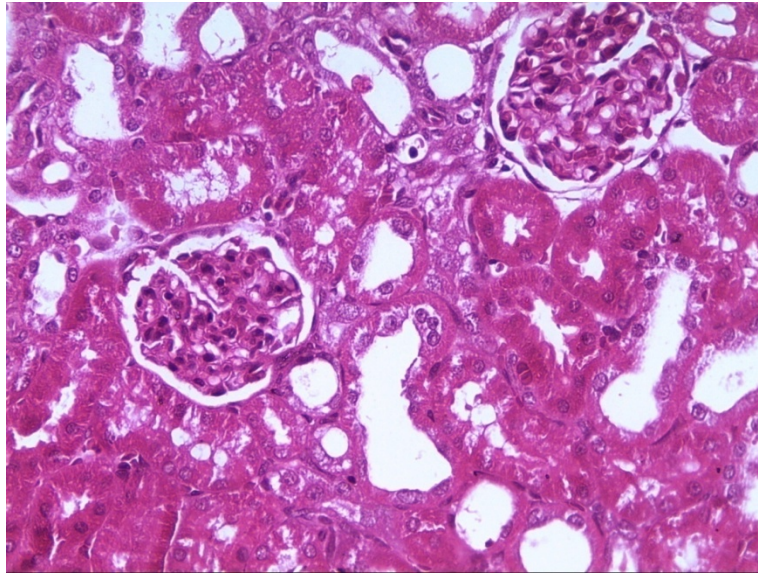
The decrease of the kidney MDA concentration by extracts though not statistically significant ( $P \geq 0.05$ ) was dose dependent. The effect of time of administration of aqueous extract of *C.citratus* on liver MDA concentration was statistically significant ( $P \leq 0.05$ ).

Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on liver MDA concentration ( $P \leq 0.05$ ) (Table 7).

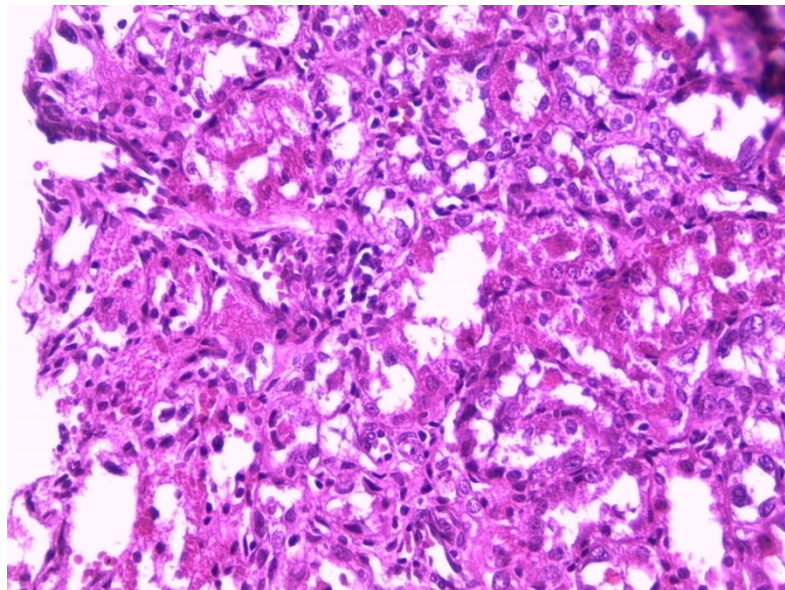
The kidney of rats in group 1 showed a normal architecture, normal organization of tubular epithelial cells and glomeruli cells (Plate 1)

In cisplatin treated kidney, drastic alterations were observed. Histopathological examination showed severe degeneration in tubular cells, congestion of glomeruli and infiltration of interstitium by inflammatory cells (Plate 2)

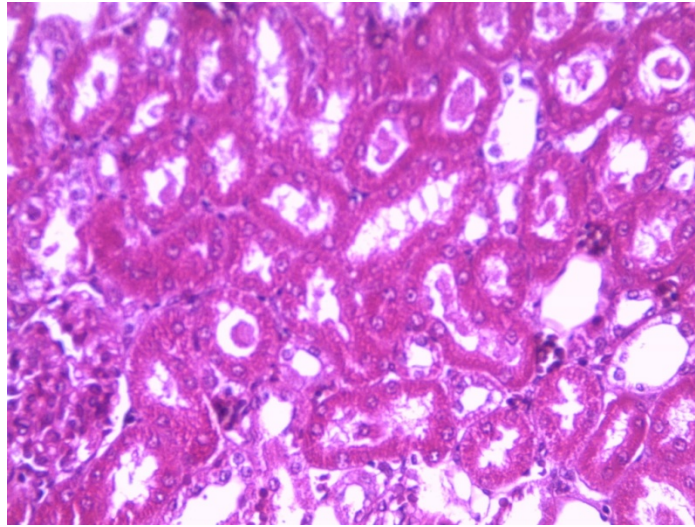
5% C.C + Cisplatin and 10 % C.C + Cisplatin treated kidney which are the test groups (3 and 4) generally showed defects observed in the cisplatin treated rats. There was significant improvement when compared with cisplatin treated kidney, (Plate 3 and 4).



**Plate 1: Photomicrograph of normal Kidney with normal organization of tubular epithelial cells and glomeruli. (H and E stained mag x100)**

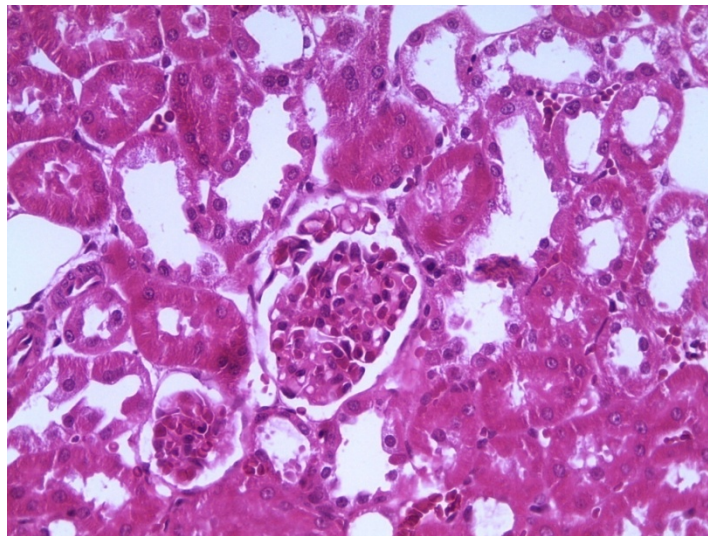


**Plate 2 Photomicrograph of Kidney treated with 5mg/kg cisplatin with severe tubular necrosis with infiltration of interstinum by inflammatory cells. (H and E stained mag x100)**



**Plate 3: photomicrograph of Kidney treated with cisplatin + 5% C.C with mild tubular necrosis.**

**Close to the normal kidney. (H and E stained mag x100)**



**Plate 4: Photomicrograph of Kidney (treated with cisplatin + 10% C.C) with mild tubular necrosis and normal glomeruli . (H and E stained mag x100)**

## DISCUSSION

Cisplatin, a heavy metal complex, is an effective chemotherapeutic agent for a wide variety of tumors (Park *et al.*, 2009). Nevertheless, it has several toxicities and side effects including hepatotoxicity (Mansour *et al.*, 2006; Pratibha *et al.*, 2006) and nephrotoxicity (Park *et al.*, 2009). Lipid peroxidation (LPO) is crucial in the pathogenesis of cisplatin-induced organ injury (Weji *et al.*, 1997; Autunes, 2000; Autunes *et al.*, 2001; Mora *et al.*, 2003). Cisplatin causes the generation of oxygen free radicals, such as hydrogen peroxide, and hydroxyl radical, which abstract a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation (Kadikoylu *et al.*, 2004). In addition, nitric oxide, with high spontaneous chemical reactivities, can react with superoxide to generate peroxynitrite (Aoyagi *et al.*, 1999), which has been suggested as a main source of hydroxyl radical in many pathological conditions (Daloz *et al.*, 1992; Obata, 2002). Lipid peroxidation is important in the pathogenesis of cisplatin-induced hepatic and renal injuries (Baliga *et al.*, 1999).

Oxidative stress injury is actively involved in the pathogenesis of cisplatin-induced acute kidney injury. Reactive oxygen species (ROS) directly act on cell components, including lipids, proteins, and DNA and destroy their structure. ROS are produced via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells. In the presence of cisplatin, ROS are produced through all these pathways and are implicated in the pathogenesis of acute cisplatin-induced renal injury. (Kawai *et al.*, 2006) Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which increased free radical production and decreased antioxidant production (Yilmaz *et al.*, 2004).

It increases intracellular calcium level which activates NADPH oxidase and also stimulates ROS production by damaged mitochondria. (Kawai *et al.*, 2006) Superoxide anion ( $O_2^{\bullet-}$ ), (Davies *et al.*, 2001) hydrogen peroxide ( $H_2O_2$ ), (Kadikolyu *et al.*, 2004) and hydroxyl radical ( $\bullet OH$ ) (Shino *et al.*, 2003) are increased in cisplatin-treated kidneys. These free radicals damage the lipid components of the cell membrane by peroxidation and denature proteins, which lead to enzymatic inactivation. Free radicals can also cause mitochondrial dysfunction. (Yilmaz *et al.*, 2004) Antioxidant enzymes are inhibited by cisplatin, and renal activities of superoxide dismutase, glutathione peroxidase, and catalase are significantly decreased. (Durak *et al.*, 2002; Bardary *et al.*, 2005) Antioxidants, melatonin, (Sener *et al.*, 2000) vitamin C, (Kadikolyu *et al.*,

2004) and vitamin E (Weiji *et al*, 2004) have been shown to prevent cisplatin-induced acute nephrotoxicity.

Many antioxidative agents have been analyzed in experimental and clinical studies searching for an agent to reduce or prevent cisplatin-induced nephrotoxicity (Autunes *et al.*, 2000; Davies *et al.*, 2001; Dillioglulil *et al.*, 2005).

Most studies reported previously, were designed to administer drugs before or at the same time of renal insult. However, most therapeutic agents are usually administered after the expression of clinical diseases. Therefore it was hypothesized that *Cymbopogon Citratus* (C.C.) might affect the course of tubular repair after the onset of cisplatin-induced renal toxicity and oxidative stress, and thus, accelerate recovery in the rats.

Chemotherapeutic levels of cisplatin known to induce renal injury in rats is thought to be a single dose of 5 mg/kg body weight which peaks in about 3 – 5days (Stein *et al.*, 1978; Singh, 1989; Okoko and Orumbo, 2008) thus the choice of a single dose of 5 mg/kg body weight, and the three days exposure before the administration of the aqueous extracts of *C. citratus* for the present study.

As predicted, administration of a single dose of cisplatin (5mg/kg) induced nephrotoxicity, manifested biochemically by a significant elevation in serum urea, creatinine and uric acid (Tables 1-3).

Oral administration of aqueous extract of *Cymbopogon citratus* (5% and 10%) after cisplatin administration caused a decline in nephrotoxicity after 15days for rats treated with cisplatin. This was evidenced by marked decrease in serum urea, creatinine and uric acid concentration of those treated with *Cymbopogon citratus* extract relative to the group treated with cisplatin alone. (Tables 1-3)

This marked decrease in serum urea, creatinine, and uric acid , with administration of aqueous leaf extracts of *Cymbopogon citratus* in cisplatin induced nephrotoxicity was in agreement with studies by other researchers (Mansour *et al.*, 2006; Nagizadeh *et al.*,2008; Ibrahim *et al.*,2010; Sreedevi *et al.*,2010; Mohamed *et al.*,2010; Noori and Mahboob,2010).

Noori and Mahboob (2010) reported that administration of cisplatin to rats caused a reduction in glomerular filtration rate, which correlated with increased creatinine and urea in plasma.

This is in agreement with the present study which showed that administration of cisplatin to rats caused a reduction in glomerular filtration rate, which correlated with alteration in the renal function as indicated by the different values of renal markers (Tables 1-3)

The alterations in glomerular function in cisplatin treated rats may also be secondary to ROS (reactive oxygen species) (Somani *et al.*, 2000 ) which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate (Aydogan *et al.*, 2008 ).

Nephrotoxicity was further confirmed by the significant decrease in superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and a significant increase in lipid peroxides measured as malondialdehyde (MDA) in kidney homogenates.

Administration of cisplatin (5 mg kg<sup>-1</sup> ip) to male wistar rats showed a strong indication of oxidative stress. However, oral administration of aqueous extract *C. citratus* (5% and 10%) after cisplatin administration caused a reduction in oxidative stress on the 15<sup>th</sup> day for rats treated with cisplatin. (Tables 4- 7) This is evidenced in marked increase in kidney SOD, catalase and GPx activities and decrease in kidney malondialdehyde (MDA) concentration of those treated with *Cymbopogon citratus* extract relative to the group treated with cisplatin alone.

The impairment in kidney function was accompanied by an increase MDA contents in kidney tissue (Somani *et al.*, 2000; Santos *et al.*, 2008) and an impaired activity of the antioxidant enzymes (Kadikoylu *et al.*, 2004).

Our results are also in agreement with previous studies as shown in Table 1-7. kidney injury develop after intraperitoneally injecting high dose of cisplatin (5mg kg<sup>-1</sup>). The kidney toxicity becomes obvious three days after injection, as indicated by marked elevations in the MDA levels and decrease in the antioxidant activities in rats' kidney tissues. (Tables 1-7)

The marked reduction in oxidative stress and lipid peroxides with the administration of *C. citratus* (Table 1-7) is in agreement with work by Ojo *et al.* (2006) who reported the antioxidative properties of *C. citratus* in paracetamol induced oxidative stress in rats. Confirming our findings, Olorunsanya *et al.* (2010) reported that *C. citratus* inhibits lipid oxidation in raw pork patties under refrigeration. Pei *et al.* (2012) also reported the alleviation of carbon tetrachloride –induced hepatic oxidative stress and toxicity as a result of the possible antioxidant potential of *C. citratus*.



This increase of the antioxidant enzymes SOD, catalase, GPx activities and decrease of lipid peroxides with administration of aqueous leaf extracts of *C.citratius* in cisplatin induced renal toxicity was in agreement with studies by other researchers on cisplatin nephrotoxicity (Nagizadeh *et al.*, 2008; Mahgoub, 2010; Sreedevi *et al.*, 2010).

These biochemical findings were further confirmed by evidences of microscopic examinations.

The kidney of rats in group 1 showed a normal architecture, normal organization of tubular epithelial cells and glomeruli cells (Plate 1)

In cisplatin treated kidney, drastic alterations were observed. Histopathological examination showed severe degeneration in tubular cells, congestion of glomeruli and infiltration of interstitium by inflammatory cells (Plate 2)

5% C.C + Cisplatin and 10 %C.C + Cisplatin treated kidney which are the test groups (3 and 4) generally showed defects observed in the cisplatin treated rats. There was significant improvement when compared with cisplatin treated kidney. Plate 3 and 4

Omotade (2009) reported that the leaves of *C. citratius* contained saponins, sesquiterpenes, lactones, steroids, flavonoids. Flavonoids are reported to exhibit antioxidant activity (Ramanathan *et al.*, 1989) and are effective scavengers of superoxide anions (Robak and Grygleuski, 1988). The aqueous extract of *C. citratius* may have exhibited hepatoprotective activity due to its possible antioxidant content attributable to flavonoids. Interestingly, saponins especially terpene glycosides are reported to enhance natural resistance and recuperative powers of the body (Singh *et al.*, 1991).

In conclusion, the results of this present study indicated that aqueous leaf extracts of *Cymbopogon citratius* has anti-nephrotoxic action against cisplatin induced renal toxicity in rats which might be ascribed to its antioxidant and free radical scavenging property .

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**Table 4: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON KIDNEY SOD ACTIVITY(U/mg protein)ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1.Control ( normal saline)	7.82 ± 0.02 <sup>a</sup>	7.81 ± 0.01 <sup>a</sup>	7.90 ± 0.01 <sup>a</sup>	7.76 ± 0.02 <sup>a</sup>	7.880 ± 0.01 <sup>a</sup>	7.83 ± 0.01 <sup>a</sup>
2.Cis (5mg/kg i.p) +2ml water	7.79 ± 0.01 <sup>b</sup>	4.98 ± 0.01 <sup>b</sup>	4.70 ± 0.02 <sup>b</sup>	4.62 ± 0.01 <sup>b</sup>	4.60 ± 001 <sup>b</sup>	4.56 ± 0.01 <sup>b</sup>
3.Cis (5mg/kg i.p) +2ml 5% C.C	7.83 ± 0.01 <sup>a</sup>	5.04 ± 0.01 <sup>b</sup>	5.92 ± 0.02 <sup>e</sup>	6.41 ± 0.01 <sup>e</sup>	6.74 ± 0.01 <sup>e</sup>	7.10 ± 0.01 <sup>e</sup>
4.Cis (5mg/kg i.p) +2ml 10% C.C	7.85 ± 0.01 <sup>a</sup>	4.98 ± 0.01 <sup>c</sup>	6.05 ± 0.01 <sup>f</sup>	6.48 ± 0.01 <sup>f</sup>	6.98 ± 0.01 <sup>f</sup>	7.45 ± 0.01 <sup>f</sup>
Results of one-way ANOVA						
F- value	48.84	18750	14510	18310	24010	24630
P- value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 27140, p<0.05. Treatment effect, p<0.05, F = 73380; time effect, p < 0.05, F = 95750

**Table 5: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON KIDNEY CATALASE ACTIVITY (U/mg protein) ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1. Control ( normal saline)	94.74 ± 0.50 <sup>a</sup>	98.07±0.15 <sup>a</sup>	97.30±0.44 <sup>a</sup>	97.90±0.46 <sup>a</sup>	94.80±0.75 <sup>a</sup>	96.30±1.57 <sup>a</sup>
2. Cis (5mg/kg i.p) +2ml water	94.06 ± 0.33 <sup>b</sup>	49.05±0.23 <sup>b</sup>	44.03±0.49 <sup>b</sup>	41.23±0.47 <sup>b</sup>	41.00±0.46 <sup>b</sup>	43.27±0.49 <sup>a</sup>
4. Cis (5mg/kg i.p) )+2ml 5% C.C	93.17±0.40 <sup>d</sup>	47.80±0.30 <sup>c</sup>	62.02±0.48 <sup>e</sup>	78.93±0.15 <sup>e</sup>	86.23±0.96 <sup>c</sup>	92.17±0.31 <sup>c</sup>
5. Cis (5mg/kg i.p) )+2ml 10% C.C	94.10±0.20 <sup>e</sup>	48.07±0.25 <sup>d</sup>	64.23±0.50 <sup>f</sup>	81.90±0.20 <sup>f</sup>	88.67±0.38 <sup>c</sup>	93.40±0.82 <sup>a</sup>
Results of one-way ANOVA						
F- value	3448	1912	3010	3400	17660	58.913
P- value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 4866, p<0.05. Treatment effect, p<0.05, F = 11780; time effect, p < 0.05, F = 17140



**Table 6: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON KIDNEY GLUTATHIONE PEROXIDASE ACTIVITY (U/mg protein) ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1.Control ( normal saline)	9.93±0.06 <sup>a</sup>	10.03±0.10 <sup>a</sup>	9.94±0.04 <sup>a</sup>	9.90±0.05 <sup>a</sup>	9.83±0.05 <sup>a</sup>	9.83±0.13 <sup>a</sup>
2.Cis (5mg/kg i.p) +2ml water	9.97±0.18 <sup>a</sup>	5.27±0.10 <sup>b</sup>	5.80±0.10 <sup>b</sup>	5.10±0.10 <sup>b</sup>	5.05±0.05 <sup>b</sup>	4.90±0.05 <sup>b</sup>
3.Cis (5mg/kg i.p) )+2ml 5% C.C	9.83±0.05 <sup>a</sup>	5.28±0.07 <sup>b</sup>	6.42±0.07 <sup>d</sup>	7.60±0.10 <sup>d</sup>	8.70±0.10 <sup>c</sup>	9.75±0.09 <sup>a</sup>
4.Cis (5mg/kg i.p) )+2ml 10% C.C	9.93±0.15 <sup>a</sup>	5.51±0.07 <sup>c</sup>	6.50±0.10 <sup>d</sup>	7.83±0.08 <sup>c</sup>	8.78±0.09 <sup>c</sup>	9.60±0.10 <sup>c</sup>
Results for one-way ANOVA						
F- value	0.772	1602	869.64	890.46	1278	1260
P- value	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 1482, p<0.05. Treatment effect, p<0.05, F = 5833; time effect, p < 0.05, F = 3154

**Table 7: EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACT OF C.C ON KIDNEY MDA LEVELS (nmol/g kidney tissue) ON CISPLATIN INDUCED RENAL OXIDATIVE STRESS AND TOXICITY IN RATS<sup>1</sup>**

Groups/Treatment	0days <sup>2</sup>	3days	6days	9days	12days	15days
1.Control ( normal saline)	375.87±1.59 <sup>a</sup>	369.77±0.35 <sup>a</sup>	367.97±0.21 <sup>a</sup>	368.07±0.35 <sup>a</sup>	369.53±0.60 <sup>a</sup>	373.70±0.30 <sup>a</sup>
2.Cis (5mg/kg i.p) +2ml water	372.37±0.59 <sup>a</sup>	1263.30±0.46 <sup>b</sup>	1288.80±0.36 <sup>b</sup>	1296.30±1.17 <sup>b</sup>	1192.90±0.25 <sup>b</sup>	1188.70±0.86 <sup>b</sup>
3.Cis (5mg/kg i.p) )+2ml 5% C.C	368.40±0.46 <sup>c</sup>	1254.40±0.44 <sup>d</sup>	1075.70±0.74 <sup>e</sup>	1039.70±0.15 <sup>e</sup>	746.00±0.62 <sup>e</sup>	617.50±0.60 <sup>e</sup>
4.Cis (5mg/kg i.p) )+2ml 10% C.C	367.10±0.20 <sup>c</sup>	1257.90±0.57 <sup>e</sup>	1054.20±0.36 <sup>f</sup>	1029.60±0.61 <sup>f</sup>	737.63±0.74 <sup>f</sup>	599.23±1.27 <sup>f</sup>
Results of one-way ANOVA						
F- value	59.228	1473000	1542000	826600	653100	419400
P- value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

<sup>1</sup>Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD). <sup>2</sup>Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 8327, p<0.05. Treatment effect, p<0.05, F = 3379; time effect, p < 0.05, F = 1564