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Effect of Pumpkin Extract (*Telfairia occidentalis*) on Routine Haematological Parameters in Acetone-Induced Oxidative Stress Albino Rats

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Abstract Plant-derived substances are becoming increasingly known for their antioxidant activity. The purpose of this study was to determine if pumpkin (*Telfairia occidentalis*) extract protects against oxidative stress using animal model. 12 albino rats were procured, grouped into four groups and allowed to acclimatize for one week. Diluted acetone was used to induce oxidative stress. The extract was prepared and administered orally. Groups 1 and 2 received 250mg/kg and 500mg/kg of the extract respectively while group 3 received only acetone and group 4 received sterile normal saline and served as control. After the administration of the extract for 7 days, 4mls of blood samples were collected from the rats through ocular puncture into EDTA blood containers. The samples were analysed for Packed Cell Volume (PCV), haemoglobin concentration (Hb) and White Blood cell count (WBC) using standard manual methods. From the results, there was significant ($p<0.05$) increase in haemoglobin concentration in the groups that received 250mg/kg and 500mg/kg of the extract when compared with control and acetone only groups. Though there was increase in PCV of the groups that received the extract, but it was not significant. From the findings of this study, the extract of *Telfairia occidentalis* may possibly improve haematological parameters and confer protection in oxidative stress conditions.

Keywords: *antioxidant activity, oxidative stress, Telfairia occidentalis, haematological parameters, acetone*

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1. Introduction

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms [1]. These include cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, Sickle Cell Disease, vitiligo, autism, and chronic fatigue syndrome. However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens [2]. Short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis [3].

Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis [4]. Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage [5]. The major portion of long term effects is inflicted by damage on DNA [6]. Most of these oxygen-derived species are produced at a low level by normal

aerobic metabolism. Normal cellular defense mechanisms destroy most of these. Likewise, any damage to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart [7]. A growing body of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play a pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer [8].

Telfairia occidentalis popularly known as fluted pumpkin is a member of Cucurbitaceae family. The plant is native to West Africa and cultivated in Southern Nigeria mainly for the leaves and seeds which are eaten because of their high content of protein, vitamins and minerals [9]. *T. occidentalis* leaf are often used as vegetable in the preparation of soups, while the seeds are eaten raw or roasted and also ground into powder and used as soup thickening. Reports of hypoglycaemic and antidiabetic activities, antioxidant and antimicrobial activities of the leaf have been published [10]. Several workers have reported on the nutritional composition, chemical characterization and functional properties of fluted pumpkin seed [11].

Nigeria has rich genetic resources of cultivated, semi-wild and wild species of crops being used as traditional vegetables and different types are consumed by the various ethnic groups for different reasons. Edible leaves from vegetable plants are eaten as supporting food or main dishes. They may be aromatic, bitter or tasteless but are the cheapest and most accessible source of proteins, vitamins, minerals, essential amino acids. Leaf vegetables are highly beneficial for maintenance of health and prevention of diseases. There are different types of vegetables and each group contributes in its own way to the diet [11]. They play prominent roles in the traditional food culture and various ethnic groups consume a variety of different indigenous types of vegetables for different reasons, some have medicinal properties reserved for the sick and recuperation. In this study, the effect of *Telfairia occidentalis* was therefore determined in oxidative stress using albino Wistar rats.

2.Materials and Methods

2.1. Animal Model

Wister strain albino rats with weight range of (95.7-202.6) obtained from the animal house, Madonna University Elele, Nigeria were used for the study. The rats were housed in wire meshed cage under standard conditions (temperature 25 - 29°C, 12 hours light and 12 hours darkness cycles) and fed with standard rat pelleted diet and water.

2.2. Experimental Design

12 albino rats were used as animal model. They were housed in 4 meshed cages containing three rats in each cage. The rats were made to acclimatize for 1 week before the experiment began. They were allowed to feed on standard feed and water freely throughout the period the experiment lasted. Administration of the aqueous pumpkin extract and diluted acetone was administered orally.

2.3. Ethical Approval

The research was approved by the ethical committee of the institution. The standard, rules and regulations of use of animal for research purposes was strictly adhered to as approved by the committee.

2.4. Order of Placement

Group1: The rats in this group were administered with feed, water, 2ml of diluted acetone and 250mg/kg of pumpkin extract.

Group2: The rats in this group were administered with feed, water, 2ml diluted acetone and 500mg/kg of pumpkin extract.

Group3: The rats in this group were administered with feed, water and 2ml of diluted acetone.

Group4: The rats in this group served as control, they were administered with feed, water and 0.5ml of normal saline.

2.5. Induction of Oxidative Stress Using Diluted Acetone

Group 1, 2, and 3 was induced with acetone (100ml of acetone diluted with 900ml of distil water). 2ml syringe was used for the administration for a period of 6days so as to induce in them oxidative stress. 2ml of the acetone was administered twice daily morning and evening to group one, two and three.

2.6. Preparation and Administration of Pumpkin Extract

The pumpkin was dried for four (4) days. The dried pumpkin was grinded into powdered form. 600ml of deionized water was added to the grinded pumpkin and was allowed to stay for 24hours. Then it was filtered using filter papers and beakers. After the filteration, it was allowed to dry in a water bath and was set at 50^{o}C and when dried was in a paste form. Different concentrations of the prepared extract was made for different groups according to their body weights. The weight of the rats was determined before induction of aqueous pumpkin extract and diluted acetone. The group 1 and 2 of the rats was given pumpkin extract, and diluted acetone, group 3 was given acetone only and group 4 normal saline. The administration of the aqueous pumpkin extract was once daily for 7 days.

2.7. Sample Collection

4ml of blood sample was collected by ocular puncture from each of the animal model using capillary tube and was dispensed into commercially prepared concentrations of ethylene diamine tetra acetic acid containers,

2.7.1. Sample Analysis

Blood samples collected were analyzed within six hours of collection for Hb, PCV and WBC analysis using standard manual methods [12].

3. Results

Table 1. Mean±SD of Hb, PCV and WBC for control, acetone only and acetone+extract

Parameters	Control (Group 4)	Acetone only (Group 3)	Acetone + 250mg/kg (Group 1)	Acetone + 500mg/kg (Group 2)	pvalue
Hb (g/dl)	14.5 ± 1.07	11.9 ± 0.52	12.5 ± 1.02	12.8 ± 0.82	p<0.05
PCV (%)	42.5 ± 1.50	39.6 ± 0.82	41.1 ± 1.35	40.2 ± 0.90	p>0.05
WBC ($x10^9$/l)	8.0 ± 0.38	7.3 ± 0.28	7.5 ± 0.42	7.5 ± 0.43	p>0.05

4. Discussion

Oxidative stress occurs when the production of reactive oxygen species (free radicals) exceeds available antioxidant systems. Interaction of these free radicals with DNA in mitochondria and the nucleus leads to mutations and deletions especially in the mitochondria where DNA repair mechanisms are less efficient [13]. In this study, oxidative stress was induced in the rats using acetone.

There was a significant difference when haemoglobin concentration (Hb) was compared among the groups. The control group and acetone only group had 14.5±1.07 and 11.9±0.52 while the 250mg/kg and 500mg/kg of the extract had 12.5±1.02 and 12.8±0.82 respectively. The acetone only group recorded a reduced haemoglobin level but was raised in the pumpkin extract group especially the 500mg/kg (Group 2). The extract was able to raise the haemoglonin level thereby conferring some form of protection in oxidative stress state. Cisplatin, a potent anti-tumour agent and selenium co-treatment have been recorded to elevate haematological parameters in oxidative stress state [14].

The Packed cell volume (PCV) was also analysed and compared. Though not significant, the PCV value was raised from 39.6±0.82 in acetone only group to 41.1±1.35 and 40.2±0.90 in 250mg/kg and 500mg/kg groups respectively.

Though much reference is unavailable to compare this with previous studies. References [15] and [16] have demonstrated that extract of *Campomanesia xanthocarpa* and ascorbate improves haematological parameters and protects against oxidative stress.

Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanism can lead to damage of cellular organelles and enzyme [17]. From the result, WBC was not significant (p>0.05) when compared. The acetone only group recorded 7.3±0.28 while the 250mg/kg group and 500mg/kg group had 7.5±0.42 and 7.5±0.43 respectively. The extract of *Telfairia occidentalis* does not impact any change or improvement of the immune system in oxidative stress.

Based on the results of this study, we can conclude that the extract of *Telfairia occidentalis* may possibly improve haematological parameters and confer protection in oxidative stress conditions.

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