

Modulatory properties of *Telfairia occidentalis* leaf extract on pancytopenia, electrolyte imbalance and renal oxidative damage in rats

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ABSTRACT: *Telfairia occidentalis* is a medicinal plant acclaimed to possess many therapeutic potential. The study was carried out to investigate the possible modulatory effects of aqueous leaf extract of *T. occidentalis* on chemical-induced oxidative stress, renal dysfunction and pancytopenia 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 of cadmium chloride to induce oxidative stress. Group III and IV were treated with 200 and 400 mg/kg body weight respectively of aqueous leaf extract of *T. occidentalis* for 14 days while rats in group II were left untreated. Results obtained showed that administration of cadmium caused significant suppression of haematopoiesis, depleted endogenous antioxidants and increased lipid peroxidation in the kidney as well as alteration of serum electrolytes. Treatment with graded doses of *T. occidentalis* leaf extract significantly reversed the cadmium induced-biochemical insults as the extract restored the normal renal integrity and boosted renal antioxidant status in rats. The leaf extract also caused significant boost in haematopoiesis in the rats. This result confirmed that aqueous extract of *T. occidentalis* effectively maintain electrolyte balance, modulates pancytopenia and oxidative renal damage in rats suggesting its protective potentials on anaemia and renal disorders.

Keywords: Electrolyte imbalance, oxidative stress, renal damage, pancytopenia, *Telfairia occidentalis*, Cadmium chloride.

INTRODUCTION

Medicinal plants possessing natural antioxidants polyphenolic compounds have been shown to have reactive oxygen species (ROS) scavenging and lipid peroxidation prevention effects. *Telfairia occidentalis* (fluted pumpkin-common name, ugu-Igbo language), is a tropical vine grown in West and Central Africa as leaf vegetable and for its edible seeds. The leaf is used locally as blood booster due to the abundance of blood enriching minerals such as iron, potassium, sodium, phosphorus, vitamins (thiamine, riboflavin, nicotinamide, ascorbic acid) and phytochemicals in the plant (Kayode

and Kayode, 2011). *T. occidentalis* leaf is also used in the treatment of infertility (Nwangwa et al., 2007), liver problem and diabetes (Eseyin et al., 2005; Adaramoye et al., 2007). Experimental evidence has claimed that the plant has positive effect on haematopoiesis (Alada, 2000). The hypolipidemic effect and the therapeutic usefulness of the leaf extract in hypercholesterolemia has also been documented (Oboh et al., 2006).

Many heavy metals such as cadmium have been considered to have deleterious effects on human health (Chounwou et al., 2012). Human exposure to cadmium

may be via medications, cigarette, diets, water, occupation and environmental pollution (Chater et al., 2008). A number of disorders including Parkinson syndrome, hepatorenal degeneration, neuropathies, pneumoconiosis and cancer have been associated with chronic exposure to cadmium (Oyewole et al., 2016).

Upon exposure, cadmium can accumulate in various organs of the body including liver, kidney, pancreas and testis eliciting adverse effects on these organs and altering their physiological and biochemical functions. The kidney is a major target of cadmium-induced toxicity due to the presence of metallothionein, a sulfhydryl group containing protein (Van Kerkhove et al., 2010). Cadmium induced anaemia has been associated with haemolysis and iron-deficient indices (Wang et al., 2012). The present study investigated the ameliorating effects of aqueous leaf extract of *T. occidentalis* on cadmium chloride-induced oxidative stress, renal dysfunction and pancytopenia in rats.

MATERIALS AND METHODS

Chemicals and reagents

Urea, creatinine and electrolytes (K^+ , Na^+ and HCO_3^-) kits are products of Randox Chemical Limited, England. Antioxidant (CAT, SOD, GST), GSH, ascorbic acid and MDA kits were obtained from Cayman Chemical Michigan, USA. Cadmium chloride is a product of British Drug House Poole, England.

Collection of plant material and aqueous extraction

Fresh leaves of *T. occidentalis* was purchased from vegetable section of Igbona Market in Osogbo, Osun State, Nigeria. The leaves were thoroughly washed and blended in water. The paste was filtered to obtain a clear aqueous extract of the leaves. The filtrate was air dried to obtain a powdery form which was used to prepare the 200 mg/kg and 400 mg/kg body weight of extract used in this experiment.

Experimental animals

Twenty-four male Wistar albino rats weighing between 130 to 140 g were used for this experiment. The rats were obtained from the Central Animal House, Osun State University Osogbo, Nigeria. The rats were kept in ventilated cage at optimum temperature and 12 hrs light/dark cycle and fed with commercial growers mash and water *ad libitum*. The experiment was carried out in accordance with current rules and guidelines established for the care of laboratory animals (NRC, 2011). The rats were acclimatized for two weeks before treatment commenced.

Experimental design and dose regimen

The 24 Wistar albino rats were sorted into four (4) different groups containing six (6) rats each. Average body weight of each animal group were taken and recorded daily. Administration of extract and cadmium chloride was done using the gavage method with the aid of oral canula. The animals were treated daily for 14 consecutive days.

Group I: received distilled water and serve as the control.

Group II: received 5 mg/kg body weight of $CdCl_2$.

Group III: received 5 mg/kg body weight of $CdCl_2$ and 200 mg/kg body weight of *T. occidentalis*.

Group IV: received 5 mg/kg body weight of $CdCl_2$ and 400 mg/kg body weight of *T. occidentalis*.

Collection of blood samples

The rats were weighed and sacrificed after 24 hrs of last dose treatment under the influence of chloroform anesthesia. The jugular vein was cut and whole blood for haematological 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 mins. The serum obtained was stored in a refrigerator at $-4^\circ C$ until it was used for biochemical analysis.

Preparation of kidney homogenates

The rats were quickly dissected and the kidneys harvested. The kidneys were rinsed with KCl and blotted with filter paper and weighed. They were then chopped into bits and homogenized in 4 volumes of the homogenizing buffer (0.1 M Tris-KCl, pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 12,500 g for 15 mins in a cold centrifuge ($4^\circ C$), 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), lymphocyte, reticulocyte and WBC differential counts were measured using the automated multiparameter blood analyzer SYSMEX KX21 as earlier described by Dacie and Lewis (1991).

Biochemical assays

Catalase activity in the kidney homogenate was determined according to the method of Sinha (1972).

Table 1. Haematological parameters in rats administered cadmium chloride and aqueous leaf extract of *T. occidentalis*.

Parameters	Group I	Group II	Group III	Group IV
PCV (%)	31.87±2.96	17.20±2.53*	26.12±3.08	32.48±3.42
Hb Conc. (g/dl)	19.75±1.54	11.87±1.07*	16.38±1.40	17.09±2.02
RBC (X 10 ⁶ µl)	8.91±0.64	3.85±0.32*	6.68±0.47	7.44±0.90
WBC (X 10 ³ µl)	16.52±1.92	10.27±1.55*	14.99±1.32	15.01±2.06
Lymphocyte (%)	18.13±3.01	10.07±2.58*	15.47±2.93	16.11±2.24
Reticulocytes (%)	12.82±1.86	8.80±1.21*	11.38±1.31	12.43±2.12
Monocyte (%)	50.70±5.85	38.88±4.92*	46.47±5.20	48.66±5.26
Eosinophil (%)	11.43±1.74	7.21±1.09*	9.96±0.79	10.21±1.25
Neutrophil (%)	34.34±3.94	22.82±4.52*	29.51±3.88	31.32±3.17
Basophil (%)	7.18±0.84	3.22±0.36*	6.33±0.64	6.97±0.51

Data presented as Mean ± SD of 6 animals. *Significantly different from normal control group at P< 0.05.

Table 2. Serum urea, creatinine and electrolytes concentrations in rats administered cadmium chloride and aqueous leaf extract of *T. occidentalis*.

Parameters	Group I	Group II	Group III	Group IV
Urea (mmol/L)	49.68±4.66	76.43±5.71*	50.88±4.53	51.35±5.21
Creatinine (mmol/L)	58.61±5.23	88.53±8.11*	62.59±4.10	60.44±5.21
Na ⁺ (mmol/L)	82.44±6.21	62.39±5.43*	75.68±6.46	78.30±5.49
K ⁺ (mmol/L)	68.77±5.10	89.24±7.12*	74.33±6.55	73.90±4.71
HCO ₃ ⁻ (mmol/L)	48.22±3.78	31.55±2.66*	34.69±3.00	38.41±2.39

Data presented as Mean ± SD of 6 animals. *Significantly different from normal control group at P< 0.05.

Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (1972). The method of Habig et al. (1974) was used in the determination of glutathione S-transferase (GST) activity. The level of reduced glutathione (GSH) in the samples was determined by the method described by Jollow et al. (1974). Lipid peroxidation (malondialdehyde) was assessed by using the procedure of Varshney and Kale (1990). The ascorbic acid concentration was determined according to the method of Jagota and Dani (1982). Serum electrolytes (Na⁺, K⁺, HCO₃⁻), urea and creatinine were measured using the appropriate kits and method described by the manufacturer (Randox).

Statistical analysis

Data were expressed as mean ± standard deviation (mean ± 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 show the haematological parameters in rats

administered cadmium chloride and aqueous leaf extract of *T. occidentalis*. Rats administered cadmium alone (group II) recorded significant reduction in haematological indices (PCV, RBC, WBC, Hb, lymphocytes reticulocytes, monocyte, eosinophil, neutrophil and basophil). This reduction was significantly (P<0.05) reversed following treatment with aqueous leaf extract of *T. occidentalis* (group III and IV).

The results of serum concentrations of urea, creatinine and electrolytes in control and experimental animals are shown in Table 2. Rats exposed to cadmium chloride (group II) showed a significant increase (P<0.05) in serum concentrations of urea and creatinine as compared to the control (group I). Cadmium also caused significant increase (P<0.05) in K⁺ while it reduced Na⁺ and HCO₃⁻ as compared to the control. The altered values of kidney function indicators were reverted significantly (P<0.05) toward normal in rats treated with 200 and 400 mg/kg body weight of leaf extract of *T. occidentalis* (group III and IV).

Table 3 shows the effect of cadmium chloride and aqueous leaf extract of *T. occidentalis* on enzymatic and non-enzymatic antioxidants as well as lipid peroxidation in the rat's kidney. Renal SOD, GST, CAT, GSH and ascorbic acid were significantly reduced while lipid peroxidation (MDA concentration) was increased in rats treated with cadmium chloride alone (group II). Adminis-

Table 3. Some oxidative stress indicators in the kidney of rats administered cadmium chloride and aqueous leaf extract of *T. occidentalis*.

Parameters	Group I	Group II	Group III	Group IV
SOD (U/mg protein)	41.31±3.55	25.65±2.68	35.29±3.24	38.52±2.98
CAT (U/mg protein)	28.16±2.22	18.47±3.11	22.82±2.36	24.43±2.54
GST (U/mg protein)	37.89±3.43	27.56±2.99	32.10±2.89	32.78±3.00
GSH (µg/ml)	8.77±0.88	4.96±0.42	7.35±0.54	7.48±0.47
Ascorbic acid (µg/ml)	5.63±0.57	2.89±0.41	5.10±0.32	5.24±0.51
MDA (units/mg protein)	5.49±0.34	10.21±0.89	6.96±0.63	6.87±0.58

Data presented as Mean ± SD of 6 animals. *Significantly different from normal control group at P< 0.05.

tration of graded doses of *T. occidentalis* leaf significantly attenuates these antioxidants anomalies in the kidney of experimental rats.

DISCUSSION

Results in Table 1 indicated a marked general decrease in blood cellular elements in the animals (pancytopenia) following administration of cadmium chloride. Exposure of rats to the metal also resulted in anaemia characterized by significant reduction in PCV and Hb concentration. However, treatment with aqueous leaf extract of *T. occidentalis* significantly increased (P<0.05) all the haematological parameters. The haematinic effect of this extract may be due to the phytochemical constituents of the leaves which consist of blood enriching minerals such as iron, potassium, sodium, phosphorus, vitamins (thiamine, riboflavin, nicotinamide, ascorbic acid) and phytochemicals (Kayode and Kayode, 2011).

The observed increased serum K⁺ level and decreased serum Na⁺ and HCO₃⁻ in rats administered cadmium alone indicates electrolyte imbalance. This result agrees with the previous reports that cadmium intoxication induced abnormal serum electrolytes and hyperkalemia (Tabassum and Bajaj, 2013). This electrolyte imbalance might have resulted due to peroxidation of the polyunsaturated fatty acids in the membrane by cadmium which delocalized Na⁺-K⁺-ATPase from basolateral to apical membrane. The mechanism of cadmium-induced organ damage has been elucidated to be through alteration of transport pathways (Patra et al., 2012), epigenetic aberrations in DNA expression, the disruption of the redox balance resulting in oxidative stress and impairment of mitochondrial functions to induce apoptosis (Matovic et al., 2012). Treatment with aqueous leaf extract of *T. occidentalis* significantly reversed the observed alterations in serum electrolytes suggesting its positive effects on homeostasis.

The observed significant increase 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 and creatinine are chiefly excreted from the blood via glomerular

filtrations and are routinely measured to assess the kidney health status (Dioka et al., 2004). Increased serum levels of these metabolites are synonymous with decreased in the rate of their excretion which is a pointer to impairment of kidney function (Patil et al., 2007). Aqueous leaf extract of *T. occidentalis* administration significantly normalized the serum levels of urea and creatinine.

The increased lipid peroxidation and depletion of antioxidants levels in the kidney of rats intoxicated with cadmium is synonymous with oxidative stress. This observation might be a consequence of increased free radical formation as well as the disruption of antioxidant status. However, treatment with aqueous extract of *T. occidentalis* significantly reversed these alterations confirming the antioxidant properties of the extract. The polyphenolic content of the leaf extract might have contributed directly to the antioxidant action. It is suggested that polyphenols have inhibitory effects on mutagenesis and carcinogenesis in human (Tsao and Akhtar, 2005). Flavonoids in plants are regarded as antioxidant molecules and could therefore reduce cellular oxidative stress (Obboh et al., 2007).

Lipid peroxidation is known to be one of the principal mechanisms of cell injury in aerobic organisms subjected to oxidative stress (Obboh and Rocha, 2007). Neurodegenerative diseases and aging processes resulting from accumulation of free radicals could be inhibited by antioxidant activities of medicinal plants such as *T. occidentalis*.

Conclusion

This study demonstrated that aqueous extract of *T. occidentalis* effectively boosted haematopoiesis, corrected electrolyte imbalance, reduced oxidative stress and attenuates renal dysfunction induced by cadmium chloride exposure in rats. This study suggests that the extract has protective potential on chemical-induced pancytopenia and renal oxidative dysfunction. Regular consumption of *T. occidentalis* leaf might therefore be a good remedy for management of anaemia while it also maintains kidney integrity.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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