Antioxidant and chelating effects of *Citrus sinensis* peel extract on wistar rats administered with lead and cadmium

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Abstract

Heavy metals possess toxic effects that leads to serious health and environmental problems, anthropogenic activities are chiefly responsible for metal exposure, and they manifest toxic effects on a biological cell. The peels of *Citrus sinensis* have been reported to have medicinal effect. However, these benefits have not been adequately investigated against metals toxicity. This study evaluated the antioxidant and chelating effect of *Citrus sinensis* peel extract (CSPE) on wistar rats administered with lead and cadmium. Forty-five (45) rats were separated into nine (9) groups of 5 rats each. The group under control was given distilled water daily, group 2 and 3 received 8mg/kg of cadmium and 15 mg/kg of lead per body weight respectively. Animals in group 4 received 8 mg/kg of cadmium and 100 mg/kg of EDTA treatment per body weight. Group 5 were given 15 mg/kg of lead and treated with 100 mg/kg of EDTA. Group 6 and 7 were respectively treated with CSPE of 250 and 500 mg/kg and administered cadmium-8 mg/kg. Lastly, group 8 and 9 received lead and 250 and 500 mg/kg of CSPE respectively. Administration lasted for 28 days afterwards the rats were sacrificed. The whole blood was separated for analysis of haematological parameters and the liver and kidney tissues harvested for metal analysis. Glutathione Peroxidase (GPx), Malondialdehyde (MDA), Superoxide dismutase (SOD) and Catalase (CAT) were also estimated. Results showed a statistically significant increase (p<0.05) of MDA levels in lead and cadmium and a significant reduction in SOD, CAT and GPx values compared to the control and treated group. The level of cadmium and lead in blood, kidney, and liver tissues of 500 mg/kg CSPE were significantly reduced. CSPE possesses antioxidant properties and exhibits chelating effects on wistar rats administered with lead and cadmium.

Keywords: Antioxidant, chelation, heavy metal, lead and cadmium

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INTRODUCTION

Heavy metals have been known to possess toxic effects that are detrimental to humans and the environment at large (Balali-Mood et al., 2021). A study conducted by the World Health Organisation (WHO) states that approximately 2.2 million people die in Africa yearly as a result of environmental risk factors (WHO, 2016). Although lead and cadmium are two major toxic metals present in the environment, anthropogenic activities are mainly responsible for their exposure (Tchounwou et al., 2012). Lead has no physiological benefit to the body, it manifests its toxic actions on the blood, liver and kidney immediately it enters the body. In addition, research has shown that exposure to lead can result to a statistically significant reduction in red blood cells count and haematocrit in the blood (Terayama, 1993). Similarly, cadmium is an environmental and industrial pollutant that has been proven to cause injury to a biological cell (Massanyi et al., 2005). For the most part, the primary sources of cadmium exposure are food and tobacco smoking due to the increased cadmium uptake by crops (Jarup and Akesson, 2009). After penetrating the body, cadmium is transported into the blood via blood protein albumin and red blood cells and cumulates in the liver and kidneys (Goyer, 1991). Cadmium exposure leads to renal dysfunction, damage to hepatic system, digestive and respiratory system disorders, testicular atrophy and anemia (Schwartz and Reis, 2000). Report by Salazar-Flores et al., 2020 revealed that toxic metals such as cadmium and lead has been implicated in oxidative stress which results in tissues and membranes damages in a biological entity. Thus, reducing the antioxidant activities of enzymes such as Catalase (CAT), Superoxide dismutase (SOD), and Glutathione Peroxidase (GPx). Although the potential of new drugs discovery from higher plants sources is still largely unexplored, about 80 percent of people in the developing countries depend on phytomedicine for primary healthcare for man and domestic animals (Oke and Hamburger, 2002). Citrus sinensis is an essential commercial fruit grown globally due to its diversified use (Manthey and Grohmann, 2001). Citrus sinensis is good source of vitamin C, it is a strong natural antioxidant and builds the body’s immune system (Etebu and Nwauzoma, 2014). Constipation, cramps, colic, diarrhea, bronchitis, TB, cough, cold, obesity, menstruation disorders, angina, hypertension, anxiety, depression, and stress are just a few of the conditions it has been used to treat locally (Milind and Chaturvede, 2012).

The availability of secondary metabolite in peels of Citrus sinensis add to the pharmacological action ascribed to this fruit. Numerous phytochemicals have been identified in the Peels of Citrus sinensis, which include the following groups: Saponins, Alkaloids, Terpenoids, Tannins, Phenols, Flavonoids and Glycosides (Favela-Hernández et al., 2026). Owning to the above-mentioned medicinal applications of Citrus sinensis peels, several studies have been conducted to scientifically validate these folk medicinal claims. Recently, many pharmacological studies (in vitro and in vivo) conducted on Citrus sinensis peels have been demonstrated to have several antibacterial, antioxidant (Hegazy and Ibrahim, 2012), larvicidal, pupicidal, repellent, and adulticidal pharmacological activities (Murugan et al., 2012). Although, studies have reported that C. sinensis peels may possess a protective potency against cadmium induced liver toxicity, its effect on lead induced nephrotoxicity and hepatotoxicity has not been investigated (Nwafor et al., 2020). Hence, this study was designed to evaluate the antioxidant and chelating effects of Citrus sinensis peel extract (CSPE) on administered lead and cadmium to wistar rats.

MATERIALS AND METHODS

All chemicals used in the present study were of analytical grade. 100 g of cadmium chloride (99.9% pure) and 100g of Ethylenediamine tetracetic acid (EDTA) manufactured by Cartival Chemical Limited, Mumbai, India was obtained from chemical store in Benin City, alongside 100 g of lead acetate, manufactured by BDH Chemical Limited, Poole, England. Equipment used for analysis are: Centrifuge and UV-VIS Spectrophotometer (Hitachi F 7000).

Extract preparation and extraction

Fresh Citrus sinensis fruits were purchased from Oba Market, Oredo Local Government Area of Edo state. The zest of the fruit (peels) was authenticated by Dr. H. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. The peels of the fresh Citrus sinensis fruits were manually separated from its fruit, washed with distilled water to remove adhering dirt and air dried for a period of three weeks until they turned crisp. The crisp leaves were dried in an incubator at 40-45°C for about two hours then pulverized to a fine powder using a mechanical grinder. The dry weight of the peel
was recorded, after which was soaked in analytical grade ethanol for 72 hours, with periodic agitation to ensure proper extraction. The resultant mixture was filtered using a cheese cloth and the filtrate obtained was further subjected to filtration by Whatman filter paper (number 1). The filtrate was concentrated in a thermostatically regulated water bath at a controlled temperature of 44°C for about 40 mins, after which it was placed in an air tight container and stored in the refrigerator.

**Experimental animals and design**

Forty-five adult wistar rats with weights ranging from 180-220g were purchased from pharmacology animal house and housed in plastic cages for experimental purposes. They were fed with standard rats feed (growers mash), distilled water and acclimatized for a period of 14 days with a constant temperature of 26 ± 2°C and 12 hours’ alternates dark and light cycles. The animals were handled in accordance with the guidelines of the Institutional Animal Ethics Committee of the Department of Science Laboratory Technology, University of Benin. The wistar rats were randomly selected and divided into nine (9) groups of five (5) rats in each group as follows:

- **Group 1 (control):** Animals received distilled water daily.
- **Group 2:** Animals received 8 mg/kg of Cadmium.
- **Group 3:** Animals received 15 mg/kg of Lead.
- **Group 4:** Animals received 8 mg/kg of Cadmium + 100 mg/kg of ethylenediamine tetracetic acid (EDTA)
- **Group 5:** Animals received 15 mg/kg of Lead + 100 mg/kg of ethylenediamine tetracetic acid (EDTA)
- **Group 6:** Animals received 8 mg/kg of cadmium + 250 mg/kg of CSPE.
- **Group 7:** Animals received 8 mg/kg of cadmium + 500 mg/kg of CSPE.
- **Group 8:** Animals received 15 mg/kg of lead + 250 mg/kg of CSPE.
- **Group 9:** Animals received 15 mg/kg of Lead + 500 mg/kg of CSPE.

The body weight of the animals was recorded at seven-day intervals. EDTA was dissolved in water and administered to the rats with the aid of an oral feeding gavage. At the end of the 28 days’ experimental period, rats were fasted overnight and sacrificed after anesthetization by chloroform inhalation, then their blood was collected and liver and kidney excised.

**Blood collection and haematological analysis**

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activity. Using a standard curve, the concentration of SOD was determined at 505 nm and represented as U/mg protein (Rasyidah et al., 2014).

Glutathione Peroxidase (GPx): The activity of GPx, which catalyzes the destruction of GSH by hydrogen peroxide, was measured and expressed as U/mg protein. GSH consumption will be assessed at 420 nm using a spectrophotometer (Flohe and Gunzler, 1984).

Catalase (CAT): Using the technique of Aebi et al the CAT activity was assessed (1984). The direct measurement of catalase’s \( \text{H}_2\text{O}_2 \) degradation is the reduction in absorbance at 240 nm. 650 ml of 50 mm phosphate buffer with a pH of 7.0 will be mixed with 50 ml of organ homogenate that has been diluted 50 times. 300 ml of 54 Mm \( \text{H}_2\text{O}_2 \) were added to start the reaction, and the absorbance change was monitored for 1 minute at 25 o C. A unit of catalase activity was established as the quantity of the enzyme required to break down 1 mol of \( \text{H}_2\text{O}_2 \) every minute. CAT was expressed in terms of U/mg protein.

Statistical analysis

All data were presented as mean ± standard error of mean (SEM) and ‘n’ represents the number of wistar rats per experimental group. Data were subjected to one-way analysis of variance (ANOVA) followed by Turkey’s multiple comparisons test. All data were analysed using Graph Pad Prism (UK) software version 6.0. \( p<0.05 \) indicates significance difference between compared data.

RESULTS

Sera antioxidant results

Table 1 shows the levels of SOD in homogenate of blood tissue as 5.05±0.24, 2.22±0.03, 5.19±1.15, 3.71±0.47 and 6.65±0.27 U/mg in control, lead, lead and EDTA, lead dose of 250 and 500 mg/kg of CSPE group respectively. There was a significant reduction in SOD level in the lead group, when compared to the control and other treatment groups (\( p<0.05 \)). The CSPE treated group enhanced the SOD level at a dose dependent manner.

CAT levels were 212.70±2.23, 208.20±13.71, 204.40±9.69, 211.10±10.33 and 216.20±5.58 U/mg in control, lead, lead and EDTA, lead dose of 250 and 500 mg/kg of CSPE respectively. The CSPE treated group enhanced the CAT level at a dose dependent manner. The blood tissue homogenates GPx levels were found to be 2.93±0.02, 1.30±0.10, 2.38±0.54, 1.65±0.16 and 2.40±0.41 U/mg in control, lead, lead and EDTA, lead dose of 250 and 500 mg/kg of CSPE group respectively. The treatment group of EDTA, 250 and 500 mg/kg of CSPE increased the GPx level when compared to the lead group which had the lowest GPx level.

Fig. 1: Effect of Citrus sinensis peel Extract on (MDA) serum level of albino rat treated /induced with lead toxicity on Lead induced toxicity.
MDA levels in homogenate of blood tissue were 23.80±2.40, 63.12±5.83, 35.89±4.40, 43.78±3.17 and 37.42±6.43 U/mg in control, cadmium, cadmium and EDTA, cadmium dose of 250 and 500 mg/kg of CSPE group respectively. The cadmium group had the highest level of MDA when compared to the control and other treatment group with a significant value of (p<0.05) as shown in Fig. 2 below.

Table 2 shows that the levels of SOD in homogenate of blood tissue was 5.05±0.24, 3.15±0.77, 4.87±1.06, 4.75±0.08 and 6.58±0.14 U/mg in control, cadmium, cadmium and EDTA, cadmium dose of 250 and 500 mg/kg of CSPE group respectively. In the cadmium group, the level of SOD significantly decreased compared to the control and other treatment groups (p<0.05). The CSPE treated group enhanced the SOD level at a dose dependent manner.

Table 1: Effect of Citrus sinensis peels on antioxidant serum level of albino rats induced with lead toxicity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± Standard Error of Mean</th>
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<tbody>
<tr>
<td></td>
<td>SOD (u/mg)</td>
</tr>
<tr>
<td>Control</td>
<td>5.05±0.24ab</td>
</tr>
<tr>
<td>Lead</td>
<td>2.22±0.03b</td>
</tr>
<tr>
<td>Lead + EDTA</td>
<td>5.19±1.15ab</td>
</tr>
<tr>
<td>Lead + CSPE (250mg/kg)</td>
<td>3.71±0.47ab</td>
</tr>
<tr>
<td>Lead + CSPE (500mg/kg)</td>
<td>6.65±0.27a</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error (n=5 per treatment). Dissimilar letters (a, b, c) are significantly different (p<0.05) from each other. CSPE= Citrus sinensis Peel extract, EDTA= Ethylenediamine tetra acetic acid.

Fig. 2: Effect of Citrus sinensis peel extract (CSPE) on MDA serum level of Albino rat induced with Cadmium toxicity.

Table 2: Effect of Citrus sinensis peel on the levels of antioxidant enzymes on cadmium induced toxicity in wistar rats.

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The effect of CSPE on haematological parameters of wistar rat

The haematological effects of Citrus sinensis peels on the wistar rats exposed to lead and cadmium heavy metals are shown in Tables 3 and 4 respectively. It was discovered that the level of WBC, LYM, MON and GRAN increased in the lead group compared to other treated groups. However, the values of RBC and HCT in the lead group, though not significant, decreased when compared to other treated groups. These reductions were ameliorated by CSPE treated groups. In addition, a significant increase (p > 0.05) in LYM level was observed in the cadmium group when compared to the control.

Results of heavy metal analysis

In Fig. 3 below, the lead level in the blood showed a significant difference between the CSPE treated groups, the EDTA group and the lead group (p<0.05). In addition, a significant difference was observed in both CSPE treated groups, this shows that the effect of CSPE was dose dependent. The control group had minute concentration of lead, this can be attributed to the presence of trace and heavy metals in the feed.

Results of lead concentration in the Liver tissue showed high significant (p<0.05) difference between the lead group and the treated groups of EDTA, 250 and 500 mg/kg CSPE group. The lead level in the kidney showed a significant decrease (p<0.05) in lead in the 500 mg/kg CSPE group compared to the lead group and other treated groups (Fig. 3). The 500 mg/kg CSPE was observed to have the lowest lead concentration in all treated groups, and there was no significant difference when compared to the control group.

Fig. 3: Effect of CSPE on Blood, Liver, and Kidney, lead level in wistar rats induced with lead toxicity.
Table 3: Effect of CSPE on the haematological parameters of lead induced toxicity in wistar rats.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± Standard Error of Mean</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>WBC</td>
</tr>
<tr>
<td>Control</td>
<td>6.30±1.80a</td>
</tr>
<tr>
<td>Lead</td>
<td>8.45±2.65a</td>
</tr>
<tr>
<td>Lead + EDTA</td>
<td>7.65±1.25a</td>
</tr>
<tr>
<td>Lead + 250mg/kg CSPE</td>
<td>6.25±2.35a</td>
</tr>
<tr>
<td>Lead + 500mg/kg CSPE</td>
<td>4.70±0.30a</td>
</tr>
</tbody>
</table>

Table 4: Effect of CSPE on the haematological parameters of cadmium induced toxicity in wistar rats.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± Standard Error of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC</td>
</tr>
<tr>
<td>Control</td>
<td>6.30±1.80a</td>
</tr>
<tr>
<td>Cadmium</td>
<td>7.85±2.25a</td>
</tr>
<tr>
<td>Cadmium + EDTA</td>
<td>9.75±0.35a</td>
</tr>
<tr>
<td>Cadmium + 250mg/kg CSPE</td>
<td>6.40±1.40a</td>
</tr>
<tr>
<td>Cadmium + 500mg/kg CSPE</td>
<td>11.10±0.30a</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error (n=5 per treatment). Different letters (a, b, c) show significant differences (p<0.05) between groups. WBC= White blood cell, LYM= Lymphocyte, MON= Monocyte, GRAN= Granulocyte, RBC= Red blood cell, HGB= Haemoglobin, HCT= Haematocrit, PLT= Platelet.

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Cadmium concentration in the blood showed a significant decrease in the CSPE treated group in comparison with the cadmium group. (p<0.05). There was also a significant difference in both CSPE treated groups, this shows that the effect of CSPE was dose dependent (Fig. 4). However, there was no significant difference between the cadmium group and the EDTA group. The control group had minute concentration of cadmium, this can be attributed to the presence of trace and heavy metals in the feed and water.

Results of cadmium concentration showed no significant (p>0.05) difference between the cadmium group and the treated groups of EDTA, 250 and 500 mg/kg CSPE. Although, reduction in cadmium concentration was observed in all treated groups, especially that of 500 mg/kg CSPE.

The cadmium level in the kidney tissue showed a significant decrease (p<0.05) in cadmium in the 500 mg/kg CSPE group compared to the cadmium group and other treated groups. The 500 mg/kg CSPE was observed to have the lowest cadmium concentration in all treated groups, and there was no significant difference when compared to the control group.

DISCUSSION

The results obtained in this study shows the antioxidant protective and chelating properties of Citrus sinensis peel extract against lead and cadmium induced toxicity in wistar rats. The biochemical mechanisms involved in the toxicity was studied by measuring the levels of Malondialdehyde (MDA) and by testing the activities of basal antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). MDA is used as an indicator of oxidative stress formed by lipid peroxidation of polyunsaturated fatty acids. Results obtained showed that the administered lead and cadmium caused a significant increase of MDA level in the blood as shown in Figures 1 and 2 respectively. Bahrami et al., (2016) suggested that the increase production of Reactive Oxygen Species (ROS) during toxicity can present a threat to biomolecules by oxidation of proteins, impairment to nucleic acids, and causing peroxidation of lipids. Studies conducted by Ndubisi et al., (2020) as well as Selmi et al., (2017) showed that CSPE inhibited lipid peroxidation in heavy metals induced rats. This present study was in agreement with findings of studies stated above, as our results showed that the CSPE was able to decrease the value of MDA to a level comparable to that of the control group in both lead and cadmium treated groups. The significant reduction in MDA levels between the CSPE treatment groups indicates that the free radical reduction and lipid peroxidation inhibitory effect is dose dependent.

Enzymatic antioxidant defence is one of the mechanism the body deploys to help guard the host cells against excess free radicals such as ROS. Examples of these antioxidants are SOD, CAT and GPx. SOD is a major enzyme that appears to act as the first line of defence against ROS, it breaks down superoxide radicals (O₂⁻) to produce hydrogen peroxide (Sudjarwo et al., 2017). The two primary enzymes involved in H₂O₂ detoxification are Glutathione peroxidase and Catalase. Hydrogen peroxide (H₂O₂) pose to be a significant ROS that results in oxidative stress. These enzymes, breakdown hydrogen peroxide.
peroxides and hydroperoxides to harmless molecules. According to our results in this study, there was no significant changes of Catalase in the lead administered group. However, in the groups administered with cadmium, expressions of catalase were significantly decreased (p<0.05). This is in correlation with studies conducted by Jun et al., (2003) who suggested that cadmium-mediated oxidative stress by hydrogen peroxide may trigger an increase in the reduction of the activity of antioxidant enzymes in conjunction with increased lipid peroxidation. The chelating effects of EDTA and CSPE were able to sustain the catalase expression in the groups they were administered. In addition, a significant difference (p<0.05) was observed in the 500 mg/kg group of CSPE, which not only sustained catalase activity in the blood, but also resulted in an increase beyond that of the control group. This indicates that the antioxidant activity of CSPE is dose dependent. Similar occurrence was observed for the GPx expressions in both lead and cadmium groups, where the levels of GPx decreased in contrast to the control group. CSPE was able to ameliorate this effect in the treatment groups, studies suggest that this might be due to its ability to reduce free radical accumulation and ROS in the blood. Bearing on the effects of toxic metals on oxidative stress condition, the level of SOD in lead and cadmium were decreased drastically to 2.22±0.03 U/mg and 3.15±0.77 U/mg respectively. CSPE treatment significantly and dose-dependently decreased free radical generation in lead induced wistar rats, thus, increasing the SOD expression in the treated groups. The same was the case for the SOD tests carried out on cadmium induce rats. Newairy and Abdou (2009) reported that lead toxicity causes numerous deleterious effects in organisms and also influences haematological parameters. Studies conducted by Bersenyi et al., (2003) shows that lead exposure in rabbits caused a significant reduction in red blood cells (RBC) and haematocrit (HCT) values. These findings suggest that red blood cells are susceptible to oxidative injury and lipid peroxidation that occurs in the membrane of RBC than other types of cells. This study revealed that HCT level decreased significantly (p<0.05) to approximately 30 % compared to the control group. The values of WBC in the blood, increased in the group administered lead. Similarly, there was an increase in the LYM, MON, and GRAN level in the lead and EDTA treatment groups as shown in Table 3. In the case of rats administered with cadmium, the number of WBC increased significantly in the cadmium and CSPE treatment groups.

Although there was a reduction in the levels of MON and GRAN of the cadmium group, it was ameliorated by the 500 mg/kg dose of CSPE. RBC, Hb, HCT and PLT values were similar in the control and 250 mg/kg CSPE groups, however, the levels were higher in the 500 mg/kg CSPE group. This finding correlates with studies conducted by Nazima et al., (2016) suggesting that some antioxidants show protective activity against breakdown of RBC by cadmium. Findings from this study revealed the phytochelating effect of CSPE on lead and cadmium as evidence by the significant decreased levels of these metals in blood, liver and kidney in the treated groups compared to the toxic group.

CONCLUSION

The results from this study have shown that Citrus sinensis peel extract, even at low doses, possesses chelating and antioxidant properties capable of reducing the deleterious effects of lead and cadmium induced toxicity in blood, kidney, and liver. Further studies are needed to evaluate long term usage of Citrus sinensis peel in metal detoxification as well as understanding its mechanism of action.

Declaration of interest

The authors declare that they have no conflict of interests to declare. All authors read and approved the manuscript.

Author contribution

EOC conceived and designed the study, wrote the original draft, and revised the paper. OEP was involved in material preparation, data collection, analysis and writing of the paper, AAB provided reagent and laboratory analysis, EJU, AON, OPO and ECM were involved in the laboratory analysis and interpretation of the data. All authors read and approved the final manuscript.

REFERENCES


