Ameliorative Effects of Chloroform Fraction of Cocos nucifera L. Husk Fiber Against Cisplatin-induced Toxicity in Rats

Oluwatosin Adekunle Adaramoye, Adesola Fausat Azeез, Olufunke Elizabeth Ola-Davies

Department of Biochemistry, Faculty of Basic Medical Sciences, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT
Background: Cisplatin (Cis) is used in the treatment of solid tumors and is known to elicit serious side effects. Objective: The present study investigated the protective effects of chloroform fraction of Cocos nucifera husk fiber (CFCN) against Cis-induced organs’ damage and chromosomal defect in rats. Quercetin (QUE), standard antioxidant, served as positive control. Materials and Methods: Thirty male Wistar rats were assigned into six groups and treated with corn oil (control), Cis alone, Cis + CFCN, CFCN alone, Cis + QUE, and QUE alone. QUE and CFCN were given at 50 and 200 mg/kg/day, respectively, by oral gavage for 7 days before the rats were exposed to a single dose of Cis (10 mg/kg, intraperitoneal) at the last 36 h of study. Results: Administration of Cis alone caused a significant (P < 0.05) increase in the levels of serum creatinine and urea by 72% and 70%, respectively, when compared with the control. The activity of serum aspartate aminotransferase was significantly (P < 0.05) increased while alanine aminotransferase and alkaline phosphatase were insignificantly (P > 0.05) affected in Cis-treated rats. Furthermore, the activities of hepatic and renal catalase, superoxide dismutase, glutathione S-transferase, glutathione peroxidase, and levels of reduced glutathione were significantly (P < 0.05) decreased in Cis-treated rats with concomitant elevation of malondialdehyde. Cis exposure increased the frequency of micronucleated polychromatic erythrocytes (mPCE) by 92%. Pretreatment with CFCN inhibited lipid peroxidation, enhanced the activities of some antioxidative enzymes and reduced the frequency of mPCE. Conclusions: Chloroform fraction of CFCN may protect against organs damage by Cis. Further studies are required to determine the component of the plant responsible for this activity. Key words: Antioxidant, Cisplatin, Clastogenicity, Cocos nucifera, Lipid peroxidation

INTRODUCTION
Cancer is a disease that is characterized with heritable disorders at cellular control levels. For the past 20 years, biochemical research has directed most of its resources in finding the exact causes and cure of cancer. Chemotherapy and radiotherapy have emerged as the most common modalities of cancer treatment. Cisplatin (Cis) is one of the most important chemotherapeutic drugs used in the treatment of a wide range of solid tumors. It was found to have anti-tumor effects in animal models, but its use is often associated with a number of side effects such as nephrotoxicity, peripheral neuropathy, ototoxicity, gastrointestinal disorder, and myelosuppression. These side effects have led to research on the development of specific agents to alleviate Cis toxicity. Oxidative stress is the hallmark of Cis-induced toxicity in both human and animal studies. Therefore, intervention studies with antioxidants showed promising results with a significant reduction in the degree of toxicity of Cis. Recent studies have shown that commonly consumed medicinal plants, which are good sources of antioxidants and are mostly polyphenols such as tannins, saponins, flavonoids, etc., can boost the endogenous antioxidant defense system and alleviate Cis toxicity. Hence, the quest for safe and effective agents to minimize Cis toxicity in animal model is still an active area of research.

Cocos nucifera (coconut) belongs to the family of the Areceaceae (Palmae). It is cultivated for its multiple utilities mainly for its nutritional and medicinal values. Its products such as coconut kernel and coconut water have numerous medicinal properties such as...
antibacterial, antifungal, antiviral, anti-dermatophytic, antioxidant, hypoglycemic, hepatoprotective, immune-stimulant, and anti-protozoal activities. Our attention was drawn to this plant when its methanol extract showed strong antioxidant, anti-lipoperoxidative, and free radicals scavenging effects in vitro in our laboratory. Quercetin (QUE) (3,5,7,3',4'-pentahydroxyflavone) is a common flavonoid found in various foodstuffs such as beverages, edible fruits, vegetables, and red wine. It has an antioxidant property which has been linked to its ability to chelate transition metal ions and scavenge free radicals. Other relevant functions ascribed to this flavonoid includes antihypertensive, antiarrhythmic, anti-inflammatory, antihypertotoxic, fertility-enhancing, and anti-tumor activities. This study exploits the protective and antioxidant potential of chloroform fraction of methanol extract of Cocos nucifera husk fiber on Cis-induced organs toxicity and chromosomal damage in male Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents
Trichloroacetic acid, 2-thiobarbituric acid, ethylenediaminetetraacetic acid, hydrochloric acid, reduced glutathione (GSH), sodium nitrite, hydrogen peroxide, 1-chloro-2, 4-dinitrobenzene, epinephrine (adrenaline), sulfosalicyclic acid, and 5,5'-dithiobis-2-nitrobenzoic acid (Ellman reagent) were purchased from Sigma Chemical Co., Saint Louis, MO, USA. Potassium chloride and sodium hydroxide were procured from British Drug House Chemical Ltd., Poole, UK. Other chemicals were of analytical grade and the purest quality available.

Collection of plant materials
Cocos nucifera husk fiber was obtained from Ojoo market in Ibadan, Nigeria. It was identified at herbarium in the Department of Botany, the University of Ibadan where voucher specimens already exist (UI 00306). It was washed and air dried in the laboratory for 4 weeks. The dried materials were ground into a coarse powder at Department of Agronomy, the University of Ibadan.

Preparation of extract
About 400 g of powdered plant was soaked in 4 L of methanol for 96 h with occasional stirring for cold extraction. This was filtered through muslin cloth and evaporated to dryness at 40°C with a rotary evaporator. The yield was 5.8%. The methanol extract was further partitioned with a separating funnel using chloroform and ethanol for 96 h and then evaporated to dryness with a rotary evaporator. The chloroform fraction was kept in the refrigerator until use.

Experimental animals
Male Wistar rats weighing between 110 and 130 g were obtained from Animal House of the Faculty of Basic Medical Sciences, the University of Ibadan, Nigeria. They were housed in plastic cages and fed on rats’ pellets and given drinking water ad libitum. The rats were allowed to acclimatize for 7 days before the commencement of the experiment and kept at 12 h light/dark cycle and temperature of 29 ± 2°C. The Faculty of Basic Medical Sciences, the University of Ibadan Animal Ethics Committee approved this study.

Study design
Thirty male rats were randomly divided into six groups of five rats each. The first group (control) received drug vehicle (corn oil), second group (Cis) received Cis alone (10 mg/kg intraperitoneal), third group (Cis + CFCN) received Cis and chloroform fraction of Cocos nucifera (200 mg/kg, peroral), fourth group (CFCN) received chloroform fraction of Cocos nucifera alone (200 mg/kg, peroral), fifth group (Cis + QUE) received Cis and QUE (50 mg/kg), while the sixth group (QUE) received QUE (50 mg/kg) alone. The CFCN and QUE were dissolved in corn oil and administered daily by oral gavage for 7 consecutive days before the rats were exposed to a single dose of Cis in the last 36 h of study.

Preparation of tissues
Rats were sacrificed 24 h after the last dose of extracts. Liver, kidney, and testes were quickly removed and washed in ice-cold 1.15% KCl solution to remove blood stains, dried, and weighed. These tissues were homogenized separately in 4 volumes (mL) of 50 mM phosphate buffer, pH 7.4, and centrifuged at 10,000 g for 15 min to obtain post mitochondrial supernatant fraction (PMF). Procedures were carried out at a temperature of 4°C.

Preparation of serum
Blood was collected from the animals by ocular puncture into plain centrifuge tubes and was allowed to stand for 1 h. Serum was prepared by centrifugation at 3000 g for 15 min in a Beckman bench centrifuge. The clear supernatant was used for the estimation of serum enzymes and other biochemical indices.

Biochemical analysis
The protein concentration of the various samples was determined by the method of Lowry et al., using bovine serum albumin as standard. Serum urea and creatinine levels were determined by the methods of Talke and Schubert, and Jaffe, respectively. The activities of alanine and aspartate aminotransferases (ALT and AST) were assayed by the combined methods of Mohun and Cook and Reitman and Frankel. Alkaline phosphatase (ALP) activity was determined according to the method described by King and Armstrong. PMF superoxide dismutase (SOD) activity was measured by the nitro blue tetrazolium reduction method of McCord and Fridovich. PMF catalase (CAT) activity was assayed spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi. Reduced GSH was determined by the method of Beutler et al. Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck et al. PMF lipid peroxidation (LPO) was determined by the method of Buege and Aust, while glutathione-S-transferase (GST) activity was determined by the method of Habig et al. Micronucleus assay was determined by the method of Haddle and Salamone.

Determination of sperm characteristics
The caudal epididymis was minced in prewarmed normal saline (37°C). One drop of sperm suspension was placed on a glass slide to analyze 200 motile sperm in 4 different fields. The motility of the epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the epididymis and data were expressed as percentage motility. Epididymal sperm was obtained by mincing the epididymis in normal saline, and filtering through a nylon mesh (80-μm pore size). The sperm were counted using a hemocytometer. The number of sperm in 5 squares (4 corners and the center) in the center grid of both sides were counted and averaged according to the method of Freund and Carol. Sperm morphology was done using two drops of Well and Awa’s stain, air-dried, and examined under the microscope. The normal sperm cells were counted and the percentage calculated.

Statistical analysis
All values were expressed as the mean ± standard deviation of five animals per group. Data were analyzed using one-way ANOVA.
followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS 10.0 (SPSS Inc., 233 South Wacker Drive, Chicago, IL). The values were considered as statistically significant at $P < 0.05$.

RESULTS

Effects of CFCN on body weight, relative weight of organs, liver, and kidney function markers in rats treated with cisplatin

Administration of Cis caused a significant ($P < 0.05$) decrease in body weight-gain of rats relative to the control. Furthermore, Cis significantly ($P < 0.05$) decreased the weight of liver and kidney of the rats. Concisely, Cis decreased the weight of liver and kidney of the rats by 35% and 39%, respectively. Pretreatment with QUE and CFCN significantly ($P < 0.05$) reversed Cis-induced decrease in body weight-gain while CFCN alone reversed the Cis-induced decrease in weight of the liver and kidney [Table 1]. In Figures 1 and 2, serum ALT and AST activities increased by 74% and 35%, respectively, in Cis-treated rats while insignificant ($P > 0.05$) increase was seen in the activity of serum ALP relative to control. On the other hand, pretreatment with CFCN and QUE (given separately to the rats) significantly ($P < 0.05$) decreased the activities of ALT and AST in Cis-treated rats when compared with the control. Furthermore, Cis exposure significantly ($P < 0.05$) increased the levels of serum urea and creatinine of the rats by 70% and 82%, respectively, relative to controls. Pretreatment with CFCN significantly ($P < 0.05$) reduced the levels of urea and creatinine when compared with Cis alone treated rats [Table 2].

Effects of CFCN on antioxidant indices and lipid peroxidation in rats treated with cisplatin

The activities of hepatic and renal antioxidative enzymes-GPx, GST, SOD, and CAT were significantly ($P < 0.05$) reduced in Cis-treated rats when compared with the controls [Figures 3, 5, 7 and Table 3]. Specifically, hepatic GPx, GST, SOD, and CAT decreased by 41%, 38%, 67%, and 44% while renal GPx, GST, SOD, and CAT decreased by 38%, 44%, 50%, and 37%, respectively. Furthermore, Cis administration caused a significant ($P < 0.05$) increase in hepatic and renal LPO levels in the animals [Figure 6]. Hepatic and renal LPO increased by 95% and 98%, respectively, in Cis-treated rats relative to controls. Likewise, the increased MDA values were accompanied by marked depletion of renal and hepatic GSH in Cis-treated rats when compared with the control [Figure 9]. However, pretreatment with CFCN or QUE significantly ($P < 0.05$) attenuated the levels of both enzymic and nonenzymic antioxidant parameters in renal and hepatic tissues of the animals. In contrast, there were no significant ($P > 0.05$) differences in the levels of testicular LPO and activities of testicular antioxidant enzymes in Cis-treated rats when compared with the control [Figures 4, 8, and 10].

Effects of CFCN on the frequency of micronucleated polychromatic erythrocytes and sperm parameters in rats treated with cisplatin

In Table 4, Cis exposure caused an insignificant ($P > 0.05$) decrease in sperm count, motility, and the live-dead ratio of the rats when compared with the controls. Likewise, pretreatment with CFCN or QUE did not affect sperm indices of Cis-treated rats relative to controls. In Table 5, Cis administration significantly ($P < 0.05$) increased the frequency of micro nucleated polychromatic erythrocytes (mPCE) by about 32 folds relative to control. However, pretreatment with CFCN or QUE led to a significant ($P < 0.05$) reduction in the frequency of mPCE.

DISCUSSION

Many chemotherapeutic drugs are limited in their effectiveness due to toxic side effects.[9] Cis, in addition to its therapeutic effects on cancer cells through its ability to bind DNA, has also been reported to induce oxidative stress,[7] which is particularly damaging to kidney and liver due to their high vulnerability to reactive oxygen species (ROS). The use of Cis in cancer therapy is limited by two factors: Acquired resistance to Cis and severe side effects in normal tissues.[37] The toxic effects by Cis can be severe and may significantly affect the quality of life, even long after the treatment has ceased. The present study shows that Cis induces oxidative stress evidenced by the elevation of malondialdehyde in the testis, renal, and hepatic tissues of rats. ROS such as superoxide anions and hydroxyl radicals are generated under normal cellular conditions and are detoxified by scavenger enzymatic and nonenzymatic antioxidants.[38]

Table 1: Effects of CFCN on body weight, organs weight, and relative weight of organs in rats treated with Cis

| Treatment | Body weight (g) | Liver weight (g) | Kidney weight (g) | Testes weight (g) | Relative weight of organs |
|-----------|----------------|-----------------|------------------|------------------|--------------------------|
|           | Initial        | Final           | Weight gain      | Liver            | Kidney                  | Testes              | Liver            | Kidney                  | Testes              |
| Control   | 123.0±2.7      | 118.0±1.5       | 21.8±1.2         | 6.3±0.2          | 0.87±0.1               | 1.64±0.4            | 4.42±0.3         | 0.6±0.03               | 1.1±0.3            |
| Cis       | 118.0±1.5      | 110.0±2.5       | −26.3±2.8        | 4.1±0.3          | 0.53±0.1               | 1.53±0.3            | 4.52±0.4         | 0.6±0.02               | 1.6±0.4            |
| Cis + CFCN| 102.0±2.5      | 115.0±1.3       | 21.0±2.0         | 5.7±0.2          | 0.79±0.2               | 1.58±0.4            | 4.62±0.5         | 0.6±0.05               | 1.3±0.5            |
| CFCN      | 116.0±2.1      | 137.0±2.8       | 22.0±2.8         | 6.5±0.4          | 0.89±0.1               | 1.70±0.1            | 4.72±0.5         | 0.6±0.05               | 1.2±0.4            |
| Cis + QUE | 116.0±2.1      | 137.0±2.8       | 20.0±2.0         | 4.5±0.3          | 0.75±0.1               | 1.56±0.3            | 3.3±0.5          | 0.6±0.07               | 1.2±0.3            |
| QUE       | 123.0±1.2      | 145.5±2.3       | 22.5±1.5         | 5.9±0.4          | 0.89±0.1               | 1.61±0.2            | 4.1±0.4          | 0.6±0.03               | 1.1±0.2            |

Values are mean±SD of 5 animals per group. *Significantly different from control ($P < 0.05$). **Significantly different from control ($P < 0.05$). CFCN = Chloroform fraction of methanol extract of Cocos nucifera husk fiber; QUE = Quercetin; Cis = Cisplatin; SD: Standard deviation.

Figure 1: Effects of CFCN on the activities of serum AST and ALT in rats treated with cisplatin. *Significantly different from control ($P < 0.05$). **Significantly different from control ($P < 0.05$). CFCN = Chloroform fraction of methanal extract of Cocos nucifera husk fiber, QUE = Quercetin, Cis = Cisplatin.
However, ROS production by Cis caused antioxidant imbalance and led to LPO.\(^\text{[39]}\) In this study, the activities of major antioxidant enzymes (SOD, GPX, and GST) and levels of GSH were significantly decreased in the liver and kidney of Cis-treated rats. These results are in agreement with findings obtained earlier by other researchers.\(^\text{[10,40]}\) GSH acts as a free radical scavenger and regenerator of alpha-tocopherol and plays a significant role in sustaining protein sulfhydryl groups.\(^\text{[41]}\) Decreased renal and hepatic GSH contents result in increased susceptibility to hepatic and renal injury through the induction of LPO.\(^\text{[42]}\) GSH is the main antioxidant, found in liver and kidney and plays a protective role in the metabolism of a large number of toxic agents. Many studies assessing the status of hepatic and renal GSH in response to Cis exposure have shown that exposure to Cis caused a marked decrease in hepatic and renal GSH contents.\(^\text{[37]}\) It is so because the liver is known to accumulate significant amounts of Cis, second only to the kidney.\(^\text{[43]}\) LPO is accepted as one of the principal causes of Cis-induced liver and renal injury mediated by the production of free radical derivatives. Both animal model and human clinical data have shown the relationship between oxidative stress and hepatic or renal LPO during Cis administration.\(^\text{[37]}\)

The free radical scavenging enzymes; SOD and CAT, are the first line of defense against oxidative injury. In this study, depletion of this enzyme (SOD) is not strange, since Cis metabolites have been shown to inactivate several proteins including antioxidant enzymes system.\(^\text{[31]}\) The inhibition of antioxidant system may cause the accumulation of \(H_2O_2\) or products of its decomposition.\(^\text{[44]}\) SOD catalyzes the conversion of superoxide anion into \(H_2O_2\) while the primary role of CAT is to scavenge \(H_2O_2\) that has been generated by free radical or by SOD. However, treatment with CFCN or QUE caused a significant decline in the levels of LPO products in Cis-treated rats. This protective effect by CFCN or QUE can be linked to its antioxidant activity, which reduced the oxidative damage by blocking the production of free radicals, and thus, inhibited LPO. Importantly, administration of CFCN or QUE restored the activities of enzymatic antioxidants in liver and kidney of Cis-treated rats.

Administration of Cis induced hepatotoxicity and nephrotoxicity evidenced by the significant elevation of serum ALT, AST, creatinine, and urea in the present study. This observation is similar to the findings of Hussain \textit{et al}.\(^\text{[45]}\) and Liu \textit{et al}.\(^\text{[46]}\) who reported the elevation of aminotransferases, urea, and creatinine in animals given Cis. Both AST and ALT are reliable makers for liver function. The elevated activities of AST and ALT, as obtained in this study, indicate an increased permeability and damage or necrosis of hepatocytes,\(^\text{[47]}\) thereby causing these enzymes to leak into the plasma. This observation is supported by the recent report of Ko \textit{et al}.\(^\text{[48]}\) in which Cis-induced hepatic damage was characterized by the elevation of these aminotransferases. The decreased activities of these enzymes in Cis-treated rats given CFCN or QUE indicate the possible hepatoprotective effects of CFCN or QUE. Therefore, our results showed that pretreatment with CFCN or QUE attenuated Cis-induced liver injury in the rats. Likewise, serum creatinine and urea levels are sensitive and reliable biochemical indices for evaluation of renal function.

### Table 2: Effects of CFCN on the levels of serum creatinine and urea of rats treated with Cis

| Treatment       | Creatinine (µmol/l) | Urea (mmol/l) |
|-----------------|---------------------|---------------|
| Control         | 2.05±0.43           | 57.74±4.50    |
| Cis             | 3.74±0.22*          | 98.02±4.81*   |
| Cis + CFCN      | 2.32±0.10**         | 60.36±3.67**  |
| CFCN            | 1.92±0.41           | 55.55±4.42**  |
| Cis + QUE       | 2.55±0.25**         | 92.22±5.04    |
| QUE             | 1.89±0.18           | 56.75±3.42**  |

Values are mean±SD of 5 animals per group. *Significantly different from control (\(P<0.05\)), **Significantly different from Cis (\(P<0.05\)). CFCN: Chloroform fraction of methanol extract of \textit{Cocos nucifera} husk fiber; QUE: Quercetin; Cis: Cisplatin; SD: Standard deviation

### Table 3: Effects of CFCN on the activities of renal, hepatic, and testicular GST in rats treated with Cis

| Treatment       | Liver GST activities (µmole/min/mg protein) | Kidney GST activities (µmole/min/mg protein) | Testis GST activities (µmole/min/mg protein) |
|-----------------|-------------------------------------------|---------------------------------------------|--------------------------------------------|
| Control         | 0.63±0.15                                 | 0.027±0.005                                 | 0.060±0.007                                |
| Cis             | 0.39±0.18*                                | 0.015±0.008*                               | 0.056±0.010*                              |
| Cis + CFCN      | 0.58±0.12**                               | 0.021±0.002**                              | 0.063±0.031*                              |
| CFCN            | 0.61±0.17                                 | 0.030±0.009                                | 0.061±0.001                               |
| Cis + QUE       | 0.64±0.18**                               | 0.023±0.001*                               | 0.057±0.007                               |
| QUE             | 0.67±0.16                                 | 0.025±0.001*                               | 0.059±0.015*                              |

Values are mean±SD of 5 animals per group. *Significantly different from control (\(P<0.05\)), **Significantly different from Cis (\(P<0.05\)). CFCN: Chloroform fraction of methanol extract of \textit{Cocos nucifera} husk fiber; QUE: Quercetin; Cis: Cisplatin; GST: Glutathione-s-transferase
The increased urea and creatinine may indicate impairment to kidney function in the animals. In the present study, it is not worthy that Cis exposure for 36 h caused significant elevation of serum urea and creatinine. This observation has also been reported in different studies. These increases could be linked to the adverse effect of Cis, which resulted in the decline of glomerular filtration rate of the renal tissue. The fact that these parameters were reversed to near normal values following pretreatment with CFCN or QUE confirmed the possible ameliorative effects of these agents against Cis-induced renal dysfunction in the rats. It is pertinent to mention that in this study, the toxic effect of Cis was very mild on the testis. Cis exposure insignificantly affected sperm characteristics and testicular antioxidant status of the rats.

Table 4: Effects of CFCN on sperm parameters in rats treated with Cis

| Treatment     | Sperm motility (%) | Sperm count (10³/g testis) | TSA        | Live-dead ratio | Volume (mL) |
|---------------|--------------------|----------------------------|------------|-----------------|-------------|
| Control       | 69.0±2.00          | 87.0±7.44                  | 11.14±1.12 | 96.7±5.8        | 5.2±0.06    |
| Cis           | 66.0±1.30          | 80.8±6.22                  | 12.50±1.52 | 90.3±5.0        | 5.1±0.04    |
| Cis + CFCN    | 68.0±2.05          | 79.0±9.90                  | 12.66±0.66 | 92.5±7.6        | 5.1±0.07    |
| CFCN          | 72.0±6.91          | 88.3±9.50                  | 11.90±1.26 | 92.0±8.1        | 5.8±0.05    |
| Cis + QUE     | 70.0±0.00          | 82.0±0.00                  | 12.35±0.00 | 91.0±3.0        | 5.2±0.03    |
| QUE           | 68.0±3.10          | 84.6±7.65                  | 11.80±0.73 | 95.0±5.0        | 5.1±0.06    |

Values are mean±SD of 5 animals per group. CFCN: Chloroform fraction of methanol extract of Cocos nucifera husk fiber; QUE: Quercetin; Cis: Cisplatin
Micronucleus induction is a key characteristic feature of genotoxic, carcinogenic compounds, which are known to induce DNA or chromosomal damage. In this study, Cis increased the frequency of mPCE from the bone marrow of the animals. The observation is consistent with the findings of Orsolic and Car, who reported clastogenic effects of Cis and the ability of QUE to reduce the frequency of mPCE in mice given Cis. In this study, pretreatment with CFCN or QUE reversed Cis-induced increase in mPCE in the animals. The reduction of mPCE by CFCN and QUE confirmed their anticlastogenic effects, which may be linked to the intrinsic antioxidant and free radical scavenging properties. CFCN has been reported to act as an effective antioxidant of great importance against diseases and degenerative processes caused by oxidative stress. The antioxidant property of CFCN has been linked to the presence of polyphenols, especially flavonoids. From this study, it can be inferred that CFCN positively modulates the antioxidant status and regenerates the liver and kidney to near normal in Cis-treated rats.

CONCLUSIONS

Our results suggest that oxidative stress, chromosomal damage, and LPO are important features in Cis-induced toxicity in liver and kidney of rats. However, treatment with CFCN was found to inhibit LPO, activates the endogenous antioxidant system, and elicits anticlastogenic effect in the animals. This may be due to antioxidant and free radical scavenging effects of CFCN. Further studies are warranted to isolate and characterize the active component in CFCN responsible for the observed effects before Cocos nucifera can be recommended for use in combination with Cis during cancer chemotherapy.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jin C, Zou T, Li J, Chen X, Liu X, Wang Y, et al. Side population cell level in human breast cancer and factors related to disease-free survival. Asian Pac J Cancer Prev 2015;16:9911-6.
2. Boulakis T. Poly (ADP-ribose) synthesis in blocked and damaged cells and its
relation to carcinogenesis. Anticancer Res 1992;12:885-98.

3. Tamzali Y, Borde L, Rois MP, Goblot M, Lavyazi F, Teissie J. Successful treatment of equine sarcoids with cisplatin electrochemotherapy: A retrospective study of 48 cases. Equine Vet J 2012;44:214-20.

4. Jin J, Ye MC, Wang LP, Li RX, Zhou Y, Wang Y, et al. Grade IV myelosuppression after induction chemotherapy of TPF on oral cancer: Clinical analysis of 29 cases. Shanghai Kou Qian Yi Xue 2014;23:219-23.

5. Prasaja Y, Sudarzno N, Andrajati R. Incidence of cisplatin-induced nephrotoxicity and associated factors among cancer patients in Indonesia. Asian Pac J Cancer Prev 2015;16:1117-22.

6. Fehrenbacher JC. Chemotherapy-induced peripheral neuropathy. Prog Mol Biol Transl Sci 2015;131:471-508.

7. Almaghrabi OA. Molecular and biochemical investigations on the effect of quercetin on oxidative stress induced by cisplatin in rat kidney. Saudi J Biol Sci 2015;22:227-31.

8. Song KJ, Park JY, Lee S, Lee D, Jang HJ, Kim SN, et al. Protective effect of tetrahydrocurcumin against cisplatin-induced renal damage: In vitro and in vivo studies. Planta Med 2015;81:286-91.

9. Kim HJ, Park DJ, Kim JH, Jeong EY, Jung MH, Kim TH, et al. Glutamine protects against cisplatin-induced nephrotoxicity by decreasing cisplatin accumulation. J Pharmacol Sci 2015;127:117-26.

10. Ko JW, Lee IC, Park SH, Moon C, Kang SS, Kim SH, et al. Protective effects of pine bark extract against cisplatin-induced oxidative stress and apoptosis in rats. Lab Anim Res 2014;30:174-80.

11. Aksoy AN, Kabil Kucur S, Batmaz G, Gözükara I, Aksoy M, Kurt N, et al. The role of antioxidant activity in the prevention and treatment of interference caused by cisplatin in rats. Gynecol Obstet Invest 2015;79:119-25.

12. Odukoya OA, Inye-Agha SI, Segun FL, Sofidiya MO, Ilori OQ. Antioxidant activity of selected Nigerian green leafy vegetables. Am J Food Technol 2007;2:169-75.

13. Calzada F, Yepes-Mulia L, Tapia-Contreras A. Effect of Mexican medicinal plant used to treat trichomoniases on Trichomonas vaginalis trophozoites. J Ethnopharmacol 2007;113:249-51.

14. Dyana JP, Kanchana G. Preliminary phytochemical screening of Cocos nucifera L. flowers. Int J Curr Pharm Res 2012;4:62-3.

15. Adaramoye OA, Azeez FA. Evaluation of antioxidant and acetylcholinesterase-inhibitory properties of methanol extracts of Nauclea latifolia, Cymbopogon citratus and Cocos nucifera. An in vitro study. Br J Med Med Res 2014;4:2156-70.

16. Moon YJ, Wang X, Morris ME. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. Toxicol In Vitro 2006;20:187-210.

17. Sestili P, Guidarelli A, Dachá M, Cantoni O. Quercetin prevents DNA single strand breakage and cytotoxicity caused by tert-butylhydroperoxide: Free radical scavenging versus iron chelating mechanism. Free Radic Biol Med 1998;25:196-200.

18. Periasamy R, Kalai IG, Krishnaswamy R, Viswanadha V. Quercetin protects human peripheral blood mononuclear cells from OTA-induced oxidative stress, genotoxicity, and inflammation. Environ Toxicol 2014; doi: 10.1002/tox.22096.

19. Al-Kahtani MA, Abdel-Moneim AM, Elmeneshawy OM, El-Kersh MA. Hemin attenuates cisplatin-induced acute renal injury in male rats. Oxyd Med. Cell Longev 2014;2014:476430.

20. Heeba GH, Mahmoud ME. Dual effects of quercetin in doxorubicin-induced nephrotoxicity in rats and its modulation of the cytotoxic activity of doxorubicin on human carcinoma cells. Environ Toxicol 2014; doi: 10.1002/tox.22075.

21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

22. Taike H, Schubert GE. Enzymatic urea determination in the blood and serum in the warburg optical test. Klin Wochenshr 1965;43:174-5.

23. Jaffe M. Above the envious strike, which piccic acid in normal urine and a new reaction of creatinine. Z Physiol Chem 1888;10:391-400.

24. Mohun AF, Cook U. Simple methods for measuring serum levels of the glutamic-oxaloacetic and glutamic-pyruvic transaminases in routine laboratories. J Clin Pathol 1957;10:394-9.

25. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.

26. King EJ, Armstrong AR. Calcium, phosphorus and phosphatases. In: Varley H, editor. Practical Clinical Biochemistry. New Delhi, India: CBS Publishers; 1988. p. 458.

27. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-55.

28. Aebi H. Catalase: Methods of enzymatic analysis. In: Bergmeyer HV, editor. Methods of Enzymatic Assays. New York: Verlag Chemie; 1974. p. 673-84.

29. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.

30. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:589-90.

31. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302-10.

32. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.

33. Haddie JA, Salamone MF. The micronucleus assay I: In vivo. In: Stich HF, San RH, editors. Topics in Environmental Physiology and Medicine. Short-Term Tests for Chemical Carcinogens. Heidelberg, Berlin: Springer-Verlag; 1981. p. 243-9.

34. Morrissey RE, Schwetz BA, Lamb JC 4th, Ross MD, Teagle JL, Morris RW. Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. Fundam Appl Toxicol 1988;11:343-58.

35. Freund M, Carol B. Factors affecting haemocytometer counts of sperm concentration in human semen. J Reprod Fertil 1964;8:149-55.

36. Wells ME, Awa OA. New technique for assessing acrosomal characteristics of spermatozoa. J Dairy Sci 1970;53:227-32.

37. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 2005;4:307-20.

38. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.

39. Sinh M, Nishanithi E, Sharma R. Nephroprotective effect of vanillic acid against cisplatin induced nephrotoxicity in wistar rats: A biochemical and molecular study. Environ Toxicol Pharmacol 2015;39:392-404.

40. Akman T, Akman L, Erbas O, Terek MC, Taskiran D, Ozsaran A. The preventive effect of oxytocin to cisplatin-induced neurotoxicity: An experimental rat model. Biomed Res Int 2015;2015:167235.

41. Giantzounis G, Salacinski HJ, Yang W, Davidson BR, Seifalian AM. The potential role of antioxidant therapy in attenuating liver ischemia-reperfusion injury: A review. Liver Transpl 2005;11:1021-47.

42. Dkhil MA, Al-Quraishy S, Diab MM, Othman MS, Aref AM, Abdel Moneim AE. Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. Int J Biol Sci 2009;5:486-73.

43. Halliwell B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet 1994;344:721-4.

44. Hussain M, Daignault S, Agarwal N, Grivas PD, Sieker-Radkte AO, Puzanov I, et al. New technique for assessing acrosomal characteristics of spermatozoa. J Dairy Sci 1970;53:227-32.

45. Smidt HC, Scheiner M, Meixenscher D, et al. Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. Int J Biol Sci 2009;5:486-73.

46. Halliwell B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet 1994;344:721-4.

47. Goldberg DM, Watts C. Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterology 1965;49:256-61.

48. el Daly ES. Influence of acute and chronic morphine or stadol on the secretion of adrenocorticotrophin and its hypothalamic releasing hormone in the rat. Life Sci 1996;59:1891-90.

49. Cheung JR, Dickinson DA, Moss J, Schuler MJ, Spellman RA, Heard PL.
Histone markers identify the mode of action for compounds positive in the TK6 micronucleus assay. Mutat Res Genet Toxicol Environ Mutagen 2015;777:7-16.

50. Oršolic N, Car N. Quercetin and hyperthermia modulate cisplatin-induced DNA damage in tumor and normal tissues in vivo. Tumour Biol 2014;35:6445-54.

51. Pinto IF, Silva RP, Filho Ade B, Dantas LS, Bispo VS, Matos IA, et al. Study of antiglycation, hypoglycemic, and nephroprotective activities of the green dwarf variety coconut water (Cocos nucifera L.) in alloxan-induced diabetic rats. J Med Food 2015;18:802-9.

52. Marimoutou M, Le Sage F, Smadja J, Lefebvre d’Hellencourt C, Gonthier MP, Robert-Da Silva C. Antioxidant polyphenol-rich extracts from the medicinal plants Antirhea borbonica, Doratoxylon apetalum and Gouania mauntiana protect 3T3-L1 preadipocytes against H2O2, TNFα and LPS inflammatory mediators by regulating the expression of superoxide dismutase and NFκB genes. J Inflamm (Lond) 2015;12:10.

53. Prabhu S, Dennison SR, Mura M, Lea RW, Snape TJ, Harris F. Cn-AMP2 from green coconut water is an anionic anticancer peptide. J Pept Sci 2014;20:909-15.

54. Silva RR, Oliveira e Silva D, Fontes HP, Alviano CS, Fernandes PD, Alviano DS. Anti-inflammatory, antioxidant, and antimicrobial activities of Cocos nucifera var. typica. BMC Complement Altern Med 2013;13:107.

ABOUT AUTHORS

Dr. Oluwatosin Adekunle Adaramoye, designed and supervised the study.

Miss. Adesola Fausat Azeez, conducted the experiments and also analysed the samples.

Dr. (Mrs). Olufunke Elizabeth Ola-Davies, was involved in sample analysis while.