

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

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## 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 micro nucleated 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

## SUMMARY

- Cisplatin (Cis) is used in the treatment of solid tumors and is known to elicit serious side effects. This study investigated the protective effects of

chloroform fraction of *Cocos nucifera* husk fiber (CFCN) against Cis-induced organs' damage while quercetin (QUE) served as standard antioxidant.

- 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 seven days before the rats were exposed to a single dose of Cis (10mg/kg, i.p.) at the last 36 h of study. Results indicate that administration of Cis caused a significant ( $P < 0.05$ ) increase in the levels of serum creatinine and urea by 72% and 70% respectively.
- 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.
- 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. The findings suggest that CFCN may protect against organs damage by cisplatin.
- Further studies are required to determine the component of the plant responsible for this activity.

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## 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.<sup>[1]</sup> Cisplatin (Cis) is one of the most important chemotherapeutic drugs used in the treatment of a wide range of solid tumors.<sup>[2]</sup> It was found to have anti-tumor effects in animal models,<sup>[3]</sup> but its use is often associated with a number of side effects such as nephrotoxicity, peripheral neuropathy, ototoxicity, gastrointestinal disorder, and myelosuppression.<sup>[4-6]</sup> 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.<sup>[7]</sup> Therefore, intervention studies with antioxidants showed promising results with a significant reduction in the degree of toxicity of Cis.<sup>[8,9]</sup> 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.<sup>[10,11]</sup> 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 *Arecaceae* (Palmae). It is cultivated for its multiple utilities mainly for its nutritional and medicinal values.<sup>[12]</sup> Its products such as coconut kernel and coconut water have numerous medicinal properties such as

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antibacterial, antifungal, antiviral, anti-dermatophytic, antioxidant, hypoglycemic, hepatoprotective, immune-stimulant, and anti-protozoal activities.<sup>[13,14]</sup> 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.<sup>[15]</sup> 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.<sup>[16]</sup> It has an antioxidant property which has been linked to its ability to chelate transition metal ions and scavenge free radicals.<sup>[17]</sup> Other relevant functions ascribed to this flavonoid includes antihypertensive, antiarrhythmic, anti-inflammatory, antihepatotoxic, fertility-enhancing, and anti-tumor activities.<sup>[18]</sup> 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), sulfosalicylic 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),<sup>[19]</sup> 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.<sup>[20]</sup> 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.*,<sup>[21]</sup> using bovine serum albumin as standard. Serum urea and creatinine levels were determined by the methods of Talke and Schubert,<sup>[22]</sup> and Jaffe,<sup>[23]</sup> respectively. The activities of alanine and aspartate aminotransferases (ALT and AST) were assayed by the combined methods of Mohun and Cook,<sup>[24]</sup> and Reitman and Frankel.<sup>[25]</sup> Alkaline phosphatase (ALP) activity was determined according to the method described by King and Armstrong.<sup>[26]</sup> PMF superoxide dismutase (SOD) activity was measured by the nitro blue tetrazolium reduction method of McCord and Fridovich.<sup>[27]</sup> PMF catalase (CAT) activity was assayed spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi.<sup>[28]</sup> Reduced GSH was determined by the method of Beutler *et al.*<sup>[29]</sup> Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck *et al.*<sup>[30]</sup> PMF lipid peroxidation (LPO) was determined by the method of Buege and Aust,<sup>[31]</sup> while glutathione-S-transferase (GST) activity was determined by the method of Habig *et al.*<sup>[32]</sup> Micronucleus assay was determined by the method of Haddad and Salamone.<sup>[33]</sup>

### 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.<sup>[34]</sup> 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.<sup>[35]</sup> 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.<sup>[36]</sup>

### 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

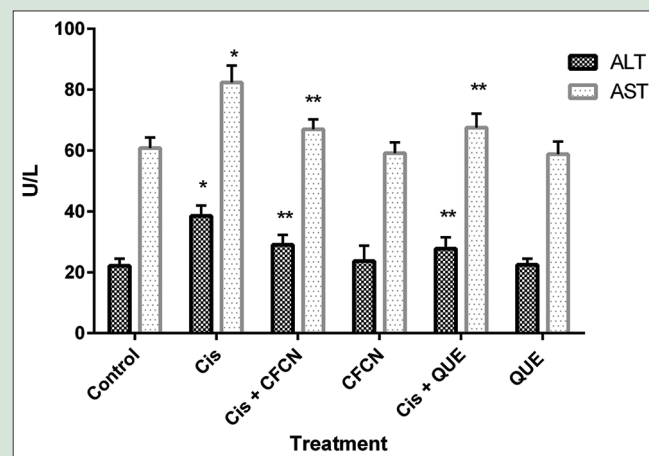
The activities of hepatic and renal antioxidative enzymes-GPx, GST, SOD, and CAT were significantly ( $P < 0.05$ ) reduced in Cis-treated rats when compared with the controls [Figures 3, 5, 7 and Table 3]. Specifically, hepatic GPx, GST, SOD, and CAT decreased by 41%, 38%, 67%, and 44% while renal GPx, 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.<sup>[5]</sup> Cis, in addition to its therapeutic effects on cancer cells through its ability to bind DNA, has also been reported to induce oxidative stress,<sup>[7]</sup> 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.<sup>[37]</sup> 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.<sup>[38]</sup>



**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 methanol extract of *Cocos nucifera* husk fiber, QUE = Quercetin, Cis = Cisplatin

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

| Treatment  | Body weight (g) |           |                        | Weight of organs (g) |                       |          | Relative weight of organs |          |         |
|------------|-----------------|-----------|------------------------|----------------------|-----------------------|----------|---------------------------|----------|---------|
|            | Initial         | Final     | Weight gain            | Liver                | Kidney                | Testes   | Liver                     | Kidney   | Testes  |
| Control    | 123.0±2.7       | 144.8±3.0 | 21.8±1.2               | 6.3±0.2              | 0.87±0.1              | 1.64±0.4 | 4.4±0.3                   | 0.6±0.03 | 1.1±0.3 |
| Cis        | 118.0±1.5       | 91.7±2.1  | -26.3±2.8 <sup>a</sup> | 4.1±0.3 <sup>a</sup> | 0.53±0.1 <sup>a</sup> | 1.53±0.3 | 4.5±0.4                   | 0.6±0.02 | 1.6±0.4 |
| Cis + CFCN | 102.0±2.5       | 123.0±1.7 | 21.0±2.0 <sup>b</sup>  | 5.7±0.2 <sup>b</sup> | 0.79±0.2 <sup>b</sup> | 1.58±0.4 | 4.6±0.5                   | 0.6±0.05 | 1.3±0.5 |
| CFCN       | 115.0±1.3       | 137.0±2.8 | 22.0±2.8               | 6.5±0.4              | 0.89±0.1              | 1.70±0.1 | 4.7±0.5                   | 0.6±0.05 | 1.2±0.4 |
| Cis + QUE  | 116.0±2.1       | 136.0±1.9 | 20.0±2.0 <sup>b</sup>  | 4.5±0.3 <sup>a</sup> | 0.75±0.1 <sup>b</sup> | 1.56±0.3 | 3.3±0.5 <sup>a</sup>      | 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. <sup>a</sup>Significantly different from control ( $P < 0.05$ ), <sup>b</sup>Significantly different from Cis ( $P < 0.05$ ). CFCN: Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber; QUE: Quercetin; Cis: Cisplatin; SD: Standard deviation



However, ROS production by Cis caused antioxidant imbalance and led to LPO.<sup>[39]</sup> 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.<sup>[10,40]</sup> GSH acts as a free radical scavenger and regenerator of alpha-tocopherol and plays a significant role in sustaining protein sulfhydryl groups.<sup>[41]</sup> Decreased renal and hepatic GSH contents result in increased susceptibility to hepatic and renal injury through the induction of LPO.<sup>[42]</sup> 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

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

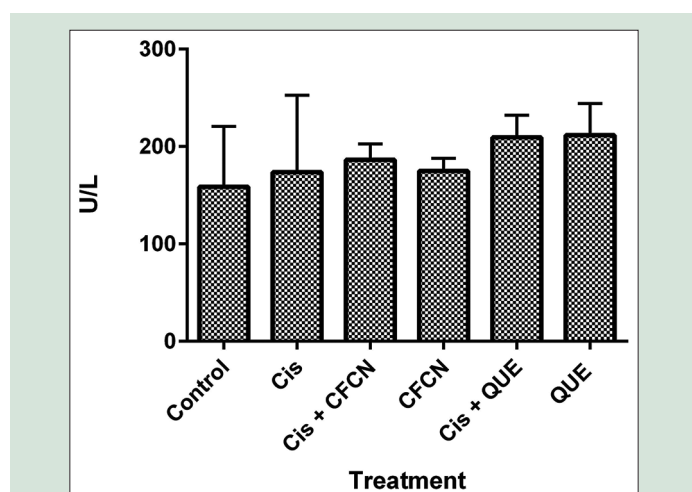
| Treatment  | Creatinine ( $\mu\text{mol/l}$ ) | Urea ( $\text{mmol/l}$ ) |
|------------|----------------------------------|--------------------------|
| Control    | 2.05 $\pm$ 0.43                  | 57.74 $\pm$ 4.50         |
| Cis        | 3.74 $\pm$ 0.22*                 | 98.02 $\pm$ 4.81*  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

Values are mean $\pm$ 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 *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  | GST activities ( $\mu\text{mole/min/mg protein}$ ) |                     |                   |
|------------|----------------------------------------------------|---------------------|-------------------|
|            | Liver                                              | Kidney              | Testes            |
| Control    | 0.63 $\pm$ 0.15                                    | 0.027 $\pm$ 0.005   | 0.060 $\pm$ 0.007 |
| Cis        | 0.39 $\pm$ 0.18*                                   | 0.015 $\pm$ 0.008*  | 0.056 $\pm$ 0.010 |
| Cis + CFCN | 0.58 $\pm$ 0.12**                                  | 0.021 $\pm$ 0.002** | 0.063 $\pm$ 0.031 |
| CFCN       | 0.61 $\pm$ 0.17                                    | 0.030 $\pm$ 0.009   | 0.061 $\pm$ 0.001 |
| Cis + QUE  | 0.64 $\pm$ 0.18**                                  | 0.023 $\pm$ 0.001** | 0.057 $\pm$ 0.007 |
| QUE        | 0.67 $\pm$ 0.16                                    | 0.025 $\pm$ 0.001   | 0.059 $\pm$ 0.015 |

Values are mean $\pm$ 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 *Cocos nucifera* husk fiber; QUE: Quercetin; Cis: Cisplatin; GST: Glutathione-s-transferase

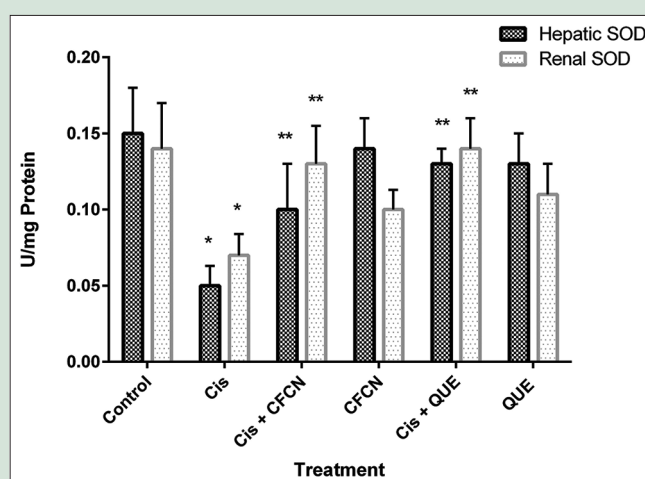


**Figure 2:** Effects of CFCN on the activity of serum alkaline phosphatase (ALP) in rats treated with cisplatin. Cis = Cisplatin, QUE = Quercetin, CFCN = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber

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.<sup>[7]</sup> It is so because the liver is known to accumulate significant amounts of Cis, second only to the kidney.<sup>[43]</sup>

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.<sup>[7]</sup> 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.<sup>[11]</sup> The inhibition of antioxidant system may cause the accumulation of  $\text{H}_2\text{O}_2$  or products of its decomposition.<sup>[44]</sup> SOD catalyzes the conversion of superoxide anion into  $\text{H}_2\text{O}_2$  while the primary role of CAT is to scavenge  $\text{H}_2\text{O}_2$  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.

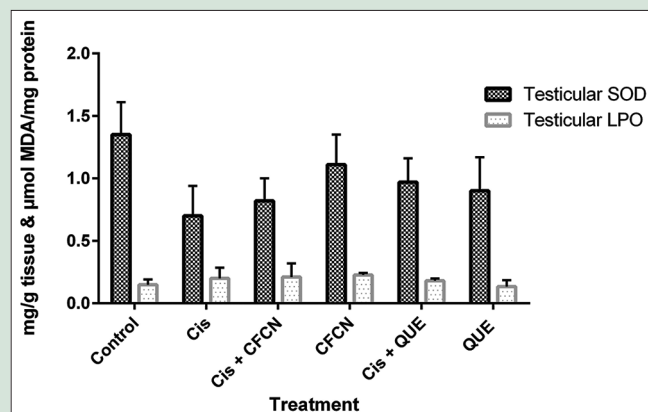
Administration of Cis induced hepatotoxicity and nephrotoxicity evidenced by the significant elevation of serum ALT, AST, creatinine, and urea in the present study. This observation is similar to the findings of Hussain *et al.*<sup>[45]</sup> and Liu *et al.*<sup>[46]</sup> who reported the elevation 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,<sup>[47]</sup> thereby causing these enzymes to leak into the plasma. This observation is supported by the recent report of Ko *et al.*<sup>[10]</sup> 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



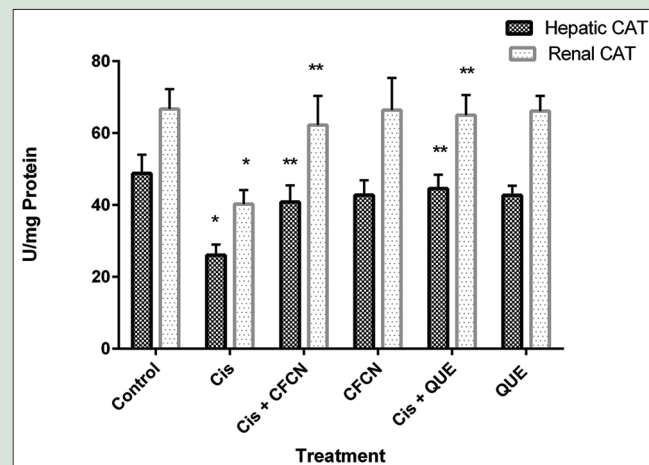
**Figure 3:** Effects of CFCN on the activities of hepatic and renal superoxide (SOD) in rats treated with cisplatin. \*Significantly different from control ( $P < 0.05$ ), \*\*Significantly different from Cis ( $P < 0.05$ ). Cis = Cisplatin, QUE = Quercetin, CF = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber

in animal models.<sup>[48]</sup> 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.<sup>[8,9]</sup> 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

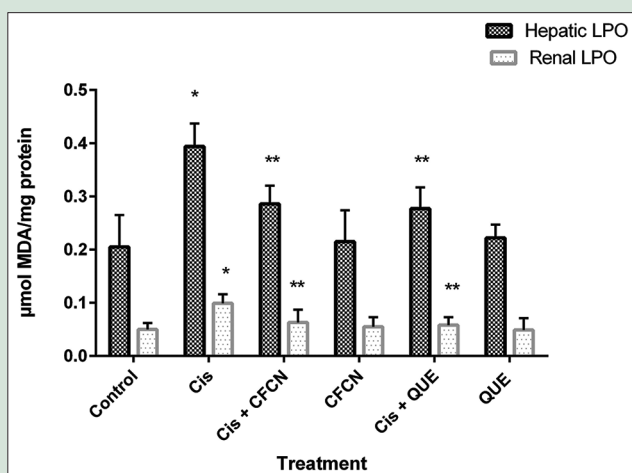
normal values following pretreatment with CFCN or QUE confirmed the possible ameliorative effects of these agents against Cis-induced renal dysfunction in the rats. It is pertinent to mention that in this study, the toxic effect of Cis was very mild on the testis. Cis exposure insignificantly affected sperm characteristics and testicular antioxidant status of the rats.



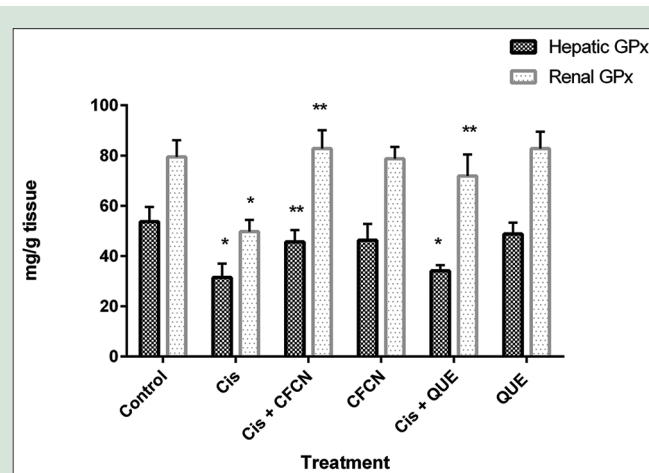
**Figure 4:** Effects of CFCN on the activities of testicular superoxide dismutase (SOD) and levels of lipid peroxidation (LPO) in rats treated with cisplatin. Cis = Cisplatin, QUE = Quercetin, CF = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber



**Figure 5:** Effects of CFCN on the activities of hepatic and renal catalase (CAT) in rats treated with cisplatin. \*Significantly different from control ( $P < 0.05$ ). \*\*Significantly different from Cis ( $P < 0.05$ ). Cis=Cisplatin, QUE= Quercetin, CFCN=Chloroform fraction of methanol extract pf *Cocos nucifera* husk fiber



**Figure 6:** Effects of CFCN on hepatic and renal lipid peroxidation (LPO) levels of rats treated with cisplatin. \*Significantly different from control ( $P < 0.05$ ). \*\*Significantly different from Cis ( $P < 0.05$ ). Cis=Cisplatin, QUE = Quercetin, CFCN = Chloroform fraction of methanol extract pf *Cocos nucifera* husk fiber

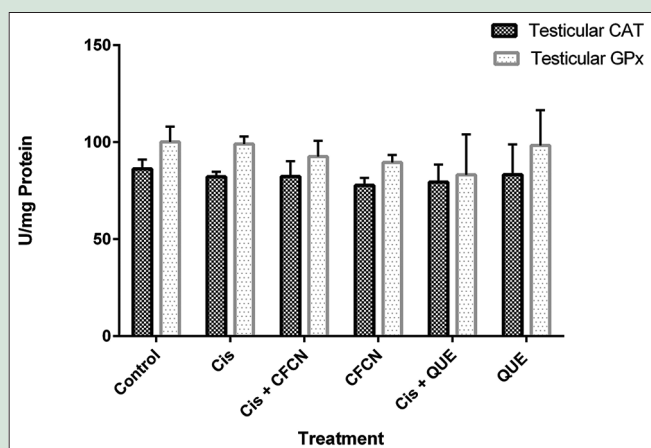


**Figure 7:** Effects of CFCN on the activities of hepatic and renal glutathione peroxidase (GPx) in rats treated with cisplatin. \*Significantly different from control ( $P < 0.05$ ). \*\*Significantly different from Cis ( $P < 0.05$ ). Cis = Cisplatin, QUE = Quercetin, CFCN = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber

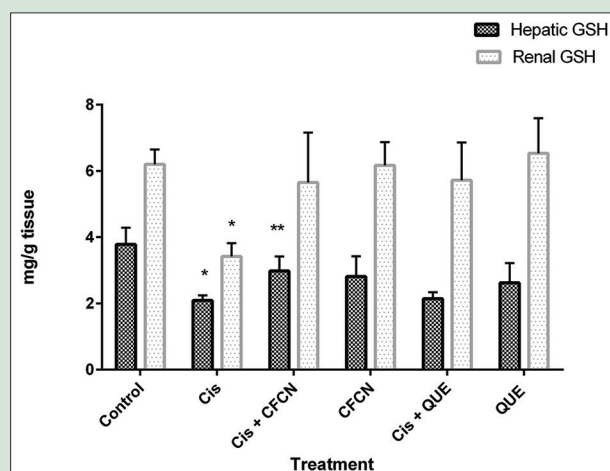
**Table 4:** Effects of CFCN on sperm parameters in rats treated with Cis

| Treatment  | Sperm motility (%) | Sperm count (10 <sup>6</sup> /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          | 80.86±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          | 88.33±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          | 84.67±6.51                              | 11.80±0.73 | 95.0±5.0        | 5.1±0.06    |

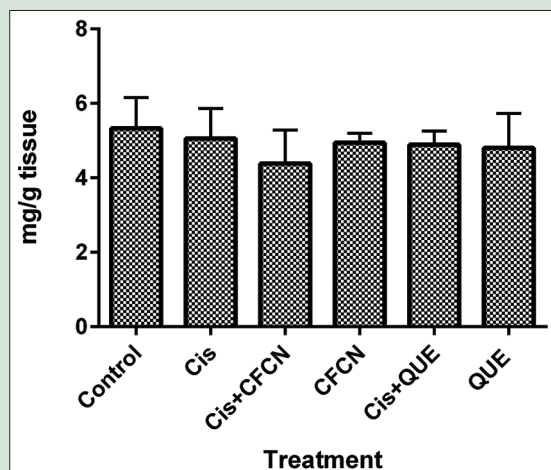
Values are mean±SD of 5 animals per group. CFCN: Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber; QUE: Quercetin; Cis: Cisplatin



**Figure 8:** Effects of CFCN on the activities of testicular catalase (CAT) and glutathione peroxidase (GPx) in rats treated with cisplatin. Cis = Cisplatin, QUE = Quercetin, CFCN = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber



**Figure 9:** Effects of CFCN on hepatic and renal GSH levels of rats treated with cisplatin. \*Significantly different from control ( $P < 0.05$ ). \*\*Significantly different from Cis ( $P < 0.05$ ). Cis = Cisplatin, QUE = Quercetin, CFCN = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber



**Figure 10:** Effects of CFCN on testicular GSH level of rats treated with cisplatin. Cis = cisplatin, QUE = Quercetin, CFCN = Chloroform fraction of methanol extract of *Cocos nucifera* husk fiber

**Table 5:** Effects of CFCN on the formation of micronuclei in polychromatic erythrocytes from bone marrow of rats treated with Cis

| Treatment  | MNPCE/1000 PCE |
|------------|----------------|
| Control    | 0.50±0.22      |
| Cis        | 15.80±1.02*    |
| Cis + CFCN | 9.00±0.45**    |
| CFCN       | 1.67±0.42**    |
| Cis + QUE  | 3.00±0.26**    |
| QUE        | 0.33±0.33**    |

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 *Cocos nucifera* husk fiber; QUE: Quercetin; Cis: Cisplatin; MNPCE/1000 PCE: Number of micronuclei per 1000 polychromatic erythrocytes; SD: Standard deviation

## 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.

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Micronucleus induction is a key characteristic feature of genotoxic, carcinogenic compounds, which are known to induce DNA or chromosomal damage.<sup>[49]</sup> In this study, Cis increased the frequency of mPCE from the bone marrow of the animals. The observation is consistent with the findings of Oršolic and Car,<sup>[50]</sup> 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.<sup>[51,52]</sup> CFCN has been reported to act as an effective antioxidant of great importance against diseases and degenerative processes caused by oxidative stress.<sup>[53]</sup> The antioxidant property of CFCN has been linked to the presence of polyphenols, especially flavonoids.<sup>[54]</sup> 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.

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