Viscum album prevents haematological changes, electrolyte imbalance, changes in liver function enzymes and histological alterations in some selected tissues in cadmium chloride-intoxicated rats

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Background: The use of Viscum album to treat different diseases is popular in the practise of alternative medicine. We investigated the ability of the aqueous extract of V. album to protect against the toxic effects of cadmium.

Methods: Thirty rats used for the experiment were treated as follows; Group 1 – no cadmium or extract. Group 2–10 mg/kg body weight of cadmium chloride. Group 3–10 mg/kg body weight of cadmium chloride and 200 mg/kg body weight of aqueous extract of V. album. Group 4–10 mg/kg body weight of cadmium chloride and 400 mg/kg body weight of aqueous extract of V. album. Group 5–10 mg/kg body weight of cadmium chloride with 800 mg/kg body weight of aqueous extract of V. album. Group 6–10 mg/kg body weight of cadmium chloride and atorvastatin (100 mg/kg body weight).

Results: Apart from WBC and platelets, other haematological parameters and electrolytes, urea and creatinine levels were not significantly affected by the administration of cadmium chloride along with the aqueous extract of V. album. Treatment with the extract caused significant decreases in the hepatosomatic index, cardiosomatic index, and increase in renosomatic index of the test rats. It also resulted in significant (P < 0.05) decrease in AST level. Histological report also shows that treatment with the extract restored the normal myocardium and vascular architecture of the heart, normal portal and vascular architecture of the liver and normal glomerular and tubular architecture of the kidney, in the cadmium-intoxicated experimental rats.

Conclusion: V. album protects against the toxic effects of cadmium chloride.

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1. Introduction

Cadmium is a heavy metal that is commonly used in the manufacturing industries and in agricultural activities. Its toxicity and ability to accumulate in living organisms is of public health impor-

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tion with sulfhydryl groups thereby modifying the activity of many enzymes (Veličković et al., 2013). The activities of cadmium involve the interaction with DNA repair mechanisms, cell proliferation and differentiation, induction of apoptosis and generation of reactive oxygen species (Rafati et al., 2017; Rani et al., 2014). It has been reported to predominantly accumulate in the kidney and liver, as well as the bone and placenta. Exposure to cadmium can present with early signs of renal dysfunction, tubular lesion, proteinuria and calcium loss (Rafati et al., 2017). Patients with cadmium toxicity have been reported to be managed by chemical decontamination using ethnomedical-based chelation therapies. These therapies often involve the use of medicinal plants like *Viscum album* (Rafati et al., 2017).

Medicinal plants are naturally endowed with varieties of phytochemicals or active principles which are important in alternative medical practices. These medicinal constituents (active principles) are known to interfere with and modify metabolic reactions and organ functions within the biological system. Mistletoe (*Viscum album*) is a medicinal plant that has gained popularity in its use in alternative medical practices among indigenous people in different cultures around the world, due to its rich content of useful active principles. *V. album* is part of the family of plants known as Loranthaceae (Polhill and Wiens, 1998). It has been reported to contain several biologically active substances that are either hydrophilic or lipophilic in nature (Orhan et al., 2006; Amer et al., 2013; Nghiém et al., 2013; Singh et al., 2016). Examples are lectins, lipids, polysaccharides, proteins, peptides, hetero-dimeric glycoproteins (mistletoe lectins I – III), viscotoxins, flavonoids, alkaloids, triterpenes, polyphenols, phytosterols, vesicles, cyclolits, amino acids and amines (Orhan et al., 2006; Amer et al., 2013; Nghiém et al., 2013; Singh et al., 2016). Some trace mineral elements such as potassium, sodium, calcium, manganese, phosphate, silica, nickel, selenium, magnesium and zinc have also been reportededly contained in *V. album* (Singh et al., 2016). Experimental and clinical studies have shown that *V. album* exhibits immunomodulatory, anti-hypertensive, anti-oxidant, cytotoxic, anti-tumor, anti-inflammatory, anti-diabetic, anti-microbial and sedative activities (Singh et al., 2016). It is widely used in various cultures in almost every continent in the treatment of various ailments like hypertension, asthma, epilepsy, infertility, cancer, diabetes, etc., and also as a diuretic agent and a tranquilizer (Burkill, 1985; Bocci, 1993; Adodo, 2004; Yakubu, 2009; JadHAV et al., 2010).

In this study, we examined the ability of *V. album* to protect against the toxic effects of cadmium chloride using experimental rat models. The effects of the co-administration of cadmium chloride and *V. album* in the experimental rats were studied by examining the changes in their haematological parameters; electrolyte, urea and creatinine levels; liver function enzyme activities; and histological alterations in key tissues (heart, liver and kidney). These effects were compared with that of the standard drug, atorvastatin.

2. Materials and methods

2.1. Plant collection and extract preparation

The leaves of *Viscum album* (Mistletoe) were harvested from a local garden within the university community and identified by a plant taxonomist from the department of Plant Biology and Biotechnology, University of Benin, Nigeria. The leaf samples were crushed into fine portions and air-dried (at room temperature) for about a week. The crushed leaves were then oven-dried at a temperature of 40 °C for about 30 min and subsequently ground into fine powder (using the British Milling Machine). About 100 g of the powdered leaf sample was macerated in 1.4 L of water for 24 h at room temperature with constant shaking and stirring (on a magnetic stirrer). The resulting mixture was filtered using a Whatman filter paper (Azmir et al., 2013). The filtrate was concentrated over hot water bath using crucibles to obtain a paste like extract which was then preserved in a sample bottle and kept inside a refrigerator for further use.

2.2. Experimental animals and experimental design

Thirty mature experimental rats of the Wister strain weighing between 180 and 250 g were used for the experiment. The rats were procured from the Animal House of the Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria. They were allowed to get used to the new environment for 2 weeks before commencement of the experiment. During this period, the rats were allowed free access to standard animal feed (Top feeds grower mash) and clean water *ad libitum*. The weight of each animal in each group was checked weekly to get the required cumulative weight. After the period of acclimatization, the rats were randomly assigned into six groups (1 to 6) consisting of 5 rats each. The different groups were treated as follows; **Group 1** - rats served as control. They were neither given cadmium nor administered with the extract. **Group 2** - rats were administered daily with 10 mg/kg body weight of cadmium chloride only. **Group 3** - rats were administered daily with 10 mg/kg body weight of cadmium chloride and also treated daily with 200 mg/kg body weight of aqueous extract of *V. album* (*Low dose*). **Group 4** - rats were administered daily with 10 mg/kg body weight of cadmium chloride and also treated daily with 400 mg/kg body weight of aqueous extract of *V. album* (*Intermediate dose*). **Group 5** - rats were administered daily with 10 mg/kg body weight of cadmium chloride and also treated daily with 800 mg/kg body weight of aqueous extract of *V. album* (*High dose*). **Group 6** - rats were administered daily with 10 mg/kg body weight of cadmium chloride and 100 mg/kg body weight of the standard drug (atorvastatin). The extract, cadmium chloride and atorvastatin were all administered simultaneously and orally with an orogastric tube to the experimental rats for a period of 60 days.

At the end of the series of treatment, the rats were weighed and then euthanized. Blood samples were collected with sterile syringes into heparinised sample bottles, for biochemical analysis. The liver, heart and kidneys of each rat were harvested and immediately fixed on 10% formalin to avoid autolysis and subsequently transported to the histopathology laboratory of University of Benin Teaching Hospital (UBTH) for tissue processing. All the animal experiments were conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and the approved Guidance of the Research and Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (CMR/REC/2014/57).

2.3. Measurements

Direct measurements of the basic parameters (body weight, hepatic weight, cardiac weight and renal weight) of the experimental rats were performed at the end of the experiment, using a Sartorius analytical balance (Sartorius Mechatronics T&H GmbH, Hamburg, Germany). The data obtained were used to calculate the respective indices as follows;

\[
\text{Hepatosomatic Index (HSI)} = \frac{\text{Weight of Liver (g)}}{\text{Weight of Body (g)}} \times 100
\]

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\text{Cardiosomatic Index (CSI)} = \frac{\text{Weight of Heart (g)}}{\text{Weight of Body (g)}} \times 100
\]
Renosomal Index (RSI) = \frac{\text{Weight of Kidney (g)}}{\text{Weight of Body (g)}} \times 100

2.4. Ethical concern and approval

The procedures and handling of the experimental rats were in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research purposes (Buzek and Chastel, 2010). Also, in accordance with the approved Guidance of the Research and Ethics Committee of the College of Medical Sciences, University of Benin (CMR/REC/2014/57).

2.5. Haematology

The haematological parameters of the test rats were analysed by aspirating about 2mls of the blood samples into the chamber of the Human Automated Hematology System Analyzer (ERMA PCE 210, ERMA, Japan). The samples were then diluted with isotonic saline solution. The levels of White Blood Cells (WBC), Lymphocytes, Mid-range Absolute Count (MID), Granulocytes, Red Blood Cells (RBC), Haemoglobin (Hb), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW-CV), Platelets, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Procalcitonin in the blood samples were subsequently determined.

2.6. Blood electrolyte, urea and creatinine determination

Potassium and sodium were determined as described by Tietz (1987), using a flame photometer (Eppendorf Flame Photometer AFM 5051, Germany). Chloride was determined as described by Skeggs and Hochstrasser (1964). Alkaline phosphatase concentration was determined as described by Van-Slyke et al. (2000), while the determination of urea was by the method described by Weatherbum (1967) (Urease Berthelot method). The determination of Creatinine was in accordance with the protocol described by Bartels and Bohmer (1972). Chloride, bicarbonate, urea and creatinine assays were done using standard assay kits from Randox (Randox Laboratory, UK).

2.7. Determination of the activities of the liver function enzymes in the blood

Aspartate aminotransferase (AST) activity and Alanine aminotransferase (ALT) activity were determined by the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) Activity was determined as described by Kochmar and Moss (1976). While Total Bilirubin (TB) and Conjugated Bilirubin (CB) were determined as described by Jendrassik and Grof (1938); AST, ALT and Bilirubin assays were done using standard assay kits from Randox (Randox Laboratory, UK) while ALP assay was done using standard assay kit from TECO (TECO Diagnostic, USA).

2.8. Histological procedures – Tissue processing and staining

Following the fixation of the harvested tissue in 10% formal saline, the tissues were processed and stained as previously described by our group (Oigbochie et al, 2018; Oigbochie et al, 2019; Innih et al, 2020; Innih et al, 2021). The photomicrograph sections of the tissues were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCCS50) attached. Digital photomicrographs of the tissue sections were taken at x40 and x100 objective magnifications.

2.9. Statistical analyses

The data obtained were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25). One-way analysis of variance (ANOVA) was carried out and data were presented as Mean ± SEM. LSD post-hoc test was used. Values of p < 0.05 were considered significant. The statistical values of some of the data obtained were converted into graphical representations in the form of bar charts.

3. Results

3.1. Aqueous extract of V. album stabilises the haematological parameters of the experimental rats under cadmium chloride toxicity

As indicated in Table 1, administration of cadmium chloride to the experimental rats resulted in significantly p < 0.05 lower WBC and significantly p < 0.05 higher Platelets in the untreated group (group 2) as compared to the control group. However, the WBC level was not significantly affected after treatment with 400 and 800 mg/kg body weight (groups 4 and 5, respectively), as compared to the untreated group (group 2). When compared to the control group (group 1), the RBC, haemoglobin, haematocrit and MCHC were not significantly affected after the administration of cadmium chloride alone (group 2). Apart from treatment with atorvastatin (group 6), treatment with the different doses of the extract (low, intermediate and high dose) along with cadmium chloride did not cause any significant changes in the RBC, haemoglobin, haematocrit and MCHC of the test rats. Other haematological parameters (lymphocytes, MID, granulocytes, MCV, MCH, RDW-CV, MPV, PDW, procalcitonin) were not significantly affected after the administration of either cadmium chloride, Viscum album extract, atorvastatin or their combination. Thus, apart from the WBC and platelets, other haematological parameters assessed were not significantly affected by either the administration of cadmium chloride alone or its administration along with the extract (irrespective of the dose).

3.2. Aqueous extract of V. album maintains the electrolyte balance, concentration of urea and creatinine in the experimental rats under cadmium chloride toxicity

In Table 2, when compared to the control group (group 1) and untreated group (group 2), the Na⁺ concentration was significantly (p < 0.05) higher in the group administered with cadmium chloride and treated with 400 mg/kg of the aqueous extract of V. album (group 4). While administration of cadmium chloride and treatment with other doses of the extract, as well as atorvastatin did not result in any significant difference in the Na⁺ concentration of the respective groups as compared to the control and untreated group. The K⁺ and HCO₃⁻ concentrations of the experimental rats were not significantly affected by either the administration of cadmium chloride alone or its administration along with the different doses of the extract, as well as its administration along with atorvastatin. Cadmium chloride administration caused significant (p < 0.05) increases in the concentration of Cl⁻ in all the test groups, as compared to the control group. The increases in Cl⁻ were maintained in all the test groups irrespective of the treatments (either with the different doses of the extract or atorvastatin). Also, when compared to the control group (group 1) and untreated group (group 2), the urea and creatinine levels were significantly (p < 0.05) higher in the group administered with cadmium chloride and treated with 800 mg/kg of the aqueous extract of V. album (group 5). Apart from the significant increase in Cl⁻, the administration of cadmium chloride and treatment with
Effects of *Viscum album* on the Haematological Parameters of the Experimental Rats with Cadmium Chloride Toxicity.

|                  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | F   | P-value |
|------------------|---------|---------|---------|---------|---------|---------|-----|---------|
| WBC (10^3/μL)    | 7.07 ± 0.67 | 4.95 ± 0.82* | 5.03 ± 0.52 | 4.76 ± 0.53* | 4.96 ± 0.57* | 5.28 ± 0.39 | 1.646 | 0.199  |
| Lymphocytes (%)  | 92.83 ± 0.72 | 93.38 ± 0.11 | 94.00 ± 1.25 | 92.82 ± 1.26 | 93.42 ± 0.79 | 92.23 ± 1.45 | 0.255 | 0.932  |
| MFD (%)          | 5.50 ± 0.61 | 4.93 ± 0.95 | 4.33 ± 0.74 | 4.46 ± 0.49 | 4.94 ± 0.63 | 5.70 ± 1.02 | 0.475 | 0.790  |
| Haemoglobin (g/dL) | 11.47 ± 0.05 | 12.23 ± 1.86 | 14.43 ± 0.35 | 14.20 ± 0.60 | 14.16 ± 0.63 | 15.35 ± 0.22* | 2.284 | 0.090  |
| Haematocrit (%)  | 31.13 ± 0.95 | 32.70 ± 2.27 | 33.23 ± 0.43 | 34.18 ± 0.75 | 34.14 ± 0.42 | 35.08 ± 0.55* | 1.403 | 0.270  |
| Mean Corpuscular Volume (MCV) (fL) | 47.77 ± 2.02 | 56.30 ± 6.31 | 49.73 ± 0.19 | 49.86 ± 0.31 | 50.24 ± 0.39 | 50.13 ± 0.42 | 1.130 | 0.380  |
| Mean Corpuscular Haemoglobin (MCH) (pg) | 18.27 ± 0.52 | 21.3 ± 4.26 | 21.53 ± 0.20 | 20.66 ± 0.92 | 20.74 ± 0.85 | 21.83 ± 0.20 | 0.353 | 0.873  |

Data are presented as Mean ± SEM. Values with * along the rows are significantly p < 0.05 different from the control group. Group 1 - Control. Group 2 - Cadmium chloride only. Group 3 - Cadmium chloride and 200 mg/kg body weight of aqueous extract of *Mistletoe* (Low dose). Group 4 - Cadmium chloride and 400 mg/kg body weight of aqueous extract of *Mistletoe* (Intermediate dose). Group 5 - Cadmium chloride and 800 mg/kg body weight of aqueous extract of *Mistletoe* (High dose) Group 6: Cadmium chloride and 100 mg/kg body weight of Atorvastatin (standard drug).

Table 2

Effects of *Viscum album* on the Electrolytes, Urea and Creatinine of the Experimental Rats with Cadmium Chloride Toxicity.

|                  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | F   | P-value |
|------------------|---------|---------|---------|---------|---------|---------|-----|---------|
| Na⁺ (mmol/L)     | 142.25 ± 0.75 | 142.00 ± 2.35 | 139.33 ± 0.67 | 150.00 ± 2.59* | 146.80 ± 0.86 | 146.20 ± 0.73 | 5.293 | 0.003  |
| K⁺ (mmol/L)      | 5.65 ± 0.71 | 4.70 ± 0.24 | 5.40 ± 0.61 | 6.06 ± 0.32 | 5.86 ± 0.29 | 6.44 ± 0.35 | 2.038 | 0.117  |
| HCO₃⁻ (mmol/L)   | 31.38 ± 0.71 | 31.45 ± 1.25 | 31.33 ± 0.71 | 31.20 ± 1.45 | 32.40 ± 1.90 | 32.00 ± 1.75 | 4.247 | 0.950  |
| Cl⁻ (mmol/L)     | 105.00 ± 1.38 | 105.00 ± 1.78 | 105.33 ± 2.19* | 108.90 ± 0.86* | 109.80 ± 0.66* | 109.80 ± 0.66* | 49.864 | 0.000  |
| Urea (mg/dL)     | 15.25 ± 10.48 | 6.17 ± 4.70 | 5.40 ± 0.53 | 5.10 ± 0.29 | 5.00 ± 0.29 | 4.90 ± 0.29 | 3.941 | 0.013  |
| Creatinine (mg/dL) | 0.80 ± 0.07 | 0.85 ± 0.06 | 0.90 ± 0.00 | 0.85 ± 0.05 | 1.06 ± 0.04* | 0.72 ± 0.05 | 0.063 | 5.237  |

Data are presented as Mean ± SEM. Values with * along the rows are significantly p < 0.05 different from the control group. Group 1 - Control. Group 2 - Cadmium chloride only. Group 3 - Cadmium chloride and 200 mg/kg body weight of aqueous extract of *Mistletoe* (Low dose). Group 4 - Cadmium chloride and 400 mg/kg body weight of aqueous extract of *Mistletoe* (Intermediate dose). Group 5 - Cadmium chloride and 800 mg/kg body weight of aqueous extract of *Mistletoe* (High dose) Group 6: Cadmium chloride and 100 mg/kg body weight of Atorvastatin (standard drug).

*V. album* extract and atorvastatin did not significantly affect the electrolyte, urea and creatinine levels of the experimental rats.

3.3. Treatment with the intermediate dose of the aqueous extract of *V. album* caused significant decreases in the body weight, cardiac weight and increase in renal weight, while treatment with the high dose caused significant decrease in the hepatic weight of the test rats under cadmium chloride toxicity

In Fig. 1; Chart (A) shows the initial and final body weights of the rats. In all the groups examined, the final body weights were significantly (p < 0.05) higher than the initial body weights. However, there were no significant differences in the final body weights between the different groups. Although that of the group given the intermediate dose of the extract (group 4) tend to be lower as compared to the other groups. Chart (B) which shows the cardiac weights of the rats in the various groups, indicates that there were decreases in the cardiac weights of the rats in all the test groups as compared to the control group. The decrease is significantly (p < 0.05) lower in group 4 (administered with cadmium chloride and treated with the intermediate dose of the aqueous extract of *Mistletoe*). In Chart (C), the hepatic weight was shown to be lower in all the test group as compared to the control group. However, treatment with the high dose of the extract (group 5) resulted in a significant (p < 0.05) decrease in the hepatic weight of the test group as compared to the control and other test groups. The renal weights of the test rats, as indicated in chart (D), shows decreases as compared to the control after cadmium chloride administration. Treatment with the intermediate dose of the extract (group 4) and atorvastatin (group 6) resulted in significant (p < 0.05) increases in the renal weight as compared to the untreated group (group 2).

3.4. Treatment with aqueous extract of *V. album* caused significant decreases in the hepatosomatic index, cardiosomatic index, and increase in renosomatic index of the test rats under cadmium chloride toxicity

In Fig. 2; Chart (E) shows the hepatosomatic index of the experimental rats. The hepatosomatic index was significantly (p < 0.05) lower in the rats administered with cadmium chloride alone (group 2) and the rats co-administered with cadmium chloride and high dose of the extract (group 5), as compared to the other groups. The resultant effects of the co-administration of the low and high doses of the extract, as well as the standard drug, were not significantly different from that of the control group. Chart (F) shows the cardiosomatic index of the experimental rats. There were no significant differences in the cardiosomatic index between the various groups, except in group 4 where the rats were administered with cadmium chloride and intermediate dose of *V. album*. Administration of the intermediate dose of the extract along with cadmium chloride resulted in a significant (p < 0.05) decrease in the cardiosomatic index as compared to the other treatments. Chart (G) shows the renosomatic index of the experimental rats. The administration of cadmium chloride alone resulted in a significant decrease in the renal weight compared to the control as shown in chart (D).
significantly (p < 0.05) lower renosomatic index as compared to the control and other groups. Treatments with the extracts and atorvastatin, along with the administered cadmium chloride also resulted in significantly (p < 0.05) lower renosomatic index in all the test groups, as compared to the control, but higher as compared to group 2 (cadmium only).

3.5. Aqueous extract of V. album possibly ameliorates the toxicity of cadmium chloride to the liver of the experimental rats

Fig. 3 shows the effects of the aqueous extract of V. album on the AST, ALP, ALT, Total and Conjugated Bilirubin of the test rats under cadmium chloride toxicity. Administration of cadmium chloride...
only resulted in significant ($p < 0.05$) decreases in the levels of AST, ALP and ALT of the experimental rats, as compared with the control and other test groups. Treatment with the aqueous extract of *V. album* or atorvastatin along with cadmium chloride administration resulted in comparative increases in the ALP level, with that of the intermediate dose of *V. album* been significant ($p < 0.05$). Treatment with the low dose of *V. album* resulted in significant ($p < 0.05$) decrease in AST level, as compared to other groups, while treatment with the intermediate or high dose or atorvastatin resulted in non-significant increases in the AST level. Treatment with the low, intermediate or high dose of *V. album* along with cadmium chloride administration resulted in significantly ($p < 0.05$) higher ALT level as compared to the other groups. However, administration of cadmium chloride alone or along with extract or atorvastatin treatment did not result in any significant differences in the levels of total and conjugated bilirubin of the experimental rats.

3.6. Aqueous extract of *V. album* shows no significant effect on the total protein and albumin concentration, but affected the globulin concentration of the experimental rats under cadmium chloride toxicity

As indicated in Fig. 4, the effects of the administration of cadmium chloride along with the different doses (low, intermediate or high) of the aqueous extract of *V. album* on the total protein and albumin concentrations were not significantly different across the groups. However, the globulin concentration was significantly ($p < 0.05$) higher in groups 2 (cadmium chloride only), 3 (cadmium chloride and low dose extract) and 4 (cadmium chloride and intermediate dose extract), as compared to the control and other groups.

3.7. Aqueous extract of *V. album* prevents perivascular infiltrations of inflammatory cells but not vascular ulceration in the heart of the test rats under cadmium chloride toxicity

Fig. 5 shows the histological sections of the heart of the experimental rats. The heart of the control rat (group 1) shows normal bundles of myocardial fibres (A), normal interstitial space (B) and normal coronary artery (C). The heart of the rat given only cadmium chloride (group 2), shows vascular ulceration (A) and perivascular infiltrates of inflammatory cells (B). The section of the heart of the rat given cadmium + 200 mg extract (group 3) shows normal myocardium (A) and focal vascular occlusion (B). Rat given cadmium + 400 mg extract (group 4) shows normal myocardium (A) and vascular architecture (B). Rat given cadmium + 800 mg extract (group 5) also shows normal myocardium (A) and focal vascular ulceration (B). The section of the heart of the rat given cadmium + atorvastatin (group 6) shows normal myocardium (A) and normal vascular architecture (B).

3.8. Aqueous extract of *V. album* prevents vascular congestion, perivascular infiltrations of inflammatory cells and vascular ulceration in the liver of the test rats under cadmium chloride toxicity

Fig. 6 shows the histological sections of the liver of the experimental rats. The liver of the control rat (group 1) shows normal hepatocytes (A), normal sinusoids (B) and normal central vein (C). The section of the liver of the rat given cadmium only (group 2) shows vascular congestion (A), heavy perivascular infiltrates of inflammatory cells (B) severe vascular ulceration (C). Liver section of rat given cadmium + 200 mg extract (group 3) shows normal hepatocyte (A) and normal portal architecture (B). Liver section of rat given cadmium + 400 mg extract (group 4) also shows normal hepatocyte (A) and normal portal architecture (B). The histological section of the rat given cadmium + 800 mg extract (group 5) shows normal hepatocyte (A) and portal vascular architecture (B). Section from liver of the rat given cadmium + atorvastatin (group 6) also shows normal hepatocyte (A) and portal vascular architecture (B).

3.9. Aqueous extract of *V. album* prevents perivascular infiltrations of inflammatory cells, vascular ulceration and interstitial oedema, but cause interstitial congestion in the kidney of the test rats under cadmium chloride toxicity

Fig. 7 shows the histological sections of the kidney of the experimental rats. The histological section of the kidney of the control rat (group 1) shows normal glomerulus (A), normal tubules (B) and normal interstitial space (C). The section of the kidney of the rat given cadmium only (group 2) shows perivascular infiltrates of inflammatory cells (A), vascular ulceration (B) and interstitial oedema (C). Kidney section of the rat given cadmium + 200 mg extract (group 3) shows normal glomerulus architecture (A), normal tubular architecture (B) and active interstitial congestion (C). Kidney section of rat given cadmium + 400 mg extract (group 4) also shows normal glomerular architecture (A), normal tubular architecture (B) and active interstitial congestion (C).
architecture (B) and active interstitial congestion (C). The histological section of the kidney of the rat given cadmium + 800 mg extract (group 5) shows normal tubular architecture (A), normal glomerular architecture (B) and active interstitial congestion (C). The section of the kidney of the rat given cadmium + atorvastatin (group 6) shows normal tubular architecture (A), normal glomerular architecture (B) and active interstitial congestion (C).

4. Discussion

Findings from this study show that *Viscum album* protects against the toxic effects of cadmium. The aqueous extract of *Viscum album* was able to stabilise the levels of the haematological parameters under examination, despite the toxic effect of cadmium chloride as indicated by the group given only cadmium chloride. It has been reported that cadmium has the capacity to cause the destruction of erythrocytes thereby reducing the level of PCV in different animal models (Omotoso et al., 2016). Our results also show increase in the number of platelets and decrease in the WBC of the experimental rats due to the effect of cadmium chloride. However, treatment with different doses of the aqueous extract of *Viscum album* possibly ameliorated the toxic effect of cadmium chloride, as indicated by the reduction in the destruction of the erythrocytes of the experimental rats. This is evident by the increase in the level of RBC in a similar fashion as observed in the group treated with the standard drug, atorvastatin. Other haematological parameters like the lymphocytes, MID, granulocytes, MCV, MCH, RDW-CV, MPV, PDW, procalcitonin were not significantly affected after the administration of either cadmium chloride, *Viscum album* extract, atorvastatin or their combination, thus ruling out any possibility of inflammation. The increase observed in the level of Cl− may be linked to the administration of the compound, cadmium chloride,
where Cl\(^-\) is a major component. Other electrolytes, like K\(^+\) and HCO\(_3^-\) ions were not significantly affected after the administration of cadmium chloride and treatment with the different doses of the aqueous extract of *V. album*. The stability of these electrolytes, as observed, is important in the maintenance of homeostasis within the biological system. Similarly, the urea and creatinine levels of the experimental animals were not significantly affected, except for the group treated with the highest dose (800 mg/kg body weight) of the extract. The increase in the concentration of urea in the group treated with the highest dose of the extract may be linked to the proteins, amino acids and amines contents of the extract, as previously reported (Orhan et al., 2006; Amer et al., 2013; Nhiem et al., 2013; Singh et al., 2016). Thus, the treatment with the different doses of the extract along with the administration of cadmium chloride did not significantly affect the urea, creatinine and electrolyte concentrations of the experimental rats.

Lu et al. (2013) earlier reported that exposure to cadmium does not affect body weight. In line with this, our findings also indicated significant weight gain across the groups despite the administration of cadmium chloride. However, we observed significant decreases in the body and cardiac weight, as well as increase in renal weight in the group treated with the intermediate dose of the extract. This may indicate excess strain or workload on the kidney as a result of its increased filtration or excretory activities, which is possibly due to increased catabolic products from the metabolism of cadmium and the extract. But, treatment with the high dose of the extract resulted in a significant decrease in the hepatic weight of the test rats. These may indicate a protective effect of the extract on the hepatocyte or reduced energy metabolism in the test animals. Thus, it may be suggested that the aqueous extract of *V. album* is effective in the maintenance or reduction in the organ weight and body weight, by stabilising energy metabolism in the biological system. Considering the ratio of the weight of the liver to that of the body, as a measure of the energy reserve of the test rats, the hepatosomatic index were significantly reduced after treatment with the extract. This may point at the possible effectiveness of *V. album* in the reduction of excess energy reserve for maintenance of steady body and organ weights. Or better still, a diversion of energy for growth away from the liver so as to be able to combat the toxic effect of cadmium. The observed decrease in the cardiosomatic index may also indicate a possible shift or diversion of energy, necessary for the growth of the cardiac tissues, away from the heart for the purpose of fighting or ameliorating the toxic effects of cadmium. On the other hand, the renosomatic index significantly increased in the test rats. The kidney which is involved in the production and filtration of blood,
as well as immune responses might have increased in weight, as observed, as a mechanism to cope with the toxic effects of cadmium. This may occur by increase in the haemolytic capacity of the kidney. As indicated above, the effect of the aqueous extract of *V. album* could be seen as it was able to reduce the destruction of erythrocytes in the treated groups.

The administration of cadmium chloride to the test rats possibly resulted in increased protein synthesis within the liver, as indicated by decreases in the liver function enzymes in the serum. The increased synthesis of proteins by the liver may be a coping mechanism against the toxic effects of cadmium or a detoxification process, with retention of the enzymes within the hepatocytes. This process was however reversed or prevented by the aqueous extract of *V. album* which, upon treatment, resulted in decreases in the activities of the enzymes within the hepatocytes and increases in their activities in the serum, although within the normal range. On the contrary, the levels of total and conjugated bilirubin were not significantly affected irrespective of the treatment, whether administration of cadmium chloride alone or along with the extract or atorvastatin. This is also in support of the reduction in the destruction of RBC occasioned by the administration of cadmium and the extract. However, the globulin concentration was significantly higher after the co-administration of cadmium and the extract. This increase may be as a result of the increased protein synthesis in the hepatocytes, as initially stated, as a coping mechanism against the toxic effects of cadmium. The high dose of the extract proved to be more effective in this case as it was able to bring about a reduction in the globulin concentration of the test rats.

Histological analysis of sections of the heart of the test rats shows that cadmium caused vascular ulceration and perivascular infiltrates of inflammatory cells. Vascular damage can occur through the generation of free radicals and their effect on endothelium and can cause an inflammatory reaction which triggers the release of inflammatory cells (Bhattacharyya et al., 2014). The group treated with atorvastatin, along with cadmium chloride administration, showed recovery as histological analysis showed normal myocardium and normal vascular architecture. Atorvastatin is a lipid-lowering medication that has been used in the primary and secondary prevention of coronary heart disease. The groups treated with *V. album*, along with cadmium administration, also showed restorative properties in a dose dependent manner. The animals treated with 400 mg/kg body weight and 800 mg/kg body weight of the extract showed complete restoration while the rats treated with 200 mg/kg of the extract showed normal myocardium with vascular occlusion. This finding is in consonance with reports indicating the cardioprotective/cardiorestorative effect of *V. album* (Poruthukaren et al., 2014; Montero et al., 2016). The Cardioprotective effect of *V. album* may be due to its antioxidant properties as previously reported. Numerous *V. album* extracts have been reported to show free radical-scavenging activity and protective effects against oxidative stress induced by free radicals, nitric oxide and superoxide anion (O$_2^-$) (Kim et al., 2017; Kusi et al., 2015). Our present study reveals that the aqueous extract of *V. album* prevents perivascular infiltrations of inflammatory cells. However, it did not prevent vascular ulceration in the heart of the cadmium-intoxicated test rats. Analysis of the histological sections of the liver of the cadmium-intoxicated test rats shows that the aqueous extract of *V. album* prevents vascular congestion, perportal infiltrations of inflammatory cells and vascular ulceration, caused by cadmium.

The protective effect of *V. album* was observed in the treated groups where the normal portal and vascular architectures of the hepatocytes were restored. The restorative effects of *V. album* in the hepatocytes may also be linked to its antioxidant and free radical scavenging ability. The effect is also similar, as observed in the histological sections of the kidney. Treatment with the aqueous extract of *V. album* prevented the perivascular infiltrations of inflammatory cells, vascular ulceration and interstitial oedema in the cadmium-intoxicated test rats. However, the extract also caused interstitial congestion in the kidney of the test rats, despite the restoration of the normal glomerular and tubular architecture. This may be connected with or responsible for the increase in the renosomal index, as discussed above. It simply points at the kidney’s fight against the toxic effects of cadmium on its tissues, with a complementary effort from the extract of *V. album*.

5. Conclusion

Our present study shows that the aqueous extract of *V. album* protects against the toxic effects of cadmium by stabilising the concentrations of the haematological parameters and electrolytes, as well as the activities of the liver function enzymes. Treatment with the extract also restored the normal myocardium and vascular architecture of the heart, normal portal and vascular architecture of the liver and normal glomerular and tubular architecture of the kidney, in the cadmium-intoxicated experimental rats.

Authors' contributions

SOI and TEL conceived, designed and performed the experiments; SOO and KO performed the analysis, interpretation of the data and prepared the draft of the manuscript. All authors have reviewed and approved the final draft of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval and consent to participate

The animal experiments were conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and the approved Guidance of the Research and Ethics Committee of the College of Medical Sciences, University of Benin (CMR/REC/2014/57). The procedures and handling of the experimental rats were also in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research purposes (Buzek and Chastel, 2010).

Consent for publication

Not Applicable.

Availability of data and material

Not Applicable
References

Adodo, A., 2003. Nature Power, A Christian Approach to Herbal Medicine. Benedictine Publication Nigeria. Edo State, pp. 103–111.

Amer, B., Jovik, O.J., Francis, C.W., Fossen, T., 2013. Novel GMH-derived natural products from European mistletoe (Viscum album). Pharm. Biol. 51 (8), 981–986.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafour, K., Norulaini, N.A.N., Omar, A.K.M., 2013. Techniques for extraction of bioactive compounds from plant materials: a review. J. Food Eng. 117 (4), 426–436.

Barany, E., 2015. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian Indigenous Medicinal Plants. J. Med. Food 13 (2), 444–451.

Bartels, H., Bohmer, M., 1972. Kinetic determination of creatinine concentration. Clin. Chem. Acta. 37–193.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., Crowe, S.E., 2014. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev. 94 (2), 329–354.

Bocci, B.A., 1993. Ingestion of economic plants in Badeggi by Tapinanthus dodoneifolius (DC) Danser and Tapinanthus globiferus (A. Rich) Van Tiegh. Nigerian J. Weed Sci. 11, 51–56.

Burkill, H.M., 1985. The useful Plants of West Tropical Africa. Royal Botanical Gardens, Kew, Vol.3 (families J-L). 548–560.

Buzeck, J., Chastel, O., 2010. Directive 2010/63/EU of the European parliament and of the Council. Protection of animals used for Scientific purposes (Text with EEA relevance). Official J. Eur. Union. L276/34.

El-Dars, F.M.S.E., Bakr, M.H.M., Gabhe, A.M., 2013. Reduction of COD in Residue ProductionWastewater Using Three Types of Activated Carbon. J. Environ. Treatment Technol. 1 (3), 126–136.

Foulkes, E., 1985. Interactions between metals in rat jejunum: Implications on the resulting hazards for human health. J. Occup. Med. Toxicol. 1 (22), 1–6.

Hyder, O., Chung, M., Cosgrove, D., Herman, J.M., Li, Z., Firoozmand, A., Gurakar, A., Oigbochie, V.E., 2013. Lead and cadmium in public health in Physicians: neglect and pitfall in patient management. N. Am. J. Med. Sci. 6 (2), 61.

Järup, L., Berglund, M., Elinder, C.G., 1998. Health effects of cadmium exposure – A review. J. Toxicol. Environ. Health 52, 15–22.

Jendrassik, L., Grof, P., 1938. Colorimetric method of determination of bilirubin. Biochem. Clin. 297, 81–82.

Kochmar, J.F., Moss, D.W., 1957. Fundamentals of clinical chemistry. W.B Saunders and Company, Philadelphia, p. 604.

Kusi, M., Shrestha, K., Malla, R., 2015. Study on phytochemical, antibacterial, antioxidant and toxicity profile of Viscum album Linn associated with Acacia catechu. Nepal J. Biotechnol. 3, 60–65.

Liu, G., Lei, Y.-X., He, C.-C., Lei, Z.-N., 2013. Blood translation elongation factor-1s is a novel marker for cadmium exposure. Int. J. Mol. Sci. 14 (3), 5189–5197.

Montero, G.D., Valladares, M.B., Tornes, C.Y.L.F., Agrapont, R.A., Calderon, J.B., Fundora, H.R., 2016. Tratamiento homeopático y convencional de la hipertensión arterial. Rev. Méd. Homeopat. 9, 53–58.

Nhiem, N.X., Kiem, P.V., Minh, C.V., Kim, N., Park, S., Lee, H.Y., et al., 2013. Diarylheptanoids and flavonoids from Viscum album inhibit LPS-stimulated production of pro-inflammatory cytokines in bone marrow-derived dendritic cells. J. Nat. Prod. 76 (4), 495–502.

Oigbochie, V.E., Omage, K., Odiaze, D.E., 2019. Aqueous Root Extract of Chrysopilium albidum caused Dose and Duration Dependent Increases in some Reproductive Hormones and Spontaneous Arrest in the Testes of Male Wistar Rats. Clin. Phytosci. 5 (3), 1–8.

Oigbochie, V.E., Osarumwense, M.O., Odiaze, D.E., Omage, K., 2018. Evaluation of the Effects of the Administration of aqueous Root Extracts of Chrysopilium albidum on Fertility in Male Wistar Albino Rats. International Journal of Pharmacology. Phytochem. Ethnomed. (IPPE) 10, 13–28.

Oigbochie, V.E., Omage, K., Odiase, D.E., 2018. Assessment of the effect of Watermelon and Aloe Vera on Cadmium induced Heart damage in adult wistar rats. Cardiol. Angiol.: Int. J. 5 (1), 1–9.

Orhan, D.D., Kupeli, E., Yesilada, E., Ergun, F., 2006. Anti-inflammatory and antinociceptive activity of flavonoids isolated from Viscum album ssp. album. Z Natur forsch C 61 (1–2), 26–30.

Polhill, R., Wiens, D., 1998. Mistletoe of Africa. The Royal Botanic Garden, Kew, U. K., 379p.

Portuhakaren, K.J., Palatty, P.L., Baliga, M.S., Suresh, S., 2014. Clinical evaluation of Viscum album mother tincture as an antihypertensive: a pilot study. J. Evid. Based Complement. Altern. Med. 19, 31–35.

Rafati, R.M., Rafati, R.M., Kazemi, S., Moghadamnia, A.A., 2017. Cadmium toxicity and treatment: An update. Caspian J. Intern. Med. 8 (3), 135–145.

Rani, A., Kumar, A., Lal, A., Pant, M., 2014. Cellular mechanisms of cadmium-induced toxicity: a review. Int. J. Environ. Health Res. 24, 378–399.

Reitman, S., Frankel, S., 1957. Glutamic pyruvate transaminase assay by colorimetric method. Am. J. Clin. Pathol. 29, 56.

Singh, B.N., Saka, C., Galun, D., Upreti, D.K., Batry, J., Kaveri, S.V., 2016. European Viscum album: a potent phytotherapeutic agent with multifarious phytochemicals, pharmacological properties and clinical evidence. EJC Adv. 6, 23837–23857.

Skeggs, L.T., Hocshtrasser, H.C., 1964. Colorimetric determination of chloride. Clin. Chem. 10, 918–920.

Tietz, N.W., 1987. Fundamentals of Clinical Chemistry. W.B. Saunders, Philadelphia.

Van-Slyke, D.D., Hastings, A.B., Murray, C.D., Sendroy Jr., J., 2000. Studies of gas and ammonia. Anal. Chem. 39, 971.

Weatherbum, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39, 971.

Yakubu, M.T., 2009. Anti-hipidaemic potentials of aqueous extract of Tapinanthus globiferus leaves in rats. Chem. Med. Value. Rpmp. 25, 1–9.