Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

CURATIVE POTENTIAL OF AQUEOUS EXTRACT OF SCENT LEAF (*OCIMUM GRATISSIMUM*) ON CISPLATIN INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

E. M. Arhoghro[1], C. Ikeh[2], A.A Uwakwe[3], K. E. Ekpo[4*], E.O. Anosike[5]
[1]Department of Medical Biochemistry, Niger Delta University, Bayelsa State, Nigeria
[2]Department of Pharmacology, Niger Delta University, Bayelsa State, Nigeria
[3]Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria
[4]Department of Biochemistry, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[5]Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria
*Email: kokoeteekpo@yahoo.com

Received on: 31/03/12 Revised on: 28/05/12 Accepted on: 15/06/12

ABSTRACT
Cisplatin is an effective chemotherapeutic agent used for a wide variety of tumors, but is reported to be hepatotoxic. In the current study, the dose dependent and time course curative potential of aqueous leaf extract of *Ocimum gratissimum* (O.G.) on cisplatin induced hepatotoxic rats using biochemical and histopathological approaches was evaluated. Male albino wistar rats weighing between 150-200g were randomly separated into four different groups of eighteen (18) rats per group. Rats in group 1 received no cisplatin. Normal saline was administered intraperitoneally (i.p). The rats in group 2 were injected with a single dose of cisplatin (5 mg/kg body weight i.p). Tissue damage was also induced in rats in groups 3 and 4 by a single intraperitoneal-administration of cisplatin (5 mg/kg body weight). After three days, 2ml/kg body weight of 5% and 10% aqueous extract of *Ocimum gratissimum* 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 while rats in group 1 were the untreated controls. They were all allowed unlimited access to tap water and growers' mash. Results showed the extract to cause significant ($P \leq 0.05$) dose and time related attenuation in the elevation of serum liver enzyme markers of acute hepatocellular injury (ALT, AST and ALP) and increase in serum protein. Cisplatin treatment caused significant increase ($P \leq 0.05$) in serum alanine aminotransferase (ALT) from 43.03 ± 1.29 to 127.90 ± 0.89 U/L and a decrease ($P \leq 0.05$) in serum protein concentration from 93.70 ± 0.61 to 50.43 ± 1.53 g/l. There were considerable decreases ($P \leq 0.05$) in body weight and liver weight to body weight ratio in the test animals. However, most of these observed changes were alleviated by prophylactic treatment with aqueous extract of O. *gratissimum* which was also found to be dose and time dependent ($P \leq 0.05$). The ameliorating effect was further evident through decreased histopathological alterations of liver tissues in the groups treated with aqueous extract of O. *gratissimum* (5% and 10%). The results from this study indicate that aqueous leaf extracts of O. *gratissimum* has anti-hepatotoxic action against cisplatin induced hepatic toxicity in rats. Hence the extracts have the potential to be used for the management of hepatopathies and as a therapeutic adjuvant in cisplatin toxicity.
KEYWORDS: *Ocimum gratissimum,* Aqueous extract**,** Cisplatin induced hepatotoxicity.

INTRODUCTION

Cisplatin [cis-diamminedichloroplatinum (II)] (CDDP), a platinum-containing anticancer drug, is one of the most commonly used potent antineoplastic agent for the treatment of a wide range of cancers [1]. Despite its excellent anticancer activity, the clinical use of cisplatin is often limited by its undesirable severe toxic side effects that interfere with its therapeutic efficacy [2, 3, 4]. 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[5,6]. While nephrotoxicity of CDDP has been recognized as the most important dose-limiting factor, little is known about CDDP induced liver injury [7]. Extensive investigations have been conducted on the hepatotoxicity as well as general organ toxicity of this anticancer drug [8,9]. These include light and electron microscopic studies of various organs and biochemical studies of liver enzymes[8,10]. There is very limited information concerning the effects of these drugs on histopathology and ultrastructure of liver cells. Further, a variety of agents including antioxidants have been shown to attenuate the hepatotoxicity of cisplatin[11]. Oxidative stress is one of the most important mechanisms involved in CDDP-induced toxicity resulting in the enhanced production of reactive oxygen species, reduction in the mitochondrial membrane potential[12] and decrease in antioxidant enzymes [13]. Therefore, antioxidants administered before cisplatin treatment act against toxicity[14].

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[15]. Over 50 % of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry.

Investigations into the chemical and biological activities of plants during the past two centuries have yielded components for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents[16].

Plant foods especially vegetables contribute to both local diets and ethno medicine in developing countries like Nigeria[17, 18].

Ocimum gratissimum (linn), family labiateae is a shrub commonly found around village huts and gardens[19]. The plant is popular among various ethnic groups in Nigeria. It is known as effin ajasin in Yoruba, ebavbokho in Bini, aaid dya ta gida in Hausa, nchanwu in Ibo, froukena in Ijaw and oran in Urhobo. Mostly a weed of the road sides and wasteland, but is also important in pastures. It prefers moist and fertile soils during growth, but will tolerate drought after flowering [20]. The plant occurs in deciduous forests and savannah and is usually cultivated for its medicinal uses and as food flavour. *Ocimum gratissimum* (O.G) is propagated by seeds or cuttings.

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it is used for medicinal, condiment and culinary purposes. The flowers and leaves of this plant are rich in essential oils, and so it is used in preparation of teas and

infusion[21]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea[22]. In the Savannah areas decoctions of the leaves are used to treat mental illness[23]. *O. gratissimum* is used by the Ibos of South Eastern Nigeria in the management of the baby's cord; to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh [24]. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye and skin, pneumonia, cough, fever and conjunctivitis[25]. The oil is known to exhibit antimicrobial, insect repellant , and antihelminthic activities[26]. Oboh[27] reported the antioxidant properties of *O. gratissimum* .The extract of *O. gratissimum* exhibited antibacterial activity [28].This extract when fed to rabbits reduced the weight and suppressed the hemopoietic system[29]. There is also report that leaf extracts from the plant are able to inhibit and even reverse carbon tetrachloride induced hepatotoxicity in rats[30]. Obianime *et al*[31] reported that the serum levels of the hepatic enzymes on prolonged administration of O.G in mouse were not significantly altered. This suggests that hepatic function in the mouse is not adversely affected by O.G. Aguiyi *et al*[32] also reported the hypoglycaemic activity of *O. gratissimum.* In this present study we report the dose and time dependent effects of the aqueous leaf extracts of O. *gratissimum* on cisplatin induced liver damage.

MATERIALS AND METHODS

Animals

Seventy two (72) adult healthy male wistar 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 and Randox from Randox Laboratories Ltd., United Kingdom were used. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

Preparation of extracts

The leaves of *O. gratissimum* 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 *O. gratissimum* 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 O *gratissimum* that were administered orally to each of the rats.

Experimental design and procedures

Cisplatin model for evaluation of antihepatotoxic activity

Cisplatin BP (50mg/50ml) was administered to the test rats intraperitoneally at a dose of 5mg/Kg body weight [41, 57].

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[41].

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 *Ocimum gratissimum* 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.

Liver as ratio of body weight

Liver was removed and weighed immediately. Liver ratio was calculated with the following formula, organ ratio(%) = organ weight (g) X 100/body weight(g)

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 [33]. Alkaline Phosphatase was estimated according to the method of Rec [34]. Serum Total Protein was estimated using the Biuret method [35].

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.

Statiscal 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 *Ocimum gratissimum*, two-way analysis of variance was used.

RESULTS

Animal model of cisplatin induced hepatotoxicity was used for the present study. There was significant ($P \leq 0.05$) decrease in body weight in the cisplatin treated rats after

15 days when compared with the normal (control) rats (Table1).

The body weights of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of *Ocimum gratissimum*, in groups 3 and 4 respectively, decreased significantly on the 3rd and 6th day but increased on the 9th , 12th and 15th day when compared to the cisplatin treated group (P≤0.05) (Table 1).

The increase of the body weights by extracts though not statistically significant (P≥0.05) was dose dependent. Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on body weight (P≤0.05) (Table 1)

Moreover a significant (P≤0.05) decrease in liver as % of body weight was noticed in cisplatin treated rats after 15 days when compared with the normal (control) (Table 2). Also, the administration of cisplatin along with various concentrations (5% and 10%) of the aqueous extracts (O.G.) increased liver weight as % of body weight significantly (P≤0.05) in all groups receiving treatment with the extracts after 15 days when compared with the cisplatin treated group (Table 2).

The increase in liver weight as % of the body weight by extracts though not statistically significant (P≥0.05) was dose dependent. Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on liver weight as % of body weight (P≤0.05) (Table 2).

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≤0.05) in the group treated with cisplatin only, after 3 days when compared with the normal (control). There were, however, slight decreases (P≥0.05) on the 12th and 15th day (Tables 3, 4 and 5)

The serum AST, ALT and ALP levels of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of *Ocimum gratissimum* 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≤0.05) (Tables 3,4 and 5). The decrease in serum AST, ALT and ALP levels by extracts were dose dependent (P≤0.05). The effect of time of administration of aqueous extract of *O. gratissimum* on ALT, AST and ALP activities was statistically significant (P≤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).

Also, there was significant (P≤0.05) decrease in serum protein in the cisplatin treated rats after 5 days when compared with the normal (control) rats (Table 6).

The serum protein of rats exposed to cisplatin and the various concentrations (5% and 10%) in groups 3 and 4 respectively, decreased significantly on the 3rd day but increased on the 6th , 9th , 12th and 15th day when compared to the cisplatin treated group (P≤0.05) (Table 6).

The increase of the serum protein by the extracts though not statistically significant (P≥0.05) was dose dependent. The effect of time of administration of aqueous extract of O. *gratissimum* on protein concentration was statistically significant (P≤0.05).

Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on serum protein (P≤0.05) (Table 6).

Table 1: Effect of administration of aqueous extract of o.g on body weight (g) on cisplatin induced hepatotoxicity in rats[1]

Body weight (g)						
Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
Control (normal saline)	165.93 ± 0.31[a]	174.33 ± 0.29[a]	175.73 ± 0.46[a]	179.43 ± 0.40[a]	190.50 ± 0.50[a]	209.17 ± 0.29[a]
Cis(5mg/kg i.p) +2ml water	215.50 ± 0.50[b]	162.83 ± 0.29[b]	155.50 ± 0.50[b]	159.77 ± 0.25[b]	162.93 ± 0.31[b]	159.97 ± 0.87[b]
Cis (5mg/kg i.p)+2ml 5% O.G	176.93 ± 0.31[c]	153.90 ± 0.26[c]	149.97 ± 0.15[c]	156.73 ± 0.31[c]	164.13 ± 0.15[c]	175.17 ± 0.40[c]
Cis (5mg/kg i.p))+2ml 10% O.G	164.10 ± 0.36[d]	137.10 ± 0.36[d]	140.67 ± 0.58[d]	149.80 ± 0.26[d]	155.87 ± 0.42[d]	162.90 ± 0.17[d]
Results of one-way ANOVA F- value P- value	14250 p< 0.05	11270 p< 0.05	4110 p< 0.05	4206 p< 0.05	7010 p< 0.05	7011 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 = 8327, p<0.05. Treatment effect, p<0.05, F = 33790; time effect, p < 0.05, F = 15640

Table 2: Effect of administration of aqueous extract of o.g on liver weight as % of body weight on cisplatin induced hepatotoxicity in rats[1]

Liver weight as % body weight (%)						
Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
Control (normal saline)	3.18 ± 0.01[a]	3.16 ± 0.00[a]	3.18 ± 0.01[a]	3.17 ± 0.02[a]	3.17 ± 0.01[a]	3.18 ± 0.01[a]
Cis (5mg/kg i.p) +2ml water	3.17 ± 0.01[a]	2.61 ± 0.00[b]	2.53 ± 0.01[b]	2.51 ± 0.01[b]	2.52 ± 0.00[b]	2.53 ± 0.01[b]
Cis (5mg/kg i.p)+2ml 5% O.G	3.17 ± 0.00[a]	2.60 ± 0.00[b]	2.75 ± 0.01[c]	2.83 ± 0.01[c]	2.91 ± 0.00[c]	3.01 ± 0.00[c]
Cis (5mg/kg i.p))+2ml 10% O.G	3.18 ± 0.01[a]	2.62 ± 0.01[b]	2.77 ± 0.01[c]	2.82 ± 0.00[c]	2.93 ± 0.01[d]	3.05 ± 0.01[d]
Results of one-way ANOVA F- value P- value	1.86 p> 0.05	365.39 p< 0.05	338.40 p< 0.05	4161 p< 0.05	4050 p< 0.05	1645 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 = 1710, p<0.05. Treatment effect, p<0.05, F = 4169; time effect, p < 0.05, F = 6500

Table 3: Effect of administration of aqueous extract of o.g on serum ast activity (u/l) on cisplatin induced hepatotoxicity in rats[1]

Serum AST (U/L)						
Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
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]
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]
Cis (5mg/kg i.p)+2ml 5% O.G	70.80 ± 0.46[a]	185.37 ± 1.19[b]	188.60 ± 0.90[b]	134.23 ± 1.68[c]	108.50 ± 1.15[c]	89.67 ± 0.55[c]
Cis (5mg/kg i.p))+2ml 10% O.G	72.83 ± 0.75[b]	181.93 ± 1.70[b]	191.70 ± 1.47[c]	127.33 ± 0.90[d]	93.97 ± 0.64[d]	83.87 ± 0.75[d]
Results of one-way ANOVA F- value P- value	49.11 p<0.05	3164 p<0.05	5024 p<0.05	2607 p<0.05	2997 p<0.05	2051 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 4: Effect of administration of aqueous extract of o.g on serum alt activity (u/l) on cisplatin induced hepatotoxicity in rats[1]

Serum ALT (U/L)						
Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
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]
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]
Cis (5mg/kg i.p)+2ml 5% O.G	46.33 ± 0.75[b]	135.37 ± 0.91[c]	139.73 ± 1.86[b]	108.03 ± 1.10[c]	91.57 ± 2.23[c]	54.47 ± 0.83[c]
Cis (5mg/kg i.p))+2ml 10% O.G	42.73 ± 0.57[a]	135.20 ± 1.31[c]	137.50 ± 0.60[b]	101.63 ± 0.40[d]	84.43 ± 1.1[d]	48.90 ± 0.30[d]
Results of one-way ANOVA F- value P- value	7.85 p<0.05	5608 p<0.05	2545 p<0.05	2260 p<0.05	1343 p<0.05	4173 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 5: Effect of administration of aqueous extract of o.g on serum alp activity (u/l) on cisplatin induced hepatotoxicity in rats[1]

ALP(U/L)						
Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
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]
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]
Cis (5mg/kg i.p)+2ml 5% O.G	247.75 ± 3.49[b]	386.77 ± 0.33[b]	375.68 ± 0.39[c]	335.50 ± 0.98[c]	289.02 ± 1.41[c]	245.72 ± 0.86[c]
Cis (5mg/kg i.p))+2ml 10% O.G	236.83 ± 1.95[a]	384.21 ± 0.25[c]	372.07 ± 1.24[d]	328.81 ± 1.93[d]	286.94 ± 1.13[c]	241.34 ± 1.19[d]
Results of one-way ANOVA F- value P- value	11.8 p<0.05	16190 p<0.05	2378 p<0.05	3418 p<0.05	7004 p<0.05	4310 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 6: Effect of administration of aqueous extract of o.g on serum total protein (g//l) on cisplatin induced hepatotoxicity in rats[1]

Serum Total protein (g/l)						
Groups/Treatment	0days[2]	3days	6days	9days	12days	15days
Control (normal saline)	97.47 ± 0.61[a]	94.17 ± 0.45[a]	93.97 ± 0.78[a]	89.40 ± 0.62[a]	95.03 ± 0.70[a]	94.37 ± 0.64[a]
Cis (5mg/kg i.p) +2ml water	93.70 ± 0.61[b]	49.27 ± 0.49[b]	49.10 ± 0.20[b]	41.00 ± 0.67[b]	44.57 ± 1.46[b]	50.43 ± 1.53[b]
Cis (5mg/kg i.p)+2ml 5% O.G	96.17 ± 0.80[c]	46.63 ± 1.40[c]	57.13 ± 0.86[c]	69.63 ± 0.64[c]	73.23 ± 1.42[c]	77.77 ± 0.81[c]
Cis (5mg/kg i.p))+2ml 10% O.G	96.03 ± 0.40[c]	47.67 ± 0.51[c]	61.73 ± 1.58[d]	73.83 ± 0.70[d]	77.90 ± 0.70[d]	79.37 ± 0.95[c]
Results of one-way ANOVA F- value P- value	18.28 p< 0.05	1668 p < 0.05	407.51 p < 0.05	1703 p < 0.05	862.58 p < 0.05	592.79 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 = 1282, p<0.05. Treatment effect, p<0.05, F = 3251; time effect, p < 0.05, F = 4991

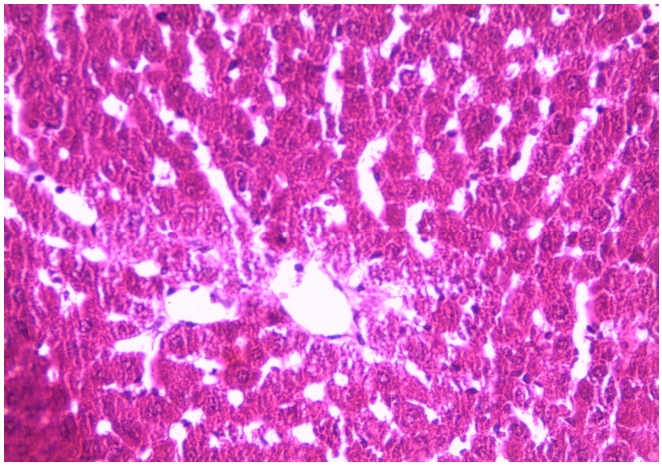

Plate 1 Liver (normal) with cords of hepatocytes well preserved cytoplasm not vacuolated.Sinusoids well demarcated no area of necrosis, no fatty change, no fatty degeneration

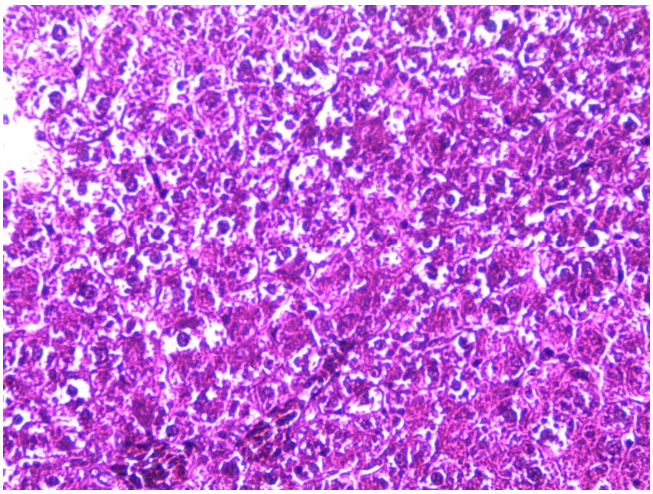

Plate 2 Liver (treated with 5mg/kg cisplatin) showing enlarged hepatocytes with vacuolation of cytoplasm.There is compression of the sinusoid

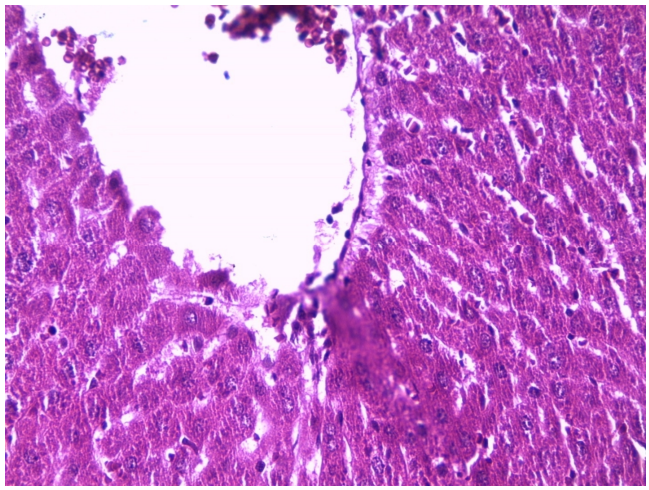

Plate 3 Liver (treated with cisplatin + 5% O.G) is essentially normal

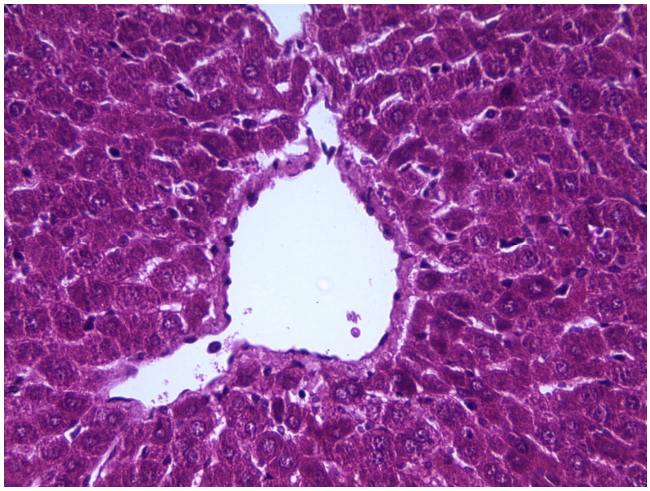

Plate 4 Liver (treated with cisplatin + 10% O.G) with normal architecture. Cords of hepatocytes well preserved cytoplasm not vacuolated.Sinusoids well demarcated no area of necrosis, no fatty change, no fatty degeneration

DISCUSSION

The consumption of plant products as complementary/ alternative medicine has been encouraged because they are relatively cheap. This is coupled with the belief that they could significantly contribute to the improvement of human health in terms of cure and the prevention of various human disorders. In addition there are less frequent side effects reported when compared to modern medicine [36].

Liver plays a very important role in the metabolism of foreign compounds entering the body. The exposure to the foreign compounds may be through consumption of alien/ contaminated foods, from exposure to chemical substances in the occupational environment or through synthetic drugs consumed for various pathological conditions. These compounds have many toxic manifestations on the human liver [37]. In humans, hepatitis or liver injury is also caused by viruses, chemicals, alcohol \and autoimmune diseases. Liver diseases remain one of the serious health problems and medicinal plants and herbs have been in use for treating these. The present modern age demands proof on a scientific basis to justify the various medicinal uses of herbs [38]. Although the biologically active compounds in most of the herbal drugs are unknown, they are being used / prescribed widely because of their effectiveness (ascertained through traditional knowledge) at a relatively low cost and fewer side effects. However, safe drugs for serious liver disorders continue to be an area of interest [39].

Cisplatin, a heavy metal complex, is an effective chemotherapeutic agent for a wide variety of tumors [40]. Nevertheless, it has several toxicities and sides effects including hepatotoxicity [41, 42] and nephrotoxicity [40]. The cytotoxicity caused by cisplatin is considered to be due to several factors viz., the peroxidation of cell membranes, mitochondrial dysfunction, inhibition of protein synthesis, and DNA damage [43, 44, 45]. *Many* antioxidative agents have been analyzed in experimental and clinical studies looking for an agent to reduce or prevent cisplatin-induced hepatotoxicity [46, 47, 48].

Much attention has been given to the possible role of dietary antioxidants in protecting liver against cisplatin-induced toxicity [49]. Phytochemicals, including flavonoids, are naturally occurring antioxidants that possess various pharmacological actions and therapeutic applications [50, 51]. So, due to their phenolic structures they inhibit free radical-mediated processes [52]. Many antioxidant compounds have been studied as chemoprotective agents such as, curcumin, selenium and other dietary components that scavenge free radicals formed by exposure to cisplatin [53, 54].

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 *Ocimum gratissimum* (O.G.) might affect the course of hepatic repair after the onset of cisplatin-induced hepatotoxicity, 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 [55, 56, 57] 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 O. *gratissimum* for the present study.

Hepatotoxicity in this study was gauged by weight loss, liver weight/ body weight ratio and ALT, AST, ALP activities and serum protein. Recent studies have been focused on the ways for protection of cisplatin hepatotoxicity [41, 58, 59, 60, 61, 62]. However, little has been reported regarding the use of *Ocimum gratissimum* against hepatotoxicity [30, 63, 64].

In the present investigation a single dose of cisplatin (5 mg kg^{-1}), in male albino rats resulted in significant body weight reduction and decreased food intake. In accordance with present results, Chirino *et al* [65] suggested that after 3 days i.p. administration of a single dose of cisplatin to male Wistar rats (7.5 mg kg^{-1}) significantly depressed their body weight. Confirming our point of view, Shimeda *et al* [66] and Norrgren *et al*. [67], stated that cisplatin has been shown to decrease total body weight in male Sprague Dawley rats and Wistar rats, respectively. Mora *et al*. [13] suggested that cisplatin induced weights loss might be due to gastrointestinal toxicity and reduced ingestion of food.

Post-treatment of aqueous extract of *Ocimum gratissimum* (O.G.) three days after cisplatin injection remarkably ameliorated the reduction in body weight induced by cisplatin; the increase was most pronounced on the 15th day.

Cisplatin induced liver damage was characterized by a significant decrease ($P \leq 0.05$) in the liver weight to body weight ratio. This change was more pronounced on the 15th day of the experiment. (Table 1) This result was consistent with Lee *et al*. [14] , who indicated that cisplatin treatment for 15 days in BALB/C mice resulted in liver weight loss manifested by significant depression as a percentage of the total body weight.

Post-treatment of aqueous extract of O.*gratissimum* returned the liver weight to body weight ratio close to normal after 9 days (Table2). However, after 15 days post-treatments of aqueous extract of O.*gratissimum* treatment resulted in higher significant elevation of these values compared to cisplatin treated groups (Table 2). Results reported by Favari and Pérez-Alvarez [68] were in agreement with our findings; where rats, receiving chronic administration of CCl_4 followed by oral intake of silymarin, 50 mg kg^{-1} for 5 days, exhibited elevation in the liver weight to body weight ratio that was more than twice that of the CCl_4 treated group.

During the present work biochemical evidence of hepatic injury has been demonstrated by elevated levels of AST, ALT, ALP and decrease in serum protein. Also, intraperitoneally administered Cisplatin (5 mg kg^{-1}) in male wistar albino rats led to notably elevated levels of these biochemical markers ($P \leq 0.05$). In contrast, oral administration of aqueous extract of O.*gratissimum* (5% and 10%) after cisplatin administration caused a decline in hepatotoxicity on the 15th day for rats treated with cisplatin. This was evidenced by marked decreases in serum AST, ALT, ALP activities and increase in serum protein level of those treated with O. *gratissimum* relative to the group treated with cisplatin alone (Table 3-6).

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 [69].

The decrease in the activities of the three most prominent marker enzyme ALT, AST and ALP with the administration of *Ocimum gratissimum* is in agreement with work by Effraim *et al*. [29] who noted that aqueous leaf extract of O.G reduced/suppressed the activities of the liver enzymes in rabbits when given orally twice a week for four weeks. Arhoghro *et al*. [30] also reported that aqueous extracts of O *gratissimum* after establishment of CCl_4-induced liver damage significantly reduced and reversed the liver damage in rats.

The reduction of the ALT, AST and ALP activities by both extract ($P \leq 0.05$) was dose dependent. The effect of time of administration of aqueous extract of O. *gratissimum* on ALT, AST and ALP activities was statistically significant ($P \leq 0.05$) (Tables 3- 6).

This marked decrease in the activities of the three marker enzymes ALT, AST and ALP with administration of aqueous leaf extracts of O.*gratissimum* 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 [70]; Silymarin [71]; Zerumbone [62]. During the present work, it could be elucidated that the decrease in protein level is likely due to the impairement of protein synthetic activity during stress conditions [72]. 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. 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 [73] and Kiceniuk *et al*. [74] who observed that xenobiotics caused an elevation of liver enzyme activity resulting to severe liver damage. O.G + 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 on other plants in cisplatin hepatotoxicity such as Silymarin [71]; Zerumbone [62].

Phytochemical screening of the leaf extract of O. gratissimum (O.G) had shown the plant to contain alkaloids,saponins,tannins,alkaloids,anthraquinone,flavonoids,steroids,terpenoids and cardiac glycosides [23, 75, 76]. Flavonoids are reported to exhibit antioxidant activity [77] and are effective scavengers of superoxide anions [78]. The aqueous extract of *O.gratissimum* 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 [79] and *O. gratissimum* has been shown to be a rich source of this compound [31, 80].

Effriam *et al.* [29] reported the presence of flavonoids and saponins in the leaf of *O. gratissimum,* while Effriam *et al.* [22] showed from histopathological studies that *O.gratissimum* can be used as a hepatoprotective agent. O.*gratissimum* is known to have hepatoprotective effect against numerous liver diseases [63, 64]. Arhoghro *et al.* [30] reported that aqueous extract of *Ocimum gratissimum* has antihepatotoxic activity against CCl_4 induced hepatotoxicity in wistar albino rats. However, no study has focused on the hepatoprotective effect of *Ocimum gratissimum* on cisplatin-induced toxicity

In conclusion, results from our studies have shown that treatment with O. *gratissimum* extracts after establishment of cisplatin induced hepatotoxicity significantly reduced the toxicity in rats.

REFERENCES

1. Wang G, Reed E and Li QQ. Molecular basis of cellular response to cisplatin chemotherapy in non-small cell lung cancer (Review). *Oncol. Rep*., 2004, 12: 955-965.
2. Aly MS, Ashour MB, El-Nahas SM and Abo-Zeid MAF. Genotoxicity and cytotoxicity of the anticancer drugs gemcitabine and cisplatin, separately and in combination: *In vivo* studies. *J. Biol. Sci*., 2003, 3: 961-972.
3. Ajani JA. Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal: Evolution of the docetaxel, cisplatin and 5-fluorouracil regimen. *Cancer*. 2008, 113: 945-955.
4. Dank M, Zaluski J, Barone C, Valvere V, Yalcin C, Peschel C, Wenczl M, Gokers E, Cisar L, Wang K, Bugat R. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5- fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach oresophagogastric junction. *Ann. Oncol*., 2008, 19: 1450-1457.
5. Stewart DJ, Benjamin RS, Luna M, Feun L, Caprioli R, Seifert W and Loo TL. Human tissue distribution of platinum after cis-diamminedichloroplatinum. *Cancer Chemother.Pharmacol*., 1982, 10:51-54.
6. Ravi R, Somani SM and Rybak LP. Mechanism of cisplatin ototoxicity: Antioxidant system. *Pharmacol. Toxicol*., 1995, 76: 386-394.
7. Zicca Z, Cafaggi S, Mariggiò MA, Vannozzi MO, Ottone M, Bocchini V, Caviglioli G and Viale M. Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats. *Eur. J. Pharmacol*., 2004, 442: 265-272.
8. Kim SH, Hong KO, Hwang JK and Park KK. Xanthorrhizol has a potential to attenuate the high dose Cisplatin 10 mg kg^{-1} platin-induced nephrotoxicity in mice. *Food Chem.Toxicol*., 2005, 43:117-122.
9. Pal S, Sadhu AS, Patra S and Mukherjea KK. Histological vs biochemical assessment on the toxic level and antineoplastic efficacy of a synthetic drug Pt-ATP on experimental animal models. *J. Exp. Clin. Cancer Res*., 2008, 27: 68-68.
10. Venkatesan PN, Rajendran P, Ekambaram G and Sakthisekaran D. Combination therapeutic effect of cisplatin along with Solanum trilobatum on benzo(a)pyrene induced experimental lung carcinogenesis. *Nat. Prod. Res*., 2008, 22: 1094-1106.
11. Yuce A, Atessahin A, Ceribasi A and Aksakal M. Ellagic acid prevents cisplatin 10 mg kg^{-1} platin-induced oxidative stress in liver and heart tissue of rats. *Basic Clin. Pharmacol. Toxicol*., 2007, 101: 345-349.
12. Saad SY, Najjar TA and Alashari M. Role of non-selective adenosine receptor blockade and phosphodiesterase inhibition in cisplatin-induced nephrogonadal toxicity in rats. *Clin. Exp. Pharmacol. Physiol*., 2004, 31: 862-867.
13. Mora LO, Antunes LM, Francescato HD and Bianchi M. The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. *Pharmacol. Res*., 2003, 47: 517-522.
14. Lee CK, Park KK, Lim SS, Park JH and Chung WY. Effects of the licorice extract against tumor growth and cisplatin-induced toxicity in a mouse xenograft model of colon cancer. *Biol. Pharm. Bull*., 2007, 30: 2191-2195.
15. Wargovich MJ, Woods C, Holis DM and Zander NE. Herbals, cancer prevention and health. *J. Nutr*., 2001, 131(11): 30345- 30365.
16. Roja G and Rao PS. Anticancer compounds from tissue cultures of medicinal plant, J. *Herbs, Spices. Med. Plants,* 2000, 7: 71-102.
17. Okafor JC. Edible indigenous woody plants in the rural economy of the Nigeria forest zone. *For. Ecol. Manage*, 1980, 3: 45-55.
18. Gbile ZO and Adesina SK. Nigerian flora and its pharmaceutical potentials. *J. Ethnopharmacol*., 1986, 19: 1-16.
19. Iwu MM. Handbook of Medicinal plants CRC Press. Boca Raton, 1993, p. 214-215.
20. Swabirk JT. Weed of the pacific islands.Technical paper No. 209. South pacific commission Nounea, New Caledonia 1244, 1997.
21. Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA and Criddle DN. Antinociceptive propertiesof the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. *Braz. J. Med. Biol. Res*., 2003, 36: 521-524.
22. Effraim KD, Jacks TW and Sodipo OA. Histopathological studies on the toxicity of *Ocimum gratissimum* leave ex tract on some organs of rabbit. *Afr J Biomed Res.,* 2003, 6: 21-25.
23. Akinmoladun AC, Ibukun EO, Emmanuel A, Obuotor EM and Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci Res Essay*, 2007, 2: 163-6.
24. Ijeh II, Omodamiro OD and Nwanna IJ. Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopia aethiopica*. *Afr. J. Biotech.,* 2005, 4: 953-956.
25. Adebola TT and Salau AO. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern *Nigeria. Afr. J. Biotech*., 2005, 4: 682-684.
26. Sofowara EA. Medicinal plants and traditional medicine in Africa. John Wiley, Chicester, 1982, p. 256.
27. Oboh G. Antioxidative potential of Ocimum gratissimum and *Ocimum canum* leaf polyphenols and protective effects on some pro-oxidants induced lipid peroxidation in rat brain: An in vitro Study. *Am. J. Food Technol*., 2008, 3: 325-334.
28. Ofokansi KC, Adikwu MU, Esimone CO and Nwodo NM. Antibacterial activity of the leaf extract of *Ocimum gratissimum* (fam labiatae) *J. of Bio. Res. and Biotech*., 2003, 1 (1): 35-42.
29. Effraim KD, Salamu HA and Osewa TS. The effect of aqueous leaf extract of *Ocimum gratissimum* on haematological and biochemical parameters in rabbits. *Afr. J. Biomed. Res*., 2000, 6: 175-179.
30. Arhoghro EM, Ekpo KE and Ibeh GO. Effects of Aqueous extracts of scent leaf (*Ocimum gratissimum linn*) on CCL_4 induced liver damage in wistar albino rat. *Afr. J. of Pharmacy and Pharmacology*, 2009, 3(11): 562-567.
31. Obianime AW, Aprioku JS and Esomonu C. The Effects of Aqueous *Ocimum gratissimum* Leaf Extract on Some Biochemical and Hematological Parameters in Male Mice. *Asian Journal of Biological Sciences*, 2011, 4: 44-52.
32. Aguiyi JC, Obi CI, Gang SS and Igweh AC. Hypoglycaemic activity Of *Ocimum gratissimum* in rats. *Fitoterapia*, 2000, 71(4): 444-446.
33. Reitman S and Frankel AS. A colometric method of determination of serum glutamic pyruvic transaminase. *Amer*. J. *Clin*. *Patho*., 1957, 28:56.
34. Rec GSCC. Optimized standard colorimetric methods. *J. Clin. Chem. Clin. Biochem.,* 1972, 10: 182.
35. Tietz NW. Textbook of clinical Chemistry, W.B. Saunders Company, Philadelphia,PA, 1983, p. 335-337.
36. Hu X, Sato J, Oshida Y, Yu M, Bajotto G and Sato Y. Effect of Goshajinki-gan (Chinese herbal medicine): Niu-che-sen-qi-wan) on insulin resistance in streptozotocin induced diabetic rats. *Diab. Res. Clin. Pract*., 2003, 59:103-111.
37. Rajesh MG and Latha MS. Protective activity of Glycyrrhiza glabra in carbon tetrachloride-induced peroxidative damage *India J. Pharmacol*., 2004, 36:284-287.
38. Pohocha N and Grampurohit ND. Antispasmodic activity of the fruits of *Helicteres isora* Linn. *Phytother. Res*., 2001, 1:49-52.
39. Mohan GK, Pallavi E, Ravi KB, Ramesh M and Venkatesh S. Activity of Carica leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *DARU;* 2007, *15:162*.
40. Park HR, Ju EJ, Jo SK, Jung U, Kim SH and Yee ST. Enhanced antitumor efficacy of cisplatin in combination with HemoHIM in tumor-bearing mice. *BMC Cancer*, 2009, 9: 85-85.
41. Mansour HH, Hafez HF and Fahmy NM. Silymarin modulates Cisplatin 10 mg kg^{-1} platin induced oxidative stress and hepatotoxicity in rats. *J. Biochem. Mol. Biol*., 2006, 39: 656-661.
42. Pratibha R, Sameer R, Rataboli PVD, Bhiwgade A and Dhume CY. Enzymatic studies of Cisplatin 10 mg kg^{-1} platin induced oxidative stress in hepatic tissue of rats. *Eur. J. Pharmacol*., 2006, 532: 290-293.

43. Brady HR, Kone BC, Stromski ME, Zeidel ML, Giebisch G and Gullans SR. Mitochondrial injury: an early event in cisplatin toxicity to renal proximal tubules. *Am. J. Physiol.,* 1999, 258: F1181–F1187.
44. Kharbanda S, Ren R, Pandey P, Shafman TD, Feller SM, Weichselbaum RR and Kufe DW. Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. *Nature,* 1995, 376:785–788.
45. Leibbrandt ME, Wolfgang GH, Metz AL, Ozobia AA and Haskins JR. Critical subcellular targets of cisplatin and related platinum analogs in renal proximal tubule cells. *Kidney Int.*, 1995, 48:761–770.
46. Antunes LMG, Darin JD and Bianchi MD. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose- dependent study. *Pharmacol. Res.,* 2000, 41:405-411.
47. Davis CA, Nick HS and Agarwal A. Manganese superoxide dismutase attenuates Cisplatin-induced renal injury: importance of superoxide. *J Am Soc Nephrol*., 2001, 12:2683–90.
48. Dillioglugil MO, Maral KH, Gulkac MD, Ozon KA, Ozdogan HK, Acar O and DillioglugilO. Protective effects of increasing vitamin E and A doses on cisplatin-induced oxidative damage to kidney tissue in rats. *Urol. Int.*, 2005, 75:340-344.
49. Behling EB, Sendao MC, Francescato HD, Antunes LM, Costa RS and Bianchi-Mde L. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. *Pharmacol. Rep*., 2006, 58: 526-532.
50. Hasanloo T, Khavari-Nejab RA, Majidi E and Ardekani MRS. Analysis of flavonolignans in dried fruits of *Silybum marianum* (L.) gaertn from Iran. *Pak. J. Biol. Sci*., 2005, 8: 1778-1782.
51. Karimi G, Ramezani M and Tahoonian Z. Cisplatin nephrooxicity and protection by milk thistle extract in rats. *Evid. Based Complement Alternat. Med*., 2005, 2: 383-386.
52. Singh D, Chander V and Chopra K. Protective effect of catechin on ischemiareperfusion-induced renal injury in rats. *Pharmacol. Rep*., 2005, 57: 70-76.
53. Antunes LM, Darin JDC and Bianchi NL. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. *Pharmacol. Res*., 2001, 43:145-150.
54. Silva CR, Antunes LM and Bianchi ML. Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. *Pharmacol. Res*., 2001, 43: 561-566.
55. Stein J, Lifschitz M and Barnes L. Current concepts on the nephrotoxicity of acute renal failure. *Am. J. Physiol*., 1978, 243:F171-F181.
56. Singh J. A possible mechanism of cisplatin-induced nephrotoxicity. *Toxicol.,* 1989, 58:71-80.
57. Okoko T and Oruambo IF. The effect of *Hibiscus sabdariffa* calyx extract on cisplatin-induced tissue damage in rats. *Biokemistri,* 2008, 20(2): 47-52.
58. Iraz M, Kalcioglu MT, Kizilay A and Karatas E. Amino guanidine prevents ototoxicity induced by Cisplatin 10 mg kg^{-1} platin in rats. *Ann. Clin. Lab. Sci*., 2005, 35: 329-335.
59. Koc A, Duru M, Ciralik H, Akcan R and Sogut S. Protective agent, erdosteine, against Cisplatin 10 mg kg^{-1} platin-induced hepatic oxidant injury in rats. *Mol. Cell.Biochem*., 2005, 278: 79-84.
60. Iraz M, Ozerol E, Gulec M, Tasdemir S, Idiz N and Fadillioglu E. Protective effect of caffeic acid phenethyl ester (CAPE) administration on cisplatin-induced oxidative damage to kidney in rat. *Cell Biochem. Funct.*, 2006, 24: 357-361.
61. Iseri S, Ercan F, Gedik N, Yuksel M and Alican I. Simvastatin attenuates Cisplatin 10 mg kg^{-1} platin-induced kidney and liver damage in rats. *Toxicology*, 2007, 230:256-264.
62. Ibrahim YM, Abdul BA, Ibrahim TT, AbdelWahab SI, Elhassan MM and Mohan S. Attenuation of cisplatin-induced hepatotoxicity in rats using zerumbone.*Res.J Bio.Sci,* 2009, 4 (7):777-784.
63. Chaturvedi R, George S and John A. Preventive and protective effects of wild basil in ethanol-induced liver toxicity in rats. *Br. J. Biomed.Sci*., 2007, 64: 10-12.
64. George S and Chaturvedi PS. Protective role of *Ocimum canum* plant extract in alcohol-induced oxidative stress in albino rats. *Br. J. Biomed. Sci*., 2008, 65: 80-85.
65. Chirino YI, Hernandez-Pando J and Pedraza-Chaveri J. Peroxynitrite decomposition catalyst ameliorates renal damage and protein nitration in cisplatin-induced nephrotoxicity in rats. 1. BMC. *Pharmacol*., 2004, 4: 20-29.
66. Shimeda Y, Hirotani Y, Akimoto Y, Shindou K, Ijiri Y, Nishihori T and Tanaka K. Protective effects of capsaicin against cisplatin-induced nephrotoxicity in rats. *Biol. Pharm. Bull*., 2005, 28: 1635-1638.
67. Norrgren K, Sjölin M, Björkman S, Areberg J, Johnsson A, Johansson L and Mattsson S. Comparative renal, hepatic and bone marrow toxicity of cisplatin and radioactive cisplatin (^{191}Pt) in wistar rats. *Cancer Biother. Radiopharm*., 2006, 21: 528-534.
68. Favari L and Pérez-Alvarez V. Comparative effects of colchicine and silymarin on CCl_4-chronic liver damage in rats. *Arch. Med. Res*., 1997, 28: 11-17.
69. Sallie R, Tredger JM and Willam R. Drugs and the Liver. *Biopharmaceutical Drug Dispos*., 1991, 12: 251-259.
70. Ashavin A, Cetin R, Erguder IB, Candir O, Ozturk HS and Durak L. Cisplatin causes oxidation in rat liver tissues: Possible Protective Effects of Antioxidant food Supplementation. *Turk J. Med. Sci.,* 2008, 38 (2):117-120.
71. Abdelmeguid NE, Chmaisse HN and Abou-Zeinab NS. Silymarin Ameliorates Cisplatin- Induced Hepatotoxicity in Rats: Histopathological and Ultrastructural Studies. *Pak. J. B. Sci.,* 2010, 13(10):463-479.
72. Rao SV, Padmovathi B, Reddy KS, Indira K.Protein metabolic response to carbon tetrachloride in a fresh fish, *Sarotherrodon mossambicus*. *Chem. Ecol*., 1995, 11:207-212.
73. Krisha AG and Veena G. 2,3,4- Triaminoazo benzene- induced hemato biochemical Anomalies in fish (Channa puntatus). *Bull Environm.Contam. Toxicol* ., 1980, 25:136 – 141.
74. Kiceniuk JW, Khan RA, Dawe M, Williams U. Examination and interaction of trypanosome infection and crude oil exposure on haematology of the Longhorn Sculphin (Myoxocephalus octodeces – pinosus). *Bull Environm, Contam- Toxicol.,* 1982, 28: 435-438.
75. Holets FB, Ueda-Nakamura T, Filho BPD, Cortez DAG, Morgado-Diaz JA and Nakamura CV. Effect of essential oil of *Ocimum gratissimum* on trypanosomatid *Herpetomonas samuelpessoai*. Acta Protonzoologica, 2003, 42:269-276
76. Akinyemi KO, Mendie UE, Smith ST, Oyefolu AO and Coker AO. Screening of some medical plants for anti-salmonella activity. *J. Herb Pharmocother.*, 2005, 5: 45-60.
77. Ramanathan R, Luu KK and Das NP. Anti –peroxidative action of flavonoids and related products in ground pork. Proceedings of III International Symposium on flavonoids in Biology and Medicine. Singapore, 1989, p. 56.
78. Robak K and Grygleuski RJ. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol*., 1988, 37: 837-841.
79. Singh N, Verma P, Misharu N and Nath R. A comparative evaluation of some antistress agents of plant origin. *Indian J. Pharmacol*., 1991, 21: 99.
80. Prabhu KS, Lobo R, Shirwaikar AA, Shirwaikar A. *Ocimum gratissimum*: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. Open Complement. *Med. J.,* 2009, 1: 1-15.

Source of support: Nil, Conflict of interest: None Declared