The Modulatory Effect of *Chrysobalanus icaco* seed on Cadmium Induced Hepatotoxicity and Nephrotoxicity in Rats

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**ABSTRACT**

The fight against cadmium induced oxidative stress has been a long battle in the field of medicine, hence a lot of research is still ongoing in search of plants based on their phytochemical properties that can ameliorate cadmium induced toxicity. This study was therefore aimed at evaluating the modulatory effect of *Chrysobalanus icaco* seed extract on some liver and kidney parameters of Wister rats exposed to cadmium. A total of twenty-four (24) rats were used for the study. The rats were divided into four (4) groups of six (6) rats as follows: Group 1: normal control; rats in this group received distilled water daily throughout the experiment. Group 2: cadmium only; the rats in this group received 4mg/kg body weight (b wt.) of cadmium in form of Cadmium chloride. Group 3: the rats in this group received 4mg/kg b wt. of cadmium in form of Cadmium chloride +100mg *Chrysobalanus icaco* (*C. icaco*) extract three times per week. Group 4: the rats in this group received 4mg/kg b wt. of cadmium in form of Cadmium chloride +500mg *C. icaco* extract three times per week. The oral administration of Cadmium and *C. icaco* extract was carried out by gavage. The experiment lasted for 21 days. The results revealed that in all groups exposed to cadmium, serum level of alanine aminotransferase (ALT) 75.00 U/L, aspartate aminotransferase (AST) 145.25 U/L and alkaline phosphatise (ALP) 70.38 U/L was significantly high (P < 0.05) when compared to the control group (ALT 28.00 U/L, AST 75.25 U/L and ALP 16.00 U/L). However, serum level of ALT, AST and ALP of the Cadmium chloride + *C. icaco* extract treated groups was significantly decreased (P < 0.05) when compared with the non-treated cadmium group. In conclusion the study revealed that aqueous extract of *Chrysobalanus icaco* fruit is able to ameliorate cadmium-induced renal and liver dysfunction due to its anti-inflammatory and antioxidant effects.

**Keywords:** Cadmium, *Chrysobalanus icaco*, liver, kidney

**Introduction**

The presence of intrinsic elements such as cadmium in the environment is unique due to the fact that their complete removal from the environment is difficult. Cadmium is found as a contaminant in foods, cigarettes and polluted air. It is also used by industries in the production of metal coatings, plastics pigments and batteries, (ATSDR, 2012; Satarug and Moore, 2012; Ige *et al.*, 2012; Satarug *et al.*, 2013). Hence, increase in its use in our daily lives and by industries is a major problem resulting in toxic metal pollution (Mahurpawar, 2015). In addition, the enhanced gastrointestinal and pulmonary absorption rates for cadmium makes children and women of...
reproductive age more susceptible to cadmium toxicity (Meltzer et al., 2010; Suh et al., 2016). Indeed, increase in chronic kidney disease is a major global problem and cadmium has been found to accumulate in the kidney and very small amounts excreted from the urine (Satarug et al., 2002). Studies have also indicated that no effective therapeutically metal-chelating agents, for cadmium reduction levels in kidney, has been identified. Hence kidney damage linked with cadmium is presumed to be caused from a reduction in re-absorption of nutrients by the tubules (Honda et al., 2010; Wallin et al., 2014). Consequently, a rise in urinary levels of these solutes is an indication of tubular malfunction. In addition, other health issues such as macular degeneration and various types of cancer has also been linked with chronic intake of cadmium in the diet (Wu et al., 2014; Kim et al., 2015).

Cadmium induces damage to the liver through an imbalance in cellular redox status leading to oxidative stress. However, the mechanism is not clear (Arroyo, 2012). It competes with other metals and many antioxidants proteins for their sites thereby inducing an abnormal activity as well as total loss of cellular defence by the antioxidant. (Ajayi et al., 2012; Ige et al., 2012; Taiwo et al., 2012).

**Materials and methods**

**Plant Collection and Identification**

The spice *Chrysobalanus icaco* (Gbafilo) was purchased from Abraka main market in Delta State, Nigeria. The Fruit was duly identified and given voucher number UBHc437 by Dr. Akinnibonsu, H.A, from the department of Botany, University of Benin, Edo State, Nigeria.

**Preparation of Plant Extract**

The seed of *C. icaco* was removed from the pod and air dried for 10days in the laboratory. The dried seed were then oven dried and grinded to a powdered form with the aid of an electric blender. Three hundred grams (300g) of powdered leaves were soaked in 900ml of water (ratio 1:3) and the mixture was stirred every six hours for 48 hours and then filtered with sieve Whatman filter paper (size no 1) to obtain a clear filtrate which was further concentrated to dryness with hot air from the oven set at 60°C. Final extract recovered weighing 11g with percentage yield of 3.6%w/w was stored in the refrigerator before use.(El-Mahmood, 2009)

**Experimental Animals**

Twenty-four, 8 weeks old albino wister rats weighing between 120g and 150g were purchased from the animal house of the College of Medicine, Delta State University. The rats were fed on growers’ mash and were housed in cages constructed from aluminium steel and wire gauze under control condition of 12h light/ 12 dark
Experimental Design
The rats were divided into Four (4) groups of six (6) rats as follows:
Group 1: normal control; rats in this group received distilled water daily throughout the experiment.
Group 2: cadmium only; the rats in this group received 4mg/kg body weight (b.wt) of cadmium in form of Cadmium chloride to induce cadmium intoxication.
Group 3: the rats in this group received 4mg/kg b wt. of cadmium in form of Cadmium chloride +100mg C. icaco extract three times per week.
Group 4: the rats in this group received 4mg/kg b wt. of cadmium in form of Cadmium chloride +500mg C. icaco extract three times per week.
The oral administration of Cadmium and C. icaco extract was carried out by gavage. The experiment lasted for 21 da ys.
On the last day the rats were allowed to starve overnight and sacrificed by cervical decapitation. Blood was collected in to heparinised tubes, and centrifuged at a speed of 3000g for 10mins. The sera were then collected and stored frozen until needed. The liver and kidney were also extracted and 0.5g was homogenised with normal saline. The supernatant was put in empty container and stored in the fridge for biochemical analysis.

Biochemical Analysis
The L-alanine aminotransferases and L-aspartate aminotransferase activities in the serum were determined by the method of Reitman and Franklin as modified by Gella et al. (1985). Alkaline Phosphatase activity was also determined in the serum by the method of Annino and Giese (1976) using the Randox kit as described in the manual. The activity of SOD in the kidney and liver was assayed for using the method of Spitz and Oberley (2001). The method of Johansson and Borg (1988) was adopted for the assay of kidney and liver catalase activity. Lipid peroxidation was determined in the organs by using the method of Gutteridge and Wilkins (1982) Creatinine activity was determined by the method of Jung et al. (1987) and Urea activity was determined by the method of Zawada et al. (2009).

Statistical Analysis
All the experimental results were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) with subsequent LSD test were used to detect further difference between groups respectively, values of p< 0.05 were considered significant.

Table 1: Ameliorating effect of aqueous extract of Chryobalanus icaco on serum hepatic markers in cadmium induced Wister rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.00 ± 8.14a</td>
<td>75.25 ± 18.70a</td>
<td>16.00 ± 1.15a</td>
</tr>
<tr>
<td>Cadmium control</td>
<td>75.00 ± 4.00b</td>
<td>145.25 ± 19.36b</td>
<td>70.38 ± 7.53b</td>
</tr>
<tr>
<td>Cadmium+100mg C icaco</td>
<td>65.00 ± 10.62c</td>
<td>111.16 ± 20.74c</td>
<td>59.92 ± 4.75c</td>
</tr>
<tr>
<td>Cadmium+500mg C icaco</td>
<td>61.004 ± 00c</td>
<td>108.15±18.62c</td>
<td>48.00 ± 7.75c</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05).

Table 2: Ameliorating effect of aqueous extract of C. icaco on serum renal indices in Cadmium induced Wister rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>15.64 ± 3.26e</td>
<td>1.07±0.74e</td>
</tr>
<tr>
<td>2-Cadmium control</td>
<td>29.48 ± 8.38e</td>
<td>5.33±0.49e</td>
</tr>
<tr>
<td>3-Cadmium+100mg C icaco</td>
<td>23.41 ± 2.66f</td>
<td>2.97±0.09f</td>
</tr>
<tr>
<td>4-Cadmium+500mg C icaco</td>
<td>18.41 ± 3.66f</td>
<td>1.97±0.09f</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05).
Table 3: Ameliorating effect of aqueous extract of *Chryobalanusicaco* on hepatic antioxidant markers in cadmium induced Wister rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units/gtissue)</th>
<th>CAT (Units/gtissue)</th>
<th>MDA (Units/gtissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>42.25 ± 1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.18 ± 4.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2- Cadmium control</td>
<td>26.34 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.23 ± 1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.81 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-Cadmium+100 mg <em>C.icaco</em></td>
<td>35.64 ± 4.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.65 ± 1.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.41 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4-Cadmium+500 mg <em>C.icaco</em></td>
<td>38.34± 4.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.65 ± 1.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.36 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05).

Table 4: Ameliorating effect of aqueous extract of *Chryobalanusicaco* on renal antioxidant markers in cadmium intoxicated Wister rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units/gtissue)</th>
<th>CAT (Units/gtissue)</th>
<th>MDA (Units/gtissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>38.18 ± 7.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.21 ± 3.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2- Cadmium control</td>
<td>26.94 ± 5.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.78 ± 4.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-Cadmium+100 mg Ci</td>
<td>32.38 ± 2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.15 ± 0.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.31 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4-Cadmium+500 mg Ci</td>
<td>34.27± 5.51&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>21.45 ± 2.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.27 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05).

**Discussion**

Cadmium has been recognized as one of the most toxic environmental and industrial pollutants. It's induced renal toxicity was reported earlier by Patra *et al.* (2011); Imafidon *et al.* (2015). Mallya *et al.* (2017).  
Recent researches have been on counteracting heavy-metal-induced toxicity by use of plant-derived components or extracts with antioxidant potential (Xu *et al.*, 2017). This present study showed that the administration of the aqueous extract of *C. icaco* reduced cadmium induced liver ALT, AST and ALP. This is consistence with the reports of Bharav *et al.* (2010); Onoshe (2014) and Imafidon *et al.* (2015). This damage may be due to direct toxic effects of the cadmium on the hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (Adefegha *et al.*, 2015).

L-alanine aminotransferases (ALT) and L-aspartate aminotransferase (AST) are well known indicators of liver damage. (Al-Habori *et al.*, 2002). The result of this study (Table 1) showed that liver dysfunctions was induced by oral exposure of rats to sub lethal concentration of cadmium as demonstrated by a significant increase in serum ALT, AST and ALP. This is consistence with the reports of Bharav *et al.* (2010); Onoshe (2014) and Imafidon *et al.* (2015). This damage may be due to direct toxic effects of the cadmium on the hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (Adefegha *et al.*, 2015).
dysfunction as verified by lowered, ALT, ALP and AST levels (Table 1), in the cadmium treated rats. One major function of the kidney is clearance of metabolites from the blood (Miners, 2017) and agents with nephrotoxicity effects may lead to a raise in serum urea level. The result of this present study indicates a significant increase in creatinine and urea levels in the serum of the rats exposed to cadmium only (group 2) as compared with the normal control (Table 2). This elevated serum urea and creatinine may be due to an imbalance in the kidney resulting in an increase in urea and creatinine levels as well as oxidative damage to the kidney (Tarasub et al., 2011; Adefegha et al., 2015). This is in line with previous works conducted by Poontawee et al. (2016) who investigated the protective effect of Cleistocalyx nervosum var. paniala fruit extract against oxidative renal damage caused by cadmium and Jafarpour et al. (2017). However, treatment of the rats with different doses of the extracts (Groups 3 and 4) as seen in Table 2 significantly reduced Serum Creatinine and urea levels when compared with the untreated Group 2 rats. Indicating that C. icaco as a nephroprotective effect.

Lipid peroxidation levels (MDA) was also significantly increased in the liver and kidney of the Cd treatment groups compared to the control groups (Table 3 and 4). This is in line with previous research by Elmallah et al. (2017) where they reported an increase in lipid peroxidation in the testes of rats treated with cadmium. The increased Lipid peroxidation in this present study (Table 3 and 4) may be due to decreased activities of Superoxide dismutase (SOD) and Catalase (CAT), the free radical scavenging enzymes as stated by Atagana and Asagba (2015) in their study on the protective effects of symbiotic diets of Bacillus coagulans, Lactobacillus plantarum and inulin against acute cadmium toxicity in rat. A significant decrease (p<0.05) was also indicated in the CAT and SOD activity of the liver and kidney of rats intoxicated with cadmium when compared with the control (Table 3and 4) indicating a damage to these organs. These finding are consistent with that of Atagana and Asagba (2015) and Elmallah et al. (2017) who recorded similar results in their work on cadmium induced toxicity in rats. Patra et al. (2011) also stated in earlier studies that significant decrease in catalase and super oxide dismutase could be due to the fact that they are part of the three endogenous antioxidant enzymes responsible for the removal of reactive oxygen species and needed in the protection of cells and tissues from oxidative damage.

However, treatment of the cadmium intoxicated rats with the aqueous extract of C. icaco reversed these trends. This confirms the antioxidant property of this spice due to its rich antioxidants content (Castillo 2011; White et al. 2016). Previous phytochemical studies of C. icaco demonstrated that it contains phytochemicals (Fernandes et al., 2003; Castilho and Kaplan, 2011). Chemical analysis of C. icaco extract identified delphinidin, anthocyanins, cyanidin, petunidin and peonidin derivatives as major components (Venancio et al., 2016). Several studies have also reported that flavonoids have anti-inflammatory and antioxidant effects (Onoche,
2014) and these antioxidants are the frontline of defence against free radicals (Onoche, 2014). It can therefore be suggested that the role of the aqueous extract of *Chrysobalanus icaco* fruit in ameliorating cadmium-induced renal and liver dysfunction as observed in this study is due to the anti-inflammatory and antioxidant effects of the different compounds of the seed.

**Conclusion**

The result in this study confirms that *Chrysobalanus icaco* seed has antioxidant properties and is able to able to protect the kidney from damage caused by cadmium toxicity in rats, which can also be inferred in man.

**REFERENCES**


