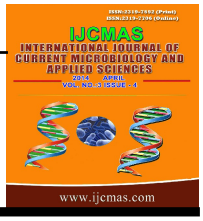
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 4 (2014) pp. 586-604**
http://www.ijcmas.com



Original Research Article

Cymbopogon citratus aqueous extract alleviates cisplatin-induced hepatic oxidative stress and toxicity in albino rats

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A B S T R A C T

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-mediated hepatic 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. Cisplatin treatment caused increase (P□0.05) in malondialdehyde (MDA) concentration, reduction (P□ 0.05) in the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in liver homogenate. Parallel to these changes, cisplatin treatment enhances hepatic damage as evidenced by sharp increase in serum biochemical parameters (alanine aminotransferase-ALT, aspartate aminotransferase-AST and alkaline phosphatase-ALP) (P□0.05). Additionally, the impairment of hepatic function corresponds histopathologically. However most of these changes were alleviated by prophylactic treatment with aqueous extract of *Cymbopogon* citratus dose and time dependently (P□ 0.05). The results of this present study indicated that aqueous leaf extracts of *Cymbopogon* citratus has anti-hepatotoxic action against cisplatin induced hepatic oxidative damage in rats which might be ascribed to its antioxidant and free radical scavenging property .

K e y w o r d s

Cymbopogon citratus; hepato-toxicity; cisplatin antioxidant anti-hepatotoxic

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).Vaccines, steroids and antiviral drugs, which are commonly used for treating liver diseases, have been found to have side effects and complications to

human health, especially when administered chronically or sub-chronically. Therefore, herbal products and traditional medicines with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants has been proposed as therapeutics agents, as well as drug co-adjuvants, to counteract liver damage.(Pei *et al*, 2012).

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 folkmedicine *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).The anti- hepatotoxic activity has also been reported(Arhoghro *et al*., 2012; Pei *et al*.,2012). 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 hepatic 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 hepatic antioxidative 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 hepatic antioxidative 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 liver tissues were immediately taken and fixed in 10% neutral buffered formalin for histopathological examination.

Preparation of liver Homogenate

The livers 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.

Serum hepatospecific markers

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman

and Frankel 1957. 0.2 ml of serum with 1 ml of substrate (aspartate and α-ketoglutarate for AST; alanine and α-keto glutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 minutes for ALT. 1 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as U/L. Alkaline Phosphatase was assayed according to the method of Rec (1972).

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 gluthathione 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 *et al*. (1963) as modified by Gutteridge and Wilkins (1980) was adopted for the assay of Malondialdehyde (MDA) concentration.

Histopathological study

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

Statistical Analysis

Data was expressed as Mean ± 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 and Discussion

Intraperitoneal administration of cisplatin (5mg/kg i.p.) caused abnormal liver function in all rats. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels increased ($P \square 0.05$) in the group treated with cisplatin only, after 6 days when compared with the normal (control). There were, however, slight decreases ($P \square 0.05$) on the 12th and 15th day (Tables 1, 2 and 3)

The serum AST, ALT and ALP levels of rats exposed to cisplatin and the various concentrations(5% and 10%) of aqueous extract of *Cymbopogon citratus* in groups 3 and 4 increased significantly on the 3rd day but decreased on the 9th ,12th and 15th day when compared to the cisplatin treated group ($P \square 0.05$) (Tables1,2 and 3). The decrease in serum AST, ALT and ALP levels by extracts were dose dependent ($P \square 0.05$). The effect of time of administration of aqueous extract of *Cymbopogon citratus* on ALT, AST and ALP activities was statistically significant ($P \square 0.05$).

Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on serum AST, ALT and ALP levels ($P>0.05$)

Intraperitoneal administration of cisplatin (5mg/kg i.p.) caused hepatic oxidative damage in all rats. Liver superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) decreased ($P \square 0.05$) in

the group treated with cisplatin only, after 15 days when compared with the normal (control) (Tables 4, 5, and 6 respectively).

The Liver 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^{rd} day but increased on the 6^{th} , 9^{th} ,12^{th} and 15^{th} day when compared to the cisplatin treated group ($P \square 0.05$) (Tables 4, 5 and 6 respectively). The increase in Liver SOD, catalase and GPx by both extracts though not statistically significant ($P \square 0.05$) were dose dependent. The effect of time of administration of aqueous extract of C.*citratus* on Liver SOD, catalase and GPx were statistically significant ($P \square 0.05$).

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

In addition, there was significant ($P \square 0.05$) increase in liver MDA concentration in the cisplatin treated rats after 15 days when compared with the normal (control) rats (Table 7).

The liver 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^{rd} day but decreased on the 6^{th} , 9^{th} ,12^{th} and 15^{th} day when compared to the cisplatin treated group ($P \square 0.05$) (Table 7).

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

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

The liver of rats in group 1 showed a normal architecture, cords of hepatocytes well preserved, cytoplasm not vacuolated, sinusoids well dermacated, no area of necrosis, no fatty change, no degeneration and no area of infiltration by inflammatory cells. Plate 1

In cisplatin treated liver, drastic alterations were observed. Histopathological examination showed extensive fatty change distended hepatocytes, vacuolated cytoplasm, compressed sinusoids, fatty degeneration, area of necrosis and infiltration by inflammatory cells. Plate 2

5% C.C + Cisplatin and 10 % C.C + Cisplatin treated liver 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 liver. Plate 3 and 4

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 sides 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 (Dalloz *et al*., 1992; Obata, 2002). Lipid peroxidation is important in the pathogenesis of cisplatin-induced hepatic injuries (Baliga *et al*., 1999).

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).

Many antioxidative agents have been analyzed in experimental and clinical studies searching for an agent to reduce or prevent cisplatin-induced hepatotoxicity(Ashavin *et al*., 2008; Ibrahim *et al*., 2009; Abdelmeguid *et al*., 2010).

Most studies reported previously, were designed to administer drugs before or at the same time of hepatic 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 hepatic repair after the onset of cisplatin-induced hepatic toxicity and oxidative stress, and thus, accelerate recovery in the rats.

Chemotherapeutic levels of cisplatin known to induce hepatic 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 Oruambo, 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 hepatotoxicity, manifested biochemically by a significant elevation in serum ALT, AST and ALP activities (Tables 1-3). Oral administration of aqueous extract of *Cymbopogon Citratus* (5% and 10%) after cisplatin administration caused a decline in hepatotoxicity after 15 days for rats treated with cisplatin. This was evidenced by marked decrease in serum ALT, AST and ALP activities of those treated with *Cymbopogon Citratus* extract relative to the group treated with cisplatin alone. (Tables 1-3)

Serum AST, ALT and ALP are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage (Sallie *et al*., 1991).

Confirming these findings, Pei *et al*. (2012) indicated the hepatoprotective effect of C. *citratus* on carbon tetrachloride- induced hepatic stress and toxicity.

Arhoghro *et al* (2012) also reported the antihepatotoxic effect of C. *citratus* on cisplatin- induced hepatotoxicity

This marked decrease in the activities of the three marker enzymes ALT, AST and ALP with administration of aqueous leaf extracts of C.*citratus* in cisplatin induced hepatotoxicity was in agreement with studies carried out by other researchers on cisplatin induced hepatotoxicity by other herbal plants such as black grape and tomato juice (Ashavin *et al*., 2008); Silymarin (Abdelmeguid *et al*., 2010); Zerumbone (Ibrahim *et al*., 2009).

Hepatotoxicity 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 liver homogenates. The increase of MDA is an indicator of liver tissue damage (Ohkwa *et al*., 1979) because MDA is an end product formed during the peroxidation of membrane polyunsaturated fatty acids.(Vaca *et al*.,1988)

Administration of cisplatin (5 mg kg^{-1} 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^{th} day for rats treated with cisplatin. (Tables 4- 7)This is evidenced in marked increase in liver SOD, catalase and GPx activities and decrease in liver malondialdehyde (MDA) concentration of those treated with *Cymbopogon Citratus* extract relative to the group treated with cisplatin alone.

Our results are also in agreement with previous studies as shown in Table 1-7. liver injury develop after intraperitoneally injecting high dose of cisplatin (5mg kg^{-1}).The liver toxicity becomes obvious three days after injection, as indicated by marked elevations in the MDA levels and decrease in the antioxidant activities in rats' liver 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.*citratus* in cisplatin induced hepatic toxicity was in agreement with studies by other researchers on cisplatin hepatotoxicity. (Ashavin *et al*., 2008; Ibrahim *et al*., 2009; Abdelmeguid *et al*., 2010).

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

Histopathological studies of the liver demonstrated that cisplatin (compared to normal) induces fatty degeneration, fatty change, distended hepatocytes, and compression of sinusoids and vacuolation of cytoplasm. This finding is correlated to the elevation of serum aminotransferase, ALP and hepatic oxidative stress markers

Table.1 Effect of C.*citratus* on serum AST activity (u/l) on Cisplatin induced hepatic oxidative damage in Rats

Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
1.Control (normal saline)	69.40 ± 0.62[a]	69.20 ± 0.95[a]	69.87 ± 1.42[a]	73.27 ± 0.47[a]	69.60 ± 1.10[a]	69.60 ± 0.62[a]
2.Cis (5mg/kg i.p) +2ml water	65.87 ± 0.21[a]	182.37 ± 2.15[b]	187.83 ± 0.95[b]	195.30 ± 0.26[b]	174.60 ± 1.15[b]	152.83 ± 0.29[b]
3.Cis (5mg/kg i.p))+2ml 5% C.C	73.30 ± 0.82[b]	178.37 ± 1.05[b]	189.67 ± 0.38[c]	123.13 ± 0.45[e]	105.27 ± 1.21[e]	94.07 ± 1.01[e]
4.Cis (5mg/kg i.p))+2ml 10% C.C	67.77 ± 0.61[a]	185.20 ± 0.95[b]	187.27 ± 1.21[b]	117.47 ± 0.84[f]	89.40 ± 1.08[f]	84.47 ± 1.66[d]
Results of one-way ANOVA		3164	5024	2607	2997	2051
F- value	49.11	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
P- value	p<0.05					

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 6082, p<0.05. Treatment effect, p<0.05, F = 28770; time effect, p < 0.05, F = 10370

Table.2 Effect of C.Citratus on Serum ALT Activity (U/L) on Cisplatin Induced Hepatic Oxidative Damage in Rats[1]

Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
1.Control (normal saline)	41.40 ± 1.59[a]	39.23 ± 0.57[a]	43.30 ± 1.14[a]	43.17 ± 1.76[a]	42.43 ± 0.65[a]	44.60 ± 1.37[a]
2.Cis (5mg/kg i.p) +2ml water	43.03 ± 1.29[a]	137.77 ± 0.71[b]	139.10 ± 1.21[b]	137.53 ± 0.57[b]	132.27 ± 0.92[b]	127.90 ± 0.89[b]
3.Cis (5mg/kg i.p))+2ml 5% C.C	44.67±0.49[a]	134.07 ± 0.83[c]	139.07 ± 0.70[b]	97.70 ± 0.78[g]	77.47 ± 1.61[e]	52.53 ± 0.91[c]
4.Cis (5mg/kg i.p))+2ml 10% C.C	43.90 ±' 0.20[a]	141.53 ± 0.50[d]	136.43 ± 1.75[b]	87.23 ± 1.66[f]	71.77 ± 1.11[f]	45.27 ± 0.35[a]
Results of one-way ANOVA		5608	2545		1343	4173
F- value	7.85	p<0.05	p<0.05	2260	p<0.05	p<0.05
P- value	p<0.05			p<0.05		

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 4461, p<0.05. Treatment effect, p<0.05, F = 8370; time effect, p < 0.05, F = 19950

Table.3 Effect of C. *citratus* on serum ALP Activity (U/L) on Cisplatin induced hepatic oxidative damage in rats[1]

Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
1.Control (normal saline)	235.22 ± 4.48[a]	232.39 ± 1.36[a]	248.70 ± 0.25[a]	245.47 ± 0.78[a]	245.53 ± 0.86[a]	233.39 ± 2.77[a]
2.Cis (5mg/kg i.p) +2ml water	245.76 ± 2.13[b]	388.56 ± 1.32[b]	388.39 ± 1.11[b]	388.91 ± 1.65[b]	386.45 ± 0.71[b]	383.76 ± 0.98[b]
3.Cis (5mg/kg i.p))+2ml 5% C.C	246.31 ± 1.47[b]	383.60 ± 0.40[c]	366.01 ± 1.37[e]	327.35 ± 1.43[d]	284.88 ± 0.98[c]	238.78 ± 0.81[d]
4.Cis (5mg/kg i.p))+2ml 10% C.C	248.99 ± 2.59[b]	381.37 ± 0.48[d]	360.17 ± 2.85[f]	324.73 ± 0.66[d]	282.87 ± 0.17[c]	236.39 ± 1.25[a]
Results of one-way ANOVA						
F- value	11.8	16190	2378	3418	7004	4310
P- value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 4461, p<0.05. Treatment effect, p<0.05, F = 8370; time effect, p < 0.05, F = 19950

Table.4 Effect of C.*citratus* on SOD Activity (U/mg protein) in Cisplatin induced hepatic oxidative damage in rats[1]

Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
1.Control (normal saline)	5.91 ± 0.02^{a}	5.91 ± 0.02^{a}	6.14 ± 0.15^{a}	6.07 ± 0.02^{a}	6.09 ± 0.01^{a}	5.85 ± 0.11^{a}
2.Cis (5mg/kg i.p) +2ml water	5.89 ± 0.02^{b}	2.86 ± 0.02^{b}	2.50 ± 0.02^{b}	2.47 ± 0.01^{b}	2.44 ± 0.12^{b}	2.46 ± 0.01^{a}
3.Cis (5mg/kg i.p) +2ml 5% C.C	5.83 ± 0.01^{e}	2.77 ± 0.01^{e}	3.74 ± 0.01^{e}	4.12 ± 0.05^{e}	5.02 ± 0.01^{d}	5.53 ± 0.01^{c}
4.Cis (5mg/kg i.p) +2ml 10% C.C	5.78 ± 0.03^{f}	2.83 ± 0.01^{f}	3.76 ± 0.01^{f}	4.31 ± 0.02^{e}	5.21 ± 0.15^{b}	5.72 ± 0.15^{c}
Results of one-way ANOVA						
F- value	29560	37550	15830	17420	25490	27.292
P- value	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 17770, $p<0.05$. Treatment effect, $p<0.05$, F = 45170; time effect, $p < 0.05$, F = 63680

Table.5 Effect of C.*citratus* on Catalase Activity (U/mg protein) in Cisplatin induced Hepatic Oxidative damage in rats[1]

Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
Control (normal saline)	63.96 ± 1.69[a]	64.37 ±1.11[a]	67.05 ± 0.57[a]	69.14 ± 0.25[a]	68.17 ± 0.90[a]	69.40 ± 0.62[a]
Cis (5mg/kg i.p) +2ml water	63.10 ± 0.62[a]	38.67 ± 0.45[b]	36.24 ± 0.61[b]	34.01 ± 0.27[b]	33.03 ± 0.61[b]	29.73 ± 0.35[b]
Cis (5mg/kg i.p) +2ml 5% C.C	63.53 ± 0.60[a]	35.94 ± 0.15[e]	51.83 ± 1.74[e]	54.20 ± 0.26[e]	62.93 ± 0.15[e]	65.94±0.15[e]
Cis (5mg/kg i.p) +2ml 10% C.C	67.14 ± 0.38[b]	36.77 ± 0.35[c]	56.33 ± 0.68[f]	59.20 ±1.05[f]	65.75 ± 0.54[f]	67.63 ± 0.45[f]
Results of One-way ANOVA					1815	2975
F-value	5.972	1288	292.92	1596	p<0.05	p<0.05
P- value	p<0.05	p<0.05	p<0.05	p<0.05		

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 924.28, p<0.05. Treatment effect, p<0.05, F = 2539; time effect, p < 0.05, F = 2814

Table.6 Effect of C.*citratus* on Glutathione Peroxidase activity (U/mg protein) on Cisplatin induced hepatic oxidative damage in rats[1]

Groups/Treatment	0days[1]	3days	6days	9days	12days	15days
1.Control (normal saline)	8.80 ± 0.10[a]	8..67 ± 0.03[a]	8.83 ± 0.06[a]	8.73 ± 0.03[a]	8.73 ± 0.08[a]	8.80 ± 0.10[a]
2.Cis (5mg/kg i.p) +2ml water	8.77 ± 0.03[a]	4.77 ± 0.12[b]	5.28 ± 0.03[b]	4.40 ± 0.10[b]	4.25 ± 0.05[b]	4.07 ± 0.08[b]
3.Cis (5mg/kg i.p) +2ml 5% C.C	8.83 ± 0.08[a]	4.97 ± 0.03[c]	5.23 ± 0.13[b]	6.77 ± 0.08[c]	7.88 ± 0.08[c]	8.82 ± 0.03[c]
4.Cis (5mg/kg i.p) +2ml 10% C.C	8.63 ± 0.06[a]	4.70 ± 0.10[b]	5.23 ± 0.06[b]	6.86 ± 0.03[c]	7.77 ± 0.08[c]	8.60 ± 0.10[c]
Results for one-way ANOVA						
F- value	5.886	1375	1272	1422	1023	1573
P- value	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 1886, p<0.05. Treatment effect, p<0.05, F = 7522; time effect, p < 0.05, F = 3798

Table.7 Effect of C. *citratus* on MDA levels (nmol/g liver tissue) on Cisplatin induced hepatic lipid peroxidation in rats[1]

Groups/Treatment	0days [2]	3days	6days	9days	12days	15days
1.Control (normal saline)	390.13 ± 1.06^{a}	397.30±0.66^{a}	402.20 ± 0.67^{a}	398.77 ± 0.31^{a}	390.20 ± 0.30^{a}	402.33 ± 1.16^{a}
2.Cis (5mg/kg i.p) +2ml water	394.17 ± 0.25^{b}	1179.30 ± 0.70^{b}	1221.70 ± 1.86^{b}	1239.90 ±1.02^{b}	1241.40 ± 0.57^{b}	1209.60 ± 0.96^{b}
3.Cis (5mg/kg i.p) +2ml 5% C.C	395.73 ± 0.55^{e}	1172.50 ± 0.50^{e}	1059.80 ± 0.81^{e}	949.27 ± 1.12^{e}	587.77 ± 0.71^{d}	507.97 ± 0.40^{c}
4.Cis (5mg/kg i.p) +2ml 10% C.C	386.03 ± 0.40^{f}	1170.50 ± 0.67^{f}	1038.90 ± 0.75^{f}	939.97 ± 0.60^{f}	564.97 ± 0.67^{e}	497.70 ± 0.92^{b}
Results of one-way ANOVA						
F- value	428580	761600	297200	221300	688600	161.931
P- value	p< 0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

[1]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). [2]Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 653200, p<0.05. Treatment effect, p<0.05, F = 2769000; time effect, p < 0.05, F = 138300

Plate 1 Photomicrograph of normal liver showing a normal architecture with central vein (arrowhead). Cords of hepatocytes well preserved (arrow) cytoplasm not vacuolated. Sinusoids well demarcated no area of necrosis, no fatty change, no fatty degeneration. **H & E stained X 100 Mag**

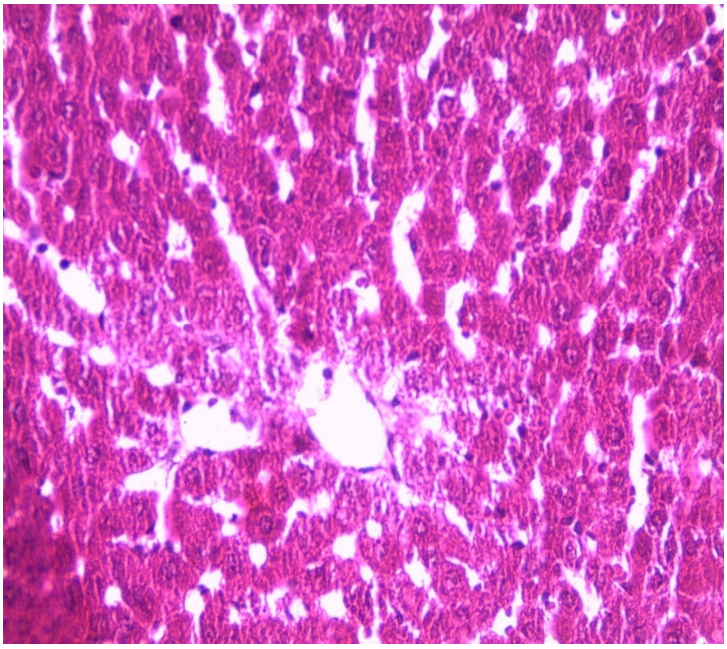


Plate 2 Photomicrograph of liver administered with cisplatin showing enlarged hepatocytes with vacuolation of cytoplasm (arrow).There is compression of the sinusoid (arrowhead). **H & E stained X 100 Mag**

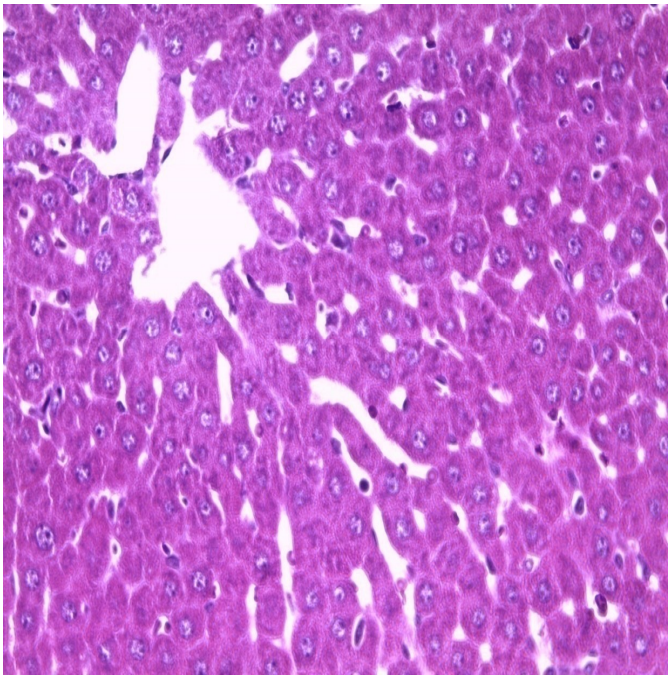


Plate 3 Photomicrograph of liver administered with cisplatin and 5% C.C. showing central vein (arrowhead) Sinusoids well demarcated (arrow).**H & E stained X 100 Mag**

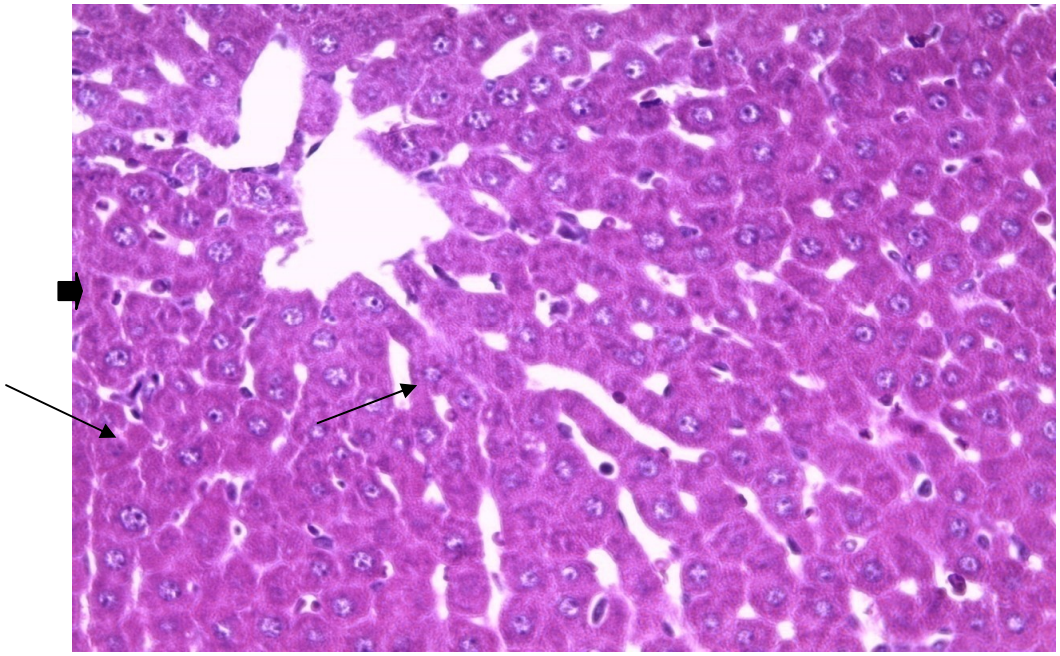
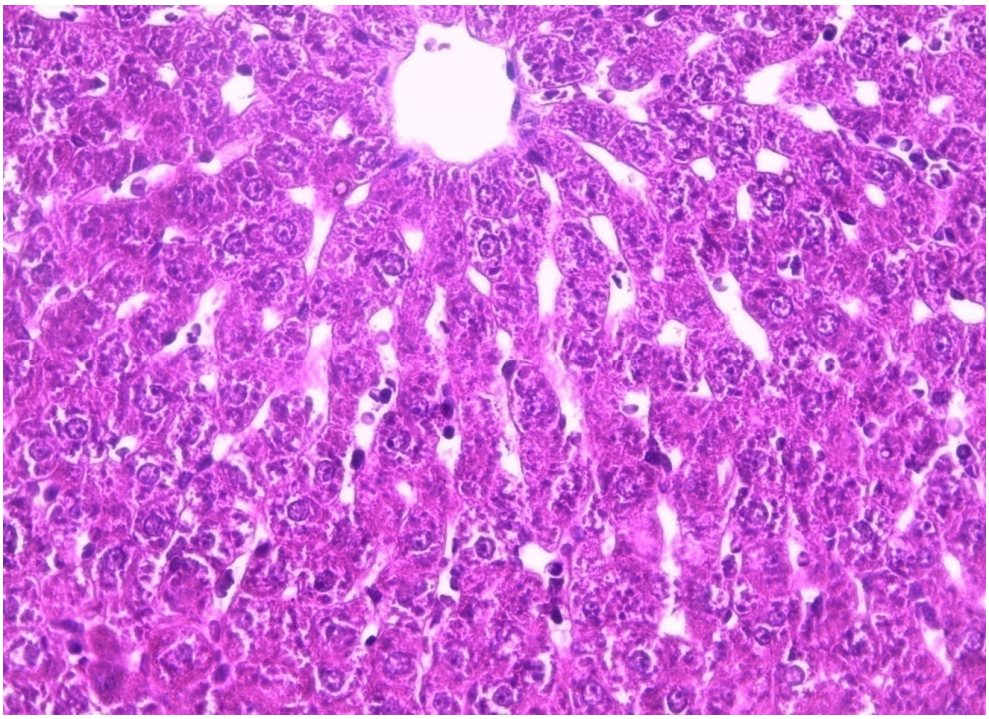


Plate 4. Photomicrograph of liver administered with cisplatin and 10% C.C showing essentially normal architecture with central vein (arrowhead) and sinusoids well demarcated (arrow) **H & E stained X 100 Mag**



which denote the progression of acute liver damage these changes could be as a result of biochemical changes that occurred in liver cells. As observed in this study, increase in the liver enzyme activities is secondary to the liver dysfunction and is also associated with disruption of cellular structure.

This is in agreement with findings of Krisha and Keena (1980) and Kiceniuk *et al*., (1982) who observed that xenobiotics caused an elevation of liver enzyme activity resulting to severe liver damage. C.C + Cisplatin treated liver which are the test groups (3 and 4) generally showed defects observed in the cisplatin treated rats. There was significant improvement when compared to liver treated with cisplatin alone. There were however, liver in some of the groups that reverted completely to normal liver (Plate 3 and 4). Results were in agreement with work done by Arhoghro *et al.*,(2012)

Omotade (2009) reported that the leaves of C. *citratus* 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. *citratus* 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* citratus has anti-hepatotoxic action against cisplatin induced hepatic oxidative damage in rats which might be ascribed to its antioxidant and free radical scavenging property.

References

Abdelmeguid NE, Chmaisse HN, Abou Zeinab NS 2010.Silymarin Ameliorates Cisplatin-Induced Hepatotoxicity in Rats: Histopathological and Ultrastructural Studies. *Pak. J. B. Sci.*,1310:463-479.

Antunes LM, Darin JDC, Bianchi NL 2001. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. *Pharmacol Res*., 43:145-150.

Antunes LMG, Darin JD, Bianchi MD 2000. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. *Pharmacol Res.*, 41:405-411.

Aoyagi, K., Akiyama, K., Shahrzad, S., Tomida. C., Hirayama, A., Nagase, S., Takemura, K., Koyama, A., Ohba, S. and Narita, M.1999. Formation of guanidinosuccinic acid, a stable nitric oxide mimic, from argininosuccinic acid and nitric oxide-derived free radicals. *Free Radic. Res.* 31:59-65.

Arhoghro, E.M.**,** Kpomah, E.D. and Uwakwe, A.A**.** 2012. Curative Potential of Aqueous Extract of lemon grass *Cymbopogon citratus* on Cisplatin - Induced Hepatotoxicity in Albino Wistar Rats. *Journal of Physiology and Pharmacology Advances* 28: 282- 294

Ashavin A, Cetin R, Erguder IB, Candir O, Ozturk HS, Durak L 2008. Cisplatin causes oxidation in rat liver tissues: Possible Protective Effects of Antioxidant food Supplementation. *Turk J. Med. Sci.*, 38 2:117-120.

Baliga, R., Ueda, N., Walker, P.D. and Shah, S.V. 1999. Oxidant mechanisms in toxic acute renal failure. *Drug Metab. Rev.* 31:971-997.

Blanco MM, Costa CA, Freire AO, Santos JG, Costa M 2009. "Neurobehavioral effect of

essential oil of Cymbopogon citratus in mice". *Phytomedicine*, 16 2-3: 265–70.

Burkill IH 1996. The useful plants of West Tropical Africa, Royal Botanic Garden, Ken 2: 29-30

Chance, B. and Maehly, A. 1955. Assay of catalases and peroxidases. In Methods Enzymology Vol 2: Chance E, Maehly A eds. pp. 764–768, New York: Academic Press.

Cohen, G.D., Dembiec and Marcus, J. 1970. Measurement of catalase activity in tissue extracts. *Anal Biochem*. 34: 30-38.

Dalloz, F., Maupoil, V., Lecour, S., Briot, F. and Rochette, L. 1997. In vitro studies of interactions of NO donor drugs with superoxide and hydroxyl radicals. *Mol Cell Biochem* 177:193-200.

Figueirinha A, Cruz MT, Francisco V, Lopes MC, Batista MT 2010.Anti-inflammatory activity of Cymbopogon citratus leaf infusion in lipopolysaccharide-stimulated dendritic cells: contribution of the polyphenols *Journal of Medicinal Food*, 133:681-90.

Gutteridge, J.M. and Wilkins, S. 1982. Copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. FEBS Letts. 137: 327-330

Hunter, F.E., Gebicki, J.M., Hoffsten, P.E, Weinstein, J. and Scott, A. 1963. Swelling and lysis of rats liver mitochondria induced by ferrous ions. *Journals* of *Biological Chemistry* 23: 828 – 835.

Ibrahim YM, Abdul BA, Ibrahim TT, AbdelWahab SI, Elhassan MM, Mohan S 2009. Attenuation of cisplatin-induced hepatotoxicity in rats using zerumbone.*Res.J Bio.Sci*, 4 7:777-784.

Kadikoylu, G., Bolanam, Z., Demir, S., Balkaya, M., Akalin, N. and Enli, Y. 2004. The effects of desferrioxamine on cisplatin-induced lipid peroxidation and the activities of antioxidant enzymes in rat kidneys. *Hum Exp Toxicol.* 23:29-34.

Kawai, Y., Nakao, T., Kunimura, N., Kohda,Y. and Gemba, M. 2006. Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. *J. Pharmacol. Sci*. 100:65–72.

Kiceniuk JW, Khan RA, Dawe M, Williams U 1982. Examination and interaction of trypanosome infection and crude oil exposure on haematology of the Longhorn Sculphin Myoxocephalus octodeces – pinosus. Bull Environm, Contam- Toxi. Col. 28 435-438.

Krishan AG, Veena G 1980. 2,3,4-Triaminoazo benzene- induced hemato biochemical Anomalies in fish Channa puntatus Bull Environm.Contam. Toxicol 25: 136 – 141.

Lee, H.J., Jeong, H.S., Kim, D.J., Noh, Y.H., Yuk, D.Y. and Hong, J.T. 2008. Inhibitory effect of citral on NO production by suppression of iNOS expression and NF-kappa B activation in RAW264.7 cells.*Archives of Pharmacal. Research* 313:342-9.

Leite, J.R., Seabra Mde, L., Maluf, E., et al. 1986. "Pharmacology of lemon grass Cymbopogon citratus Stapf. III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans". J. Ethnopharmacol. 17 1: 75–83.

Mansour HH, Hafez HF, Fahmy NM 2006. Silymarin modulates Cisplatin 10 mg kg^{-1} platin induced oxidative stress and hepatotoxicity in rats. *J. Biochem. Mol. Biol*., 39: 656-661.

Mora LO, Antunes LM, Francescato HD, Bianchi M 2003. The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. *Pharmacol. Res*., 47: 517-522.

Obata, T.2002. Nitric oxide and depolarization induce hydroxyl radical generation. *Jpn. J. Pharmacol.* 88:1-5.

Ohkawa, H., Ohishi, N. and Yagi, K.1979. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem .95: 351–358.

Ojo,O.O., Kabutu, F.R., Bello, M. and Babayo, U. 2006. Inhibition of paracetamol-induced oxidative stress in rats by extracts of lemongrass *Cymbropogon citratus* and green tea *Camellia sinensis* in rats, *Afr. J. Biotechnol*. 5: 1227-1232.

Okoko T, Oruambo IF 2008. The effect of *Hibiscus sabdariffa* calyx extract on

cisplatin-induced tissue damage in rats. *Biokemistri,* 202: 47-52.

Olorunsanya AO, Olorunsanya EO, Bolu SAO, Adejumobi CT, Kayode RM 2010. Effect of graded levels of lemongrass *Cymbopogon citratus* on oxidative stability of raw or cooked pork patties. *Pak.J.Nutr*. 95: 467-470.

Omotade IO 2009. Chemical profile and antimicrobial activity of *Cymbopogon citratus* leaves.*Journal of Natural Products* ,2:98-103.

Park HR, Ju EJ, Jo SK, Jung U, Kim SH, Yee ST 2009. Enhanced antitumor efficacy of cisplatin in combination with HemoHIM in tumor-bearing mice. *BMC Cancer*, 9: 85-85.

Pei HK, Mokhtar RA, Mohammad I 2012. Antioxidant potential of *Cymbopogon citratus* extract: alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. *Hum. Exp. Toxicol.* 311*:81-91*

Pohocha N, Grampurohit ND 2001. Antispasmodic activity of the fruits of *Helicteres isora* Linn *Phytother Res*., 1:49-52.

Pratibha R, Sameer R, Rataboli PVD, Bhiwgade A, Dhume CY 2006. Enzymatic studies of Cisplatin 10 mg kg^{-1} platin induced oxidative stress in hepatic tissue of rats. *Eur. J. Pharmacol*., 532: 290-293.

Ramanathan R, Luu K K, Das NP 1989. Anti –peroxidative action of flavonoids and related products in ground pork Abstracts proceedings of III International Symposium on flavonoids in Biology and Medicine. Singapore. p.56.

Rec GSCC 1972.l Optimized standard colorimetric methods. *J. Clin.Chem. Clin. Biochem.,* 10: 182.

Reitman S, Frankel AS 1957. A colometric method of determination of serum glutamic pyruvic transaminase. *Amer*. J. *Clin*. *Patho*.28: 56.

Robak k, Grygleuski RJ 1988. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol*., 37: 837-841.

Rodrigues E, Carlini EA 2006. Plants with possible psychoactive effects used by the Krahô Indians, Brazil. *Revista Brasileira de Psiquiatria* 284: 277-282.

Sallie R, Tredger M, Willam R 1991. Drugs and the Liver, *Biopharmaceutical Drug Dispos*., 12: 251-259.

Singh, N., Verma, P., Misharu, N. and Nath R 1991. A comparative evaluationof some antistress agents of plant origin. *Indian J. Pharmacol*. 21: 99.

Singh J 1989. A possible mechanism of cisplatin-induced nephrotoxicity. *Toxicol.*, 58:71-80.

Stein J, Lifschitz M, Barnes L,1978. Current concepts on the nephrotoxicity of acute renal failure. *Am. J. Physiol*. 243:F171-F181.

Tiwari M, Dwivedi UN, Kakkar P 2010. Suppression of oxidative stress and pro-inflammatory mediators by Cymbopogon citratus D. Stapf extract in lipopolysaccharide stimulated murine alveolar macrophages. *Food Chem Toxicol*., 4810:2913-9.

Vaca, C.E.,Wilhelm, J. and Harms-Rihsdahl M.1988 Interaction of lipid peroxidation product with DNA. A review. Mut Res: Rev Gen Toxicol 195: 137–149.

Wargovich, M.J., Woods, C., Holis, D.M. and Zander, N.E. 2001. Herbals, cancer prevention and health. *J. Nutr*. 13111: 30345- 30365.

Weijl, N.I., Cleton, F.J. and Osanto, S. 1997. Free radicals and antioxidants in chemotherapy-induced toxicity. *Cancer Treat. Rev*. 23:209-240.

Yilmaz, H.R., Iraz, M., Sogut, S., Ozyurt, H., Yildirim, Z., Akyol, O. and Gergerlioglu, S. 2004 The effects of erdosteine on the activities of some metabolic enzymes during cisplatin-induced nephrotoxicity in rats. *Pharmacol.*.