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Effect Of Methanolic Leaves Extract of *Telfairia occidentalis* on 2,4-Dinitrophenylhydrazine Induced Anaemic Rats

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Abstract

Anaemia constitutes a serious health problem in many tropical countries including Nigeria because of the high prevalence of malaria and other parasitic infections which possibly leads to a decrease in hemoglobin. Fluted pumpkin has been reported to be very good in building the constituents of the blood and also replacing them. This study investigated the effects of the methanolic leaves extract of fluted pumpkin on some hematological parameters in 2, 4-dinitrophenylhydrazine-induced anaemic rats. Thirty Wister rats of both sexes were separated into five groups of six rats each. Group 1 serves as normal control, while groups 2-5 were induced with anaemia using 40mg/kg body weight of 2,4-dinitrophenylhydrazine. Group 2 serves as the negative control without treatment, group 3 were given 20 mg/kg (b.w) of standard hematinic drug astymin, and group 4 and 5 were administered with 200 and 400 mg/kg w/w methanolic leaves extract of Telfairia occidentals respectively. All rats were fed with grower's mash and water ad libitum for 21 days. The rats were sacrificed and blood samples were collected for hematological analysis. All analysis was done using standard methods. The result of this study demonstrated that oral administration of 200 mg/kg and 400mg/kg of the extract increased the haematological parameters under investigation. A significant ($p<0.05$) increase in haematinic activity was observed in group 3 and 5, and it was concluded that methanolic leaves extract of fluted pumpkin could be effective in ameliorating anaemia.

Keywords: Anemia; Telfairia occidentalis; 2, 4-dinitrophenylhydrazine; Haematological

INTRODUCTION

Anaemia is the decrease in blood haemoglobin (Hb) concentration in red blood cells which is related to the age, sex, and geographical specifications of the study population (Omote *et al.*, 2020). Chopra *et al.* (2020) also defined anemia as Hb concentration of less than 13 mg/dl and 12 mg/dl in male and female adults respectively. Haemoglobin functions to bind and transport oxygen across the tissue of the body for normal metabolic processes and therefore decrease in its levels would alter the ability of the blood to efficiently transport oxygen. The Iron in the Hb provides the spaces for oxygen binding during transportation (Shukla *et al.*, 2012). Iron deficiency is the major cause of anaemia in individuals with heart failure (Chopra *et al.*, 2020). Anaemia is recognized as one of the most common nutritional problems throughout the world affecting approximately 1.6 billion people (Andriastuti *et al.*, 2020).

Telfairia occidentalis is a vegetable belonging to the family of plants Curcubitacea. It is locally called pumpkin or ugu and is mostly cultivated in southern-eastern Nigeria. The leaves are rich in iron, potassium, magnesium, vitamin c, and carotene. The vegetable has been investigated to affect blood production in humans and thus used for treatment and prevention of anemia (Dick *et al.*, 2017). Blood transfusion is a fast and reliable technique for patients with severe anaemia, but due to the cost implications and disease conditions such as hepatitis, AIDS, and ebola which have remained a great threat to blood transfusion. This is due to the fact that blood screening techniques are poorly handled especially in rural areas, mainly due to absence of necessary equipment. Also, psychological trauma, blood incompatibility, and clinical complications at times pose a challenge for this procedure. It is therefore imperative to implore more affordable, convenient, and less traumatic strategies for the treatment or prevention of anaemia (Barro *et al.*, 2018).

MATERIALS AND METHODS

Sample Collection and Preparation

T. occidentalis leaves were collected from the National Biotechnology Development Agency North-West zone of Katsina State. The plant was identified by a botanist in the Department of Biology, Umaru Musa Yaradua University, Katsina with the voucher number; UMUUH 2270. The leaves were thoroughly washed with distilled water to remove soluble impurities; air-dried inside the laboratory at room temperature and then crushed into fine powder using mortar and pestle.

Extraction of Plant Material

A known quantity, 45gram of the powdered form of the leaves of *Telfairia occidentalis* was blended in 500 ml of methanol and was allowed to stand for 48hrs. The solution was filtered with ADARSH filter paper.

Experimental Animal

A total of twenty (30) albino male and female rats were obtained and kept in well-aerated laboratory cages in the Biochemistry Laboratory, Department of Biochemistry, Umaru Musa Yar'adua University, Katsina. The animals were allowed to acclimatise to the laboratory environment for two weeks before the commencement of the experiment. They were fed with water and growers' mash (Vital Feeds).

Induction of Anaemia

Induction was done by the modified method of Allahmoradi *et al*. (2020). Rats from groups 2 to 5 were injected intraperitoneally for two days with 40mg/kg phenyl-hydrazine hydrochloride. Anaemia was established 24 hours after the second induction. Packed Cell volume of < 35% was considered as an index for anaemia.

Experimental Design

Thirty Wistar rats of both sexes with an average weight of 120-130 g were divided into five groups of six animals each as follows;
Group I: Normal Control
Group II: Negative control
Group III: administered with 20 ml/kg of astymin/day.
Group IV: administered with 200 mg/kg of the extract daily.
Group V: administered with 400 mg/kg of the extract daily.
All the rats were maintained with grower's mash and water *ad libitum* for twenty-one (21) days.

Sample Collection And Analysis

After 21 days, the rats were anesthetized with chloroform and sacrificed by cutting the jugular vein. The blood samples were collected in EDTA-treated bottles and stored until analysis of hematological parameters.

Statistical Analysis

Results were presented as mean ± standard error of the mean (SEM). Within and between groups, comparisons were performed by the analysis of variance (ANOVA) (using SPSS 17.0 for windows Computer Software Package). Significant differences were compared by Duncan's New Multiple Range tests; a probability level of less than 5% ($P< 0.05$) was considered significant.

RESULTS

The effect of methanolic leave extract of *T. Occidentalis* on parked cell volume and hemoglobin concentration of hydrazine-induced anemic rats as presented in table 1, shows that the anemic control (Negative control) has significantly ($p>0.05$) lower hematological indices as compared to the control and treated groups.

Table 1: Effect of methanolic leave extract of *T. Occidentalis* on parked cell volume and hemoglobin concentration of hydrazine-induced anemic rats

GROUPS	PCV (%)	Hb (g/dl)
NC	47.67 ± 1.67^{b}	$15.87\pm.55^{b}$
AN	23.00 ± 2.51^{a}	$7.67\pm.83^{a}$
DC	42.33 ± 2.18^{b}	$14.11\pm.72^{b}$
A + 200mg/kg	$41.00\pm.57^{b}$	$13.67\pm.19^{b}$
A + 400mg/kg	44.67 ± 2.33^{b}	$14.89\pm.78^{b}$

Results are expressed as mean ± of five determinations. Values with different superscript down the column are significantly ($p<0.05$) different. NC=Normal control, AC = Anaemic control, DC = drug control (standard drug), A + 200mg/kg = Anaemic rats treated with 200mg/kg of the methanolic leaf extract, A + 400mg/kg = Anaemic rats treated with 400mg/kg of methanolic leaf extract. PCV=Packed cell volume, Hb= Hemoglobin concentration

Table 2 present the effect of methanolic leave extract of *T. Occidentalis* on hematological indices of hydrazine induced anemic rats. The result shows that the anemic control (Negative control) has values more related to anemia as compared to the normal control and treated groups.

Table 2: Effect of methanolic leave extract of *T. Occidentalis* on hematological indices of hydrazine induced anemic rats

GROUPS	RBC (10^6/µL)	WBC (10^3/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)
NC	8.00±.57^c	13.00±.57^a	59.95±2.88^a	19.98±.96^a	33.32±.00^a
AC	2.26±.37^a	34.67±2.60^d	103.89±11.23^b	34.64±3.75^b	33.33±.00^a
DC	6.67±.33^c	20.67±1.20^c	64.06±6.00^a	21.36±2.00^a	33.33±.00^a
A+200mg/kg	6.33±.67^b	22.33±.88^c	66.38±7.85^a	22.12±2.62^a	33.33±.00^a
A+400mg/kg	7.33±.33^c	17.00±.57^a	61.01±3.10^a	20.34±1.03^a	33.33±.00^a

Results are expressed as mean ± SEM of five determinations. Values with different superscript down the column are significantly ($p<0.05$) different. NC=Normal control, AC = Anaemic control, DC = drug control (standard drug), A + 200mg/kg = Anaemic rats treated with 200mg/kg of the methanolic leaf extract, A + 400mg/kg = Anaemic rats treated with 400mg/kg of methanolic leaf extract. WBC=White blood cell count, RBC = Red blood cell count, MCV=Mean Corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration.

DISCUSSION

Plants have been used for a long time for the treatment of diseases (Ankita *et al.*, 2012). About 65-80% of the populations of the developing countries are presently using natural medicinal products as remedies to multiple ailments (WHO, 2011).Although many have been displaced by conventional pharmaceutical approaches; there is currently a renewal of interest in the use of natural products by the public(Hano, and Tungmunnithum, 2020; Ghosh and Playfield, 2003).

This study demonstrated that methanolic extract of *T. occidentalis* leaves causes a significant increase in packed cell volume, hemoglobin concentration, and other hematological indices observed in this study. The result shows that rats administered with 400 mg/kg leaf extract of *T. Occidentalis* have significantly higher ($P<0.05$) PCV, RBC, and hemoglobin concentration compared to the negative control and other groups with different treatments. Similar results were obtained in the work of (Salman *et al.*, 2008; Salman *et al.*, 2018; Salman *et al.*, 2021). Also, this study was consistent with the observations of Toma *et al.* (2015) who had reported a significant increase in heamatological parameters of 2,4-dinitrophenylhydrazine-induced anaemic rats treated with several natural products such as *Jatropha tanjorensis* and Alligator Pepper.

Phenylhydrazine causes anaemia by induction of oxidative stress in the rats, superoxide anion radical and hydrogen peroxides are the main products that lead to peroxidation of RBC membrane lipids and Heinz body formation, these effects result in the depletion of the RBC levels and cause anemia (Shukla *et al.*, 2012). *T. Occidentalis* has high antioxidant potential due to the presence of bioactive compounds called phenols. Eseyin *et al.*, (2014), Oladele *et al.* (2020) reported that *T. Occidentalis* leaves extract could scavenge hydrogen peroxide due to the possession of phenolic groups that serve as electron donors to hydrogen peroxide neutralizing it to water. Furthermore, the increase in the hematological parameters could be due to the availability of proteins, fats, vitamin A, vitamin C, vitamin B complexes, and iron in the leaf extract of *T. occidentalis* (Salman *et* al., 2008; Chiamaka *et al.*, 2020).

CONCLUSION

The result of this study showed that methanolic extract of *T. occidentalis* elevated hematological parameters in anaemic rats induced with 2, 4-dinitrophenyl-hydrazine. Hence, *T. occidentalis* could have erythropoietic potential leading to anti-anaemic activities.

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