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CADMIUM CHLORIDE INDUCED ELECTROLYTE IMBALANCE, PANCYTOPENIA, OXIDATIVE STRESS AND RENAL DAMAGE: AMELIORATING EFFECTS OF AQUEOUS EXTRACT OF *TELFAIRIA OCCIDENTALIS*

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ABSTRACT: Cadmium is a known nephrotoxic metal with other toxicities including pancytopenia. *Telfairia occidentalis* is a medicinal plant with antioxidant and chemoprotective properties. This study was carried out to investigate the ameliorating effects of aqueous extract of *Telfairia occidentalis* on cadmium chloride-induced oxidative stress, renal damage and hematological alterations in rats. Twenty four (24) adult male rats were divided into 4 groups of 6 rats each. Group I received distilled water, group II, III, and IV were administered 5 mg/kg body weight CdCl₂, group III; IV was treated with 200 and 400 mg/kg bw respectively of aqueous extract of *Telfairia occidentalis* for 14 days while rats in group II were left untreated. Results obtained showed that administration of cadmium caused significant reduction in all the hematological parameters, reduced GSH, ascorbic acid, Superoxide dismutase (SOD), glutathione S-transferase (GST), Catalase (CAT), serum Sodium ion and bicarbonate ions as well as increase in MDA (malondialdehyde) level and serum level of potassium ion, serum urea, and creatinine. However, treatment with various doses of aqueous extract of *Telfairia occidentalis* significantly ameliorate the adverse effects of Cadmium as it increased the cellular components of the blood while it also reverses serum potassium, urea, creatinine, and redox status. In conclusion, aqueous extract of *Telfairia occidentalis* effectively suppressed Cadmium-induced electrolyte imbalance, pancytopenia, oxidative stress, and renal damage, suggesting its protective potentials in anemic and renal-related disorders.

INTRODUCTION: Many heavy toxic metals such as cadmium have been considered to have deleterious effects on human health. Cadmium is highly toxic even at very low exposure levels; it has acute and chronic effects on health and the environment.

Human exposure to cadmium may be via medications, cigarette, diets, water, occupation and environment ^{1, 2}. Several disorders have been associated with chronic exposure to these heavy metals which include Parkinsonian syndromes as a result of manganese toxicity, minimata associated with arsenic poisoning; others include hepatorenal degeneration, neuropathies, pneumoconiosis, and cancers ^{3, 4}. Upon exposure, cadmium can be accumulated in various organs of the body including liver, kidney, pancreas, and testis; eliciting adverse effects on these organs and altering their physiological and biochemical

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functions. The kidney is known as a major target of cadmium-induced toxicity due to the presence of metallothionein, a sulfhydryl group containing protein^{5, 6}. Oyewole et al.,⁷ has earlier reported that exposure of rats to cadmium compound resulted into pancytopenia, a medical condition where there is a deficiency of all cellular components of the blood. Also, cadmium-induced anemia has been associated with hemolysis and iron-deficient indices⁸.

The mechanisms of cadmium-induced organ damage have been elucidated to be through the alteration of transport pathways^{9, 10}, epigenetic aberrations in DNA expression¹¹, the disruption of the redox balance resulting in oxidative stress^{12, 13}, and impairment of mitochondrial functions to induce apoptosis¹⁴. *Telfairia occidentalis* is a medicinal plant widely grown in parts of West and Central Africa, used locally as blood booster due to its abundance in blood enriching minerals such as iron, potassium, sodium, phosphorus, vitamins (thiamine, riboflavin, nicotinamide, ascorbic acid), and phytochemicals¹⁵. *Telfairia occidentalis* also used in the treatment of reproductive and fertility issues¹⁶, liver and high blood sugar problems^{17, 18}. Experimental evidence has claimed increase hematological parameters benefits of this plant¹⁹. Hypolipidemic effect and the therapeutic usefulness of the extraction hypercholesterolemia have also been documented¹⁸. The extracts of this plant have possessed the ability to lower lipid peroxidation, free radical scavenging and antioxidant properties^{18, 20}.

This present study investigated the ameliorating effects of aqueous extract of *Telfairia occidentalis* on cadmium chloride - induced oxidative stress, renal damage and hematological alterations in rats.

MATERIALS AND METHODS:

Chemicals and Reagents: Potassium ion (K⁺), Sodium ion (Na⁺), Bicarbonate ions (HCO₃⁻), Urea and creatinine kits are products of Randox Chemical Limited, England. All other chemicals were of analytical grade.

Collection of Plant Material and Aqueous Extraction: The fresh leaves of *Telfairia occidentalis* were purchased from the vegetable section of Igbona market in Osogbo, Osun State.

The leaves were thoroughly washed and blended, the paste was filtered to obtain a clear aqueous extract of the leaves. The sediment filtrate was air-dried to obtain a powdery form which was used to prepare the 200 mg/kg and 400 mg/kg body weight used in this experiment.

Experimental Animals: Twenty four male wistar albino rats weighing between 130-140 g were used for this experiment. The rats were obtained from Central Animal House, Osun State University Osogbo, Nigeria. The rats were kept in a ventilated cage at optimum temperature and 12 h light / dark cycle and fed with commercial grower mash and water *ad libitum*. The experiment was carried out by current rules and guidelines that have been established for the care of the laboratory animals²¹. The rats were acclimatized for two weeks before treatment commenced.

Experimental Design and Dose Regimen: The twenty-four Wistar albino rats were sorted into four (4) different groups containing six (6) rats each. The body weight and the average weight of each group were taken and recorded daily. Administration of aqueous extract of *Telfairia occidentalis* leaf (AETO) was done using the gavage method using oral cannula. The animals were treated daily for 14 consecutive days.

- **Group I:** received distilled water daily and serve as the Control.
- **Group II:** received 5mg/kg body weight CdCl₂
- **Group III:** received 5mg/kg body weight CdCl₂ and 200mg/kg body weight of AETO.
- **Group IV:** received 5mg/kg body weight CdCl₂ and 400mg/kg body weight of AETO.

A Sacrifice of Experimental Animals and Sample Collection: The rats were weighed and sacrificed after 24 h of last dose treatment under the influence of chloroform anesthesia. The kidneys were harvested immediately and stored on ice. Whole blood for hematological analysis collected into labeled EDTA bottles to prevent clotting. Serum for biochemical analysis were obtained by collecting blood from the jugular vein into separate plain bottles, allowed to clot and centrifuged at 4000 rpm for 30 min. The serum obtained was stored in a refrigerator at -4 °C until it was used for biochemical analysis.

Preparation of Homogenates: The kidney were rinsed with KCl and blotted with filter paper and weighed. They were then chopped into bits and homogenized in four volumes of the homogenizing buffer (0.1M Tris-KCl, pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 12,500 g for 15 min in a cold centrifuge (4 °C), to obtain the post-mitochondrial fraction. The supernatant was collected and used for biochemical analyses.

Measurement of Haematological Parameters:

Haematological parameters including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), lymphocyte, platelet counts and reticulocyte count were measured using the automated multiparameter blood analyzer SYSMEX KX21 as earlier described Dacie and Lewis²². Fifty micro liters of blood samples were introduced into the equipment, and it automatically employs the differences in characteristics possessed by each of the blood components to distinguish and estimate those⁷.

Biochemical Assays: Catalase activity in the kidneys was determined according to the method of Sinha²³. Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich²⁴. The method of Habig et al.,²⁵ was used in the determination of Glutathione S-transferase (GST)

activity. The level of reduced glutathione (GSH) in the kidney samples was determined by the method described by Jollow et al.²⁶ Lipid peroxidation (malondialdehyde) was assessed by using the procedure of Varshney and Kale²⁷. The ascorbic acid concentration was determined according to the method of Jagota and Dani²⁸. Sodium ion, Potassium ion, Bicarbonate Concentration, Urea, creatinine in the serum of rats using the appropriate kits and method described by the manufacturer (Randox).

Statistical Analysis: Data were expressed as mean \pm standard deviation (mean \pm SD) and analyzed using one-way analysis of variance (ANOVA) with the aid of SPSS 12.0 computer software package (SPSS Inc; Chicago, U.S.A). Student's t-test was employed for comparison between two sets of data and differences at P<0.05 were considered significant.

RESULTS: Table 1 shows the hematological parameters in rats administered cadmium chloride and aqueous leaf extract of *Telfairia occidentalis*. Rats administered cadmium alone (group II) recorded significant reduction in hematological indices (PCV, RBC, WBC, Hb, Monocyte, Eosinophil, Neutrophil, MCV, and MCHC). This reduction was significantly (P<0.05) reverse following treatment with aqueous leaf extract of *Telfairia occidentalis* (group III and IV).

TABLE 1: HAEMATOLOGICAL PARAMETERS IN RATS ADMINISTERED CADMIUM CHLORIDE AND AQUEOUS LEAF EXTRACT OF TELFAIRIA OCCIDENTALIS

Parameters	Group I	Group II	Group III	Group IV
PCV (%)	31.87 \pm 2.96	17.20 \pm 2.53*	26.12 \pm 3.08 [#]	32.48 \pm 3.42 [#]
Hb Conc. (g/dl)	19.75 \pm 1.54	11.87 \pm 1.07*	14.38 \pm 1.40 [#]	17.09 \pm 2.02 [#]
RBC (X 10 ⁶ μ l)	8.91 \pm 0.64	3.85 \pm 0.32*	5.68 \pm 0.47 [#]	7.44 \pm 0.90 [#]
WBC (X 10 ³ μ l)	16.52 \pm 1.92	10.27 \pm 1.55*	12.99 \pm 1.32 [#]	15.01 \pm 2.06 [#]
MCV (fl)	65.06 \pm 4.76	47.34 \pm 5.10*	52.85 \pm 4.94 [#]	60.79 \pm 5.30 [#]
MCHC (g/dl)	42.37 \pm 4.66	30.99 \pm 3.86*	35.21 \pm 4.07*	39.45 \pm 4.28 [#]
Lymphocyte (%)	18.13 \pm 3.01	12.07 \pm 2.58*	14.47 \pm 2.93 [#]	16.11 \pm 2.24 [#]
Reticulocyte (%)	12.82 \pm 1.86	10.80 \pm 1.21	11.38 \pm 1.31*	12.43 \pm 2.12 [#]
Monocyte (%)	50.70 \pm 5.85	38.88 \pm 4.92*	43.47 \pm 5.20 [#]	48.66 \pm 5.26 [#]
Eosinophil (%)	11.43 \pm 1.74	8.63 \pm 1.09*	9.96 \pm 0.79	10.21 \pm 1.25*
Neutrophil (%)	34.34 \pm 3.94	22.82 \pm 4.52*	26.51 \pm 3.88 [#]	30.32 \pm 3.17 [#]
Basophil (%)	7.18 \pm 0.84	3.22 \pm 0.36*	5.48 \pm 0.64 [#]	6.97 \pm 0.51

Data presented as Mean \pm SD of 6 animals each per group. PCV: Packed cell volume, Hbconc: Hemoglobin concentration, RBC: Red blood cell count, WBC: White blood cell count, MCHC: mean cell hemoglobin concentration, MCV: mean cell volume. * Significantly different from normal control group at P<0.05, [#] significantly different from group II at P<0.05.

Fig. 1 shows that rats exposed to 5 mg/kg body weight of Cadmium Chloride (group II) showed a significant increase (P<0.05) in serum concentration levels of urea and creatinine as

compared to the control (group I). These altered values were reverted significantly ($P < 0.05$) toward normal in a dose-dependent manner in rats treated with 200 and 400 mg/kg body weight of aqueous leaf extract of *Telfairia occidentalis* (group III and IV respectively).

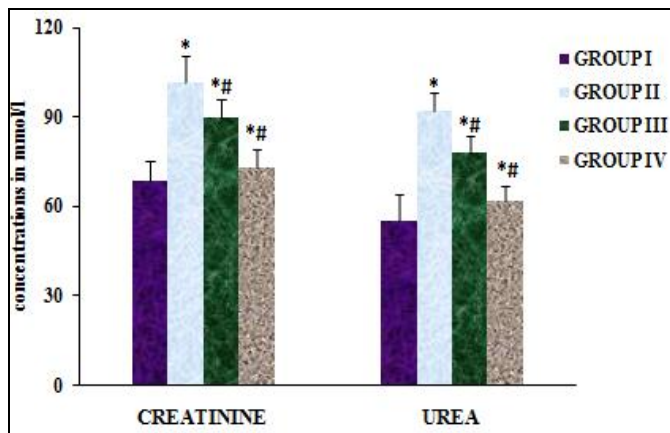


FIG. 1: THE EFFECT OF CADMIUM CHLORIDE AND AQUEOUS LEAF EXTRACT OF *TELFAIRIA OCCIDENTALIS* ON SERUM RENAL METABOLITES IN EXPERIMENTAL RATS. Data presented as Mean \pm SD of 6 animals each per group. * Significantly different from normal control group at $P < 0.05$, # significantly different from group II at $P < 0.05$.

Serum electrolytes levels in the experimental animals are shown in Fig. 2. Cadmium chloride exposure caused a significant increase ($P < 0.05$) in potassium ion but significantly reduced sodium ion and Bicarbonates as compared to the control. However, treatment with 200 and 400 mg/kg body weight of aqueous leaf extract of *Telfairia occidentalis* reversed these changes.

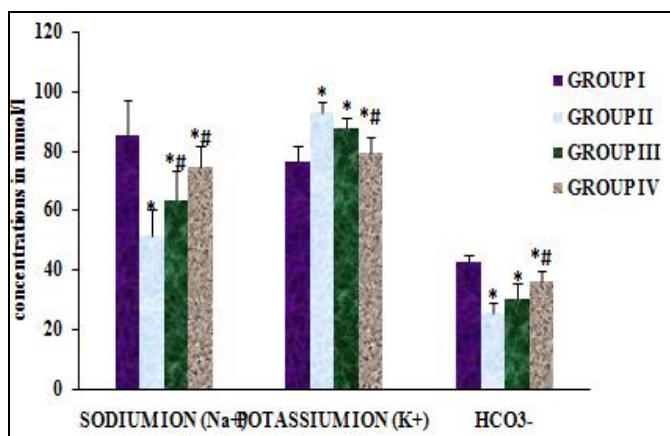


FIG. 2: THE EFFECT OF CADMIUM CHLORIDE AND AQUEOUS LEAF EXTRACT OF *TELFAIRIA OCCIDENTALIS* ON SOME SERUM ELECTROLYTES IN RATS. Data presented as Mean \pm SD of 6 animals each per group. * Significantly different from normal control group at $P < 0.05$, # significantly different from group II at $P < 0.05$.

Fig. 3 and 4 show the effect of Cadmium Chloride and aqueous leaf extract of *Telfairia occidentalis* on the antioxidant status in the kidney of the experimental rats. Renal SOD, GST, CAT, GSH, and Ascorbic acid were significantly reduced ($P < 0.05$) as compared to the control, as well as significantly increase ($P < 0.05$) in Lipid peroxidation (MDA) in rats treated with Cadmium chloride alone (group II).

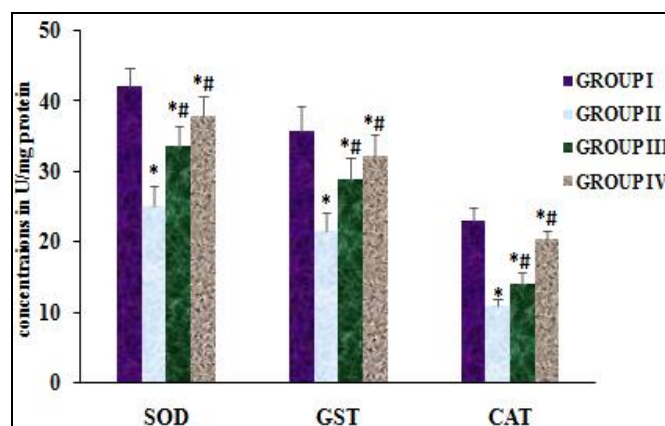


FIG. 3: THE EFFECT OF CADMIUM CHLORIDE AND AQUEOUS LEAF EXTRACT OF *TELFAIRIA OCCIDENTALIS* ON ANTIOXIDANT ENZYMES: SUPEROXIDE DISMUTASE (SOD), GLUTATHIONE S-TRANSFERASE (GST), AND CATALASE (CAT) IN RATS. Data presented as Mean \pm SD of 6 animals each per group. * Significantly different from normal control group at $P < 0.05$, # significantly different from group II at $P < 0.05$.

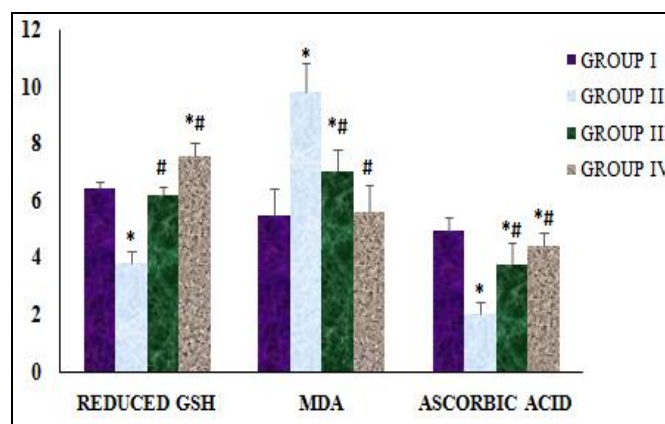


FIG. 4: THE EFFECT OF CADMIUM CHLORIDE AND AQUEOUS LEAF EXTRACT OF *TELFAIRIA OCCIDENTALIS* ON REDUCED GLUTATHIONE (GSH), LIPID PEROXIDATION (MDA) AND ASCORBIC ACID. Reduced GSH is measured in ug/ml, MDA (malondialdehyde) in units/mg protein and ascorbic acid in ug/ml. Data presented as Mean \pm SD of 6 animals each per group. * Significantly different from normal control group at $P < 0.05$, # significantly different from group II at $P < 0.05$.

Administration of aqueous leaf extract of *Telfairia occidentalis* significantly ($P < 0.05$) attenuate this anomaly in a dose-dependent manner.

DISCUSSION: This study investigated the protective potentials of aqueous leaf extract of *Telfairia occidentalis* against cadmium chloride-induced haemotoxicity, oxidative stress and renal dysfunction in male wistar albino rats. The results in **Table 1** indicated that there is marked general decrease in the levels of hematological parameters (PCV, RBC, WBC, Monocyte, Eosinophil, Neutrophil, MCV, and MCHC) following the administration of cadmium chloride. This result further strengthens the previous findings that exposure of rats to cadmium chloride leads to a disease condition called pancytopenia which there is a general decrease in the cellular elements in the blood of animal ⁷. Also, exposure of rats to cadmium chloride resulted in anemia followed by significant reduced ($P<0.05$) in the level of hemoglobin and red blood cells **Table 1**.

This is in tandem with the report of Horiguchi, *et al.*, ⁸ that cadmium-induced anaemia has been associated with hemolysis and iron-deficient indices. However, treatment with aqueous leaf extract of *Telfairia occidentalis* was found to increase significantly ($P<0.05$) all the hematological parameters. The heamato-protective effect of this extract may be due to the phytochemical constituents of leaves which consist blood enriching minerals such as iron, potassium, sodium, phosphorus, Vitamins (thiamine, riboflavin, nicotinamide, ascorbic acid), and phytochemicals ¹⁵. The observed elevated level of MCV and MCHC together with the increase in total WBC count by the extract suggests that it might also be immune-protective.

Administration of cadmium chloride in this study significantly ($P<0.05$) increased serum Potassium ion (K^+) level and significantly ($P<0.05$) decreased serum Sodium ion (Na^+) and bicarbonate ions (HCO_3^-) resulting to an electrolyte imbalance in the experimental animals. This result agrees with the previous reports that cadmium intoxication induced abnormal serum electrolytes and hyperkalemia ^{29, 30}. Treatment with aqueous leaf extract of *Telfairia occidentalis* was found to significantly reverse these alterations in the serum electrolyte homeostasis. This suggests the ameliorative effect of the aqueous extract on per oxidation of the polyunsaturated fatty acids in the membrane which

delocalized Na-K ATPase from basolateral to apical membrane resulting in electrolyte imbalance.

The observed significant increase ($P<0.05$) in the level of urea and creatinine in the serum of cadmium-treated rats (group II) as compared to the normal control (group I) is an indication of renal dysfunction. Urea is an indicator of renal function which is routinely measured to assess the kidney health status while creatinine is an important marker of the filtration function of the kidneys because it is chiefly excreted from the blood via glomerular filtration. The increased level of these serum renal metabolites may have resulted from a decrease in the rate of their excretion which may be as a result of impairment in kidney ability to carry out the biochemical process ^{31, 32}. Aqueous leaf extract of *Telfairia occidentalis* administration significantly ameliorate the alterations in kidney functions. Mechanism of Cadmium toxicity includes oxidative stress ^{12, 13}. This was established in this study by the significant increase ($P<0.05$) in MDA (malondialdehyde) level, an end product of lipid peroxidation in the kidney as well as significant decrease ($P<0.05$) in the reduced GSH content, ascorbic acid level and in the activities level of antioxidant enzymes: Superoxide dismutase (SOD), glutathione S-transferase (GST), and Catalase (CAT). The increased lipid peroxidation level observed in this study might be a consequence of increased free radical formation as well as the disruption of antioxidant status. However, treatment with aqueous extract of *Telfairia occidentalis* significantly reversed and ameliorated these alterations confirming the antioxidant properties of the extract.

CONCLUSION: Taken together, the results in this study demonstrated that aqueous extract of *Telfairia occidentalis* effectively suppress hematological alterations, electrolyte imbalance, oxidative stress, and renal dysfunctions induced by Cadmium chloride exposure in rats. This study suggests the protective potentials of aqueous extract of *Telfairia occidentalis* in anemic and renal-related disorders.

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CONFLICT OF INTEREST: Nil**REFERENCES:**

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