



Stimulation of haemopoiesis and up-regulation of T-helper cells production in mice by different fractions of *Telfairia occidentalis* Hook F and *Tectona grandis* Linn.

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ABSTRACT

The effect of different solvent fractions of *Telfairia occidentalis* and *Tectona grandis* on immunological and haematological parameters of normal mice was investigated. Dynamogen administered at the dose of 50 mg/kg body weight served as the standard drug control. The administration of the different fractions of *Telfairia occidentalis* and *Tectona grandis* at doses of 200 and 400 mg/kg body weight for 14 days resulted in stimulation of increased production of CD₄⁺ cells, total white blood cell (tWBC) and packed cell volume (PCV) in mice. The result showed that there was a significant ($p < 0.05$) increase in the CD₄⁺ counts, tWBC and percent PCV when compared to the group not given any extract which served as negative control and the group administered dynamogen. Although the different fractions of the extracts exhibited certain levels of stimulation, crude extract and ethyl acetate fraction showed the highest stimulation of immune cells investigated. These results therefore suggest that the different fractions of *Telfairia occidentalis* and *Tectona grandis* are capable of boosting immunological and haematological status in normal and may be very useful in infections and disease states.

Keywords: *Telfairia occidentalis*, *Tectona grandis*, Dynamogen, Haematological, Immunological.

INTRODUCTION

The reality of our modern society is the avalanche of activities that elevate free radicals generation, engender stress, ultimately weaken the immune system and increase susceptibility to infections and diseases. The immune system is known to be involved in the etiology as well as the pathophysiologic mechanism of many diseases [11]. The immune system can be influenced by nutritional/metabolic status [17]. Agents that alter the immune system either by stimulating or suppressing it are of great significance in managing immunological disorders and are known as immunomodulators [22]. Immunostimulatory therapy is now recognized as an alternative to conventional chemotherapy for a variety of disease conditions involving the impaired immune response of the host [7]. Immunostimulators are known to support T-cell function, activate macrophages and granulocytes and complement natural killer cells apart from the production of various effector molecules generated by activated cells [32]. Helper T-cells are arguably the most important cells in adaptive immunity as they are required for almost all adaptive immune responses. They do not only activate B-cells to secrete antibodies and macrophages to destroy ingested

microbes, but they also help activate cytotoxic T-cells to kill infected target cells. Helper T-cells regulate both innate and adaptive immune responses and help determine which immune response the body makes to a particular pathogen [15]. As dramatically demonstrated in AIDS patients, without helper T-cells the body becomes defenceless even against many microbes that are normally harmless.

Anaemia is one of the most common haematological problems affecting people of all ages. It results from the decrease in the oxygen carrying capacity of the blood due to a reduction in the number of circulating red blood cells, and also the decrease in the amount of haemoglobin present in RBCs [14, 9]. It reflects an abnormality in the number, structure, or function of RBCs [19]. Reduction of haemoglobin is usually accompanied by a fall in red cell count and packed cell volume (PCV).

White blood cells (leukocytes) are the cells of the immune system. They defend the body against pathogens, infections and foreign materials [23]. White blood cells are central to the effectiveness of both innate and adaptive immune responses of the host.

Telfairia occidentalis leaves are rich sources of protein, oil, vitamins and minerals, enhances, nourishes, protects and heals the body. The leaves are low in crude fibre but rich sources of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C and K [2] and are cheap nitrogen and mineral sources [6]. Many researchers have observed free radical scavenging ability and antioxidant property in *Telfairia occidentalis* [21, 20, 1, 14].

Tectona grandis on the other hand is a medicinally important plant. Various parts of this plant are used to treat many kinds of disease conditions such as bronchitis, constipation, hyperacidity, dysentery, burning sensation, diabetes, leprosy, skin diseases, leucoderma, headache, piles, indigestion, and as worm expeller [15, 16] and have been shown to possess anti-inflammatory activity. Both plants are known to be rich in phytochemicals and antioxidant compounds which are vital sources of immunomodulating agents [9]. *Telfairia occidentalis* Hook F and *Tectona grandis* Linn are well known for their medicinal properties however there is no much documented evidence of any investigation on this aspect of immune response.

EXPERIMENTAL SECTION

Collection and identification of plant materials

The leaves of *Telfairia occidentalis* Hook F and *Tectona grandis* Linn used for this study were bought from the Nsukka main market in Enugu State, Nigeria and were identified by Mr. Alfred Ozioko of the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka. The fresh leaves were first washed with distilled water and subsequently, normal saline to remove dirt and possible mycotoxins. The samples were dried under shade for several days and then pulverized into fine powder.

Extraction of plant materials

A quantity, 400g each of the leaves *Telfairia occidentalis* and *Tectona grandis* were macerated in 1.2 L of ethanol for 48h. The solutions were filtered with Whatman no. 4 filter paper and the filtrates were concentrated to a powdered residue using a rotary evaporator.

Fractionation of the Extract

The dried ethanol extract (20 g) was fractionated in a glass column (150 cm x 1.5 cm) packed with 200 g of a slurry of silica gel (70-230 mesh). The column was eluted in succession with 500 mL chloroform, 600 mL ethyl acetate 500 mL ethanol and 500 mL methanol to obtain ethyl acetate (EAF), ethanol (EF) and methanol (MF) fractions, respectively. Chloroform did not give any yield of extract.

Column chromatographic separation

About 5 g of the methanol fraction was chromatographed on silica gel (70-230 mesh, 200 g) packed into a glass column (1.5 x 150 cm) with the bed of 100 cm height. The elution was performed with gradient mixtures of hexane:chloroform 1:3 (400 mL); 1:6 (400 mL); 1:9 (400mL); chloroform: ethyl acetate 1:1 (400 mL), 1:2 (400 mL), 1:5 (400 mL) and finally ethyl acetate: methanol 1:1 (400 mL), 1:3 (400 mL), 1:5 (400 mL), 1:7 (400 mL). Aliquots of 20 mL were collected and monitored with TLC. Similar fractions were combined to get the major column fractions.

EXPERIMENTAL ANIMAL

Adult mice weighing between 22-32g were obtained from the Department of Veterinary Medicine University of Nigeria, Nsukka. The animals were acclimatized for 7 days under standard environmental conditions, with a 12 hour light / dark cycle maintained on a regular feed (Top feed; grower mash) and water ad libitum.

Experimental Design

The animals were divided into eighteen (18) groups of four (4) mice group as follows:

- Group 1: Received 200 mg/kg body weight of crude ethanol extract of *Telfairia occidentalis*
- Group 2: Received 400 mg/kg body weight of crude ethanol extract of *Telfairia occidentalis*
- Group 3: Received 200 mg/kg body weight of ethanol fraction of *Telfairia occidentalis*
- Group 4: Received 400 mg/kg body weight of ethanol fraction of *Telfairia occidentalis*
- Group 5: Received 200 mg/kg body weight of methanol fraction of *Telfairia occidentalis*
- Group 6: Received 400 mg/kg body weight of methanol fraction of *Telfairia occidentalis*
- Group 7: Received 200 mg/kg body weight of ethyl acetate fraction of *Telfairia occidentalis*
- Group 8: Received 400 mg/kg body weight of ethyl acetate fraction of *Telfairia occidentalis*
- Group 9: Received 200 mg/kg body weight of crude ethanol extract of *Tectona grandis*
- Group 10: Received 400 mg/kg body weight of crude ethanol extract of *Tectona grandis*
- Group 11: Received 200 mg/kg body weight of ethanol fraction of *Tectona grandis*
- Group 12: Received 400 mg/kg body weight of ethanol fraction of *Tectona grandis*
- Group 13: Received 200 mg/kg body weight of methanol fraction of *Tectona grandis*
- Group 14: Received 400 mg/kg body weight of methanol fraction of *Tectona grandis*
- Group 15: Received 200 mg/kg body weight of ethyl acetate fraction of *Tectona grandis*
- Group 16: Received 400 mg/kg body weight of ethyl acetate fraction of *Tectona grandis*
- Group 17: Received 50 mg/kg body weight of Dynamogen (standard drug)
- Group 18: Normal mice received only normal saline.

At the end of the experimental period the mice were starved for 12 h and then sacrificed under ether anaesthesia. Blood samples were received into clean EDTA tubes for immunological and hematological studies.

Determination of CD4⁺ count

This was done using Flow Cytometry technique according to the method of [10].

Principle

CD4-PE fluorescence is analysed on a cyflow cytometer with an excitation light source of 488nm or 532nm.

Procedure

A volume 20 µl of well mixed anticoagulated blood was added to a test tube. To this was added 20 µl of CD4 mAb PE mixed gently and incubated for 15 mins at room temperature protected from light. 800 µl of no lyse buffer was added and shaken. The mixture was loaded into the cyflow device and analysed.

Determination of packed cell volume

The percentage packed cell volume was determined according to hematocrit method as described by [22].

Principle: When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.

Method: Using microhaematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until they are approximately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with plastic seal and placed in the medial grooves of the centrifuge, head exactly opposite each other, with the sealed end away from the centre of the centrifuge. All tubes were spun for five minutes at 1000 rpm. The tubes were removed as soon as the centrifuge had stopped spinning.

Calculation: PCV was obtained for each tube using microhaematocrit-reader by measuring the height of the RBC column and expressing this as a ratio of the height of the total blood column.

$$\text{PCV (\%)} = \frac{\text{Height of cell column}}{\text{Height of total blood column}} \times 100$$

Determination of total leucocyte count by Haemocytometry

The total leucocyte count was determined by haemocytometry following the method described by [22]. The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leucocyte. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid and the cells were counted under low power microscope by using a counting chamber. The number of cells in undiluted blood was reported as the number of white cells/cu.mm of whole blood. The additional requirements are the following:

1. WBC pipette
2. WBC diluting fluid.

This contains 1% acetic acid solution tinged with gentian violet stain. Acetic acid facilitates haemolysis of RBC and gentian violet stains the nuclei of RBC.

Procedure

An aliquot (0.02ml) of blood was added to 0.38ml of diluting fluid (Acetic acid, tinged with gentian violet) and mixed. The counting chamber was charged with the well-mixed diluted blood (after discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 minutes. The four corners of the chamber was visualised under a low power (10X) objective and the cells were counted in all the four marked corner squares.

Calculation

Total WBC / cu mm = Number of cells counted x dilution factor

Area counted x depth of fluid

Where

(1) Dilution = 1:200

(2) Area counted = 1/5 sq.mm

(3) Depth of fluid= 1/10mm

(4) Number of red cells counted = N x 200

Total WBC = N x 200 x 50 = N x 10,000

1/5x1/10

Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean \pm standard deviation. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Telfairia occidentalis and *Tectona grandis* leaf extracts in this study caused significant ($P < 0.05$) increases in percentage packed cell volume (PCV) and total white blood cell (tWBC) count of mice treated with crude extracts and different fractions compared to the untreated normal control mice. The results equally showed a dose dependent effect with the higher doses of the extract giving a more significant increase than the lower doses. The result is consistent with the report of other researchers [5, 31] who had earlier observed increase in haematological indices of rat by leaf extract of *Telfairia occidentalis* and bark extract of *Tectona grandis*. The increases in these parameters suggest an increased production of majority of the cells involved in the immune system which are produced in the stem cells of the bone marrow. These extracts could be considered to have a stimulatory effect on the immune responses considering that increased production of the immune system cells may imply an enhanced immune system function. Lymphocytes are usually depleted during active infection. *Telfairia occidentalis* and *Tectona grandis* has been shown to possess haematonic abilities as they stimulate the activity of the bone marrow and has been reported to manage experimental induced haemolytic anaemia [27].

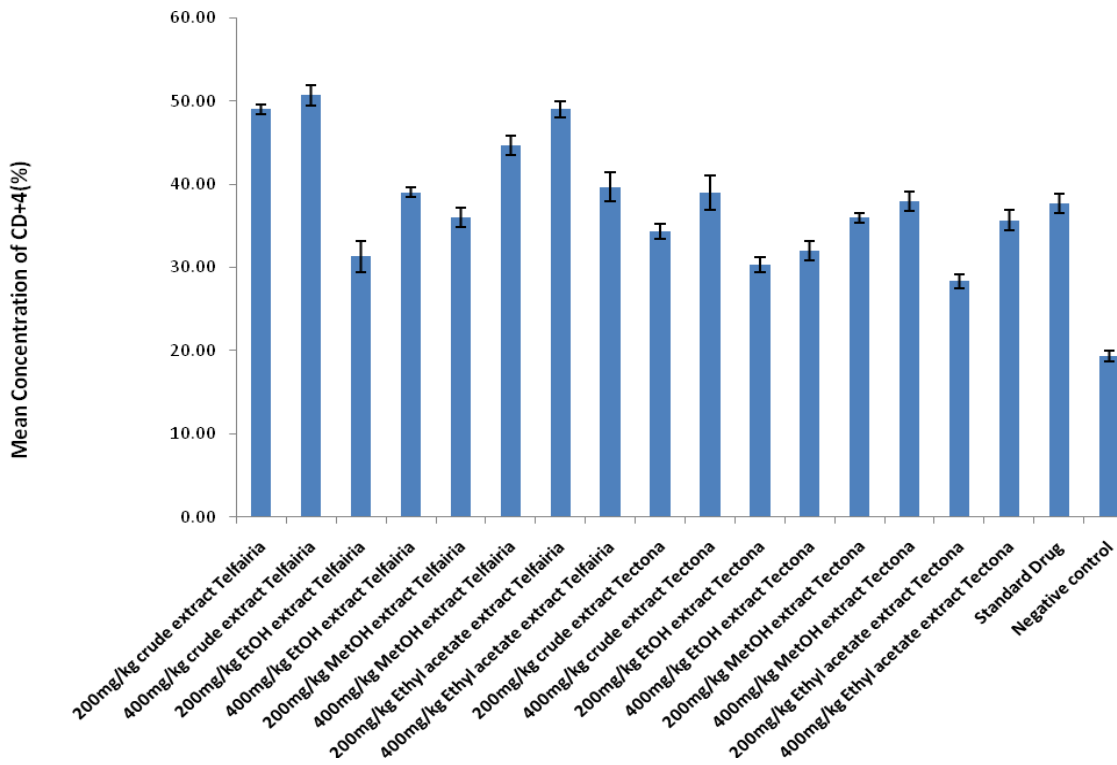


Fig. 1: Effects of crude extract solvent fractions of *Telfairia occidentalis* and *Tectona grandis* on CD⁺4 Count in Mice
Key: EtOH: Ethanol, MetOH: Methanol

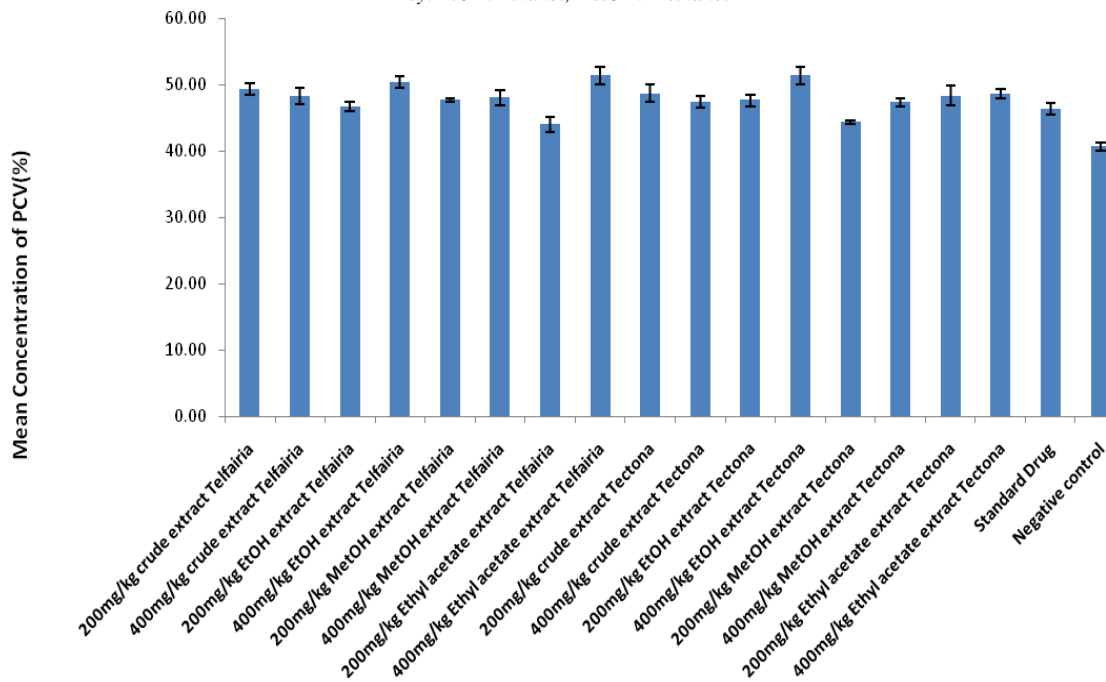


Fig.2: Effects of crude extract solvent fractions of *Telfairia occidentalis* and *Tectona grandis* on percentage packed cell volume (PCV) in Mice
Key: EtOH: Ethanol, MetOH: Methanol

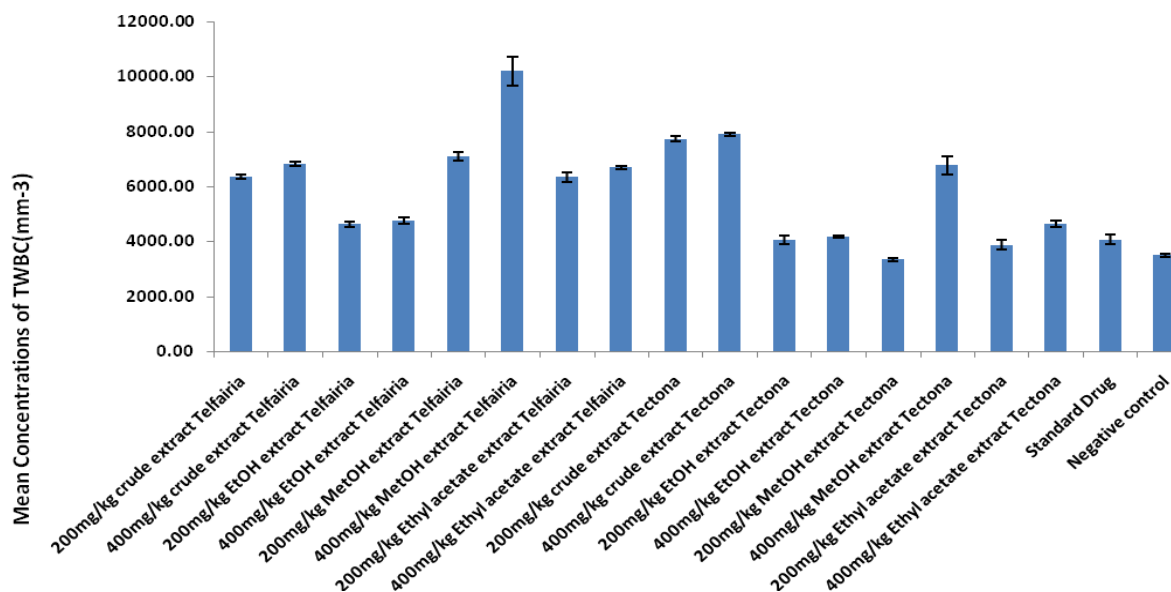


Fig.3: Effects of crude extract solvent fractions of *Telfairia occidentalis* and *Tectona grandis* on tWBC count in Mice
Key: EtOH: Ethanol, MetOH: Methanol

Helper T-cells are arguably the most important cells in adaptive immunity as they are required for almost all adaptive immune responses. They do not only activate B-cells to secrete antibodies and macrophages to destroy ingested microbes, but they also help activate cytotoxic T-cells to kill infected target cells. Helper T-cells regulate both innate and adaptive immune responses and help determine which immune response the body makes to a particular pathogen [18]. Without helper T-cells the body becomes defenceless even against many microbes that are ordinarily harmless.

In this study, the extracts stimulated the increased production of T-helper cells in mice significantly ($P < 0.05$) when compared to the untreated normal control and mice and those administered dynamogen, a standard immune boosting drug. CD_4^+ is an established marker of T-helper cells status and this was significantly ($P < 0.05$) increased in the study. This result suggests that stimulation of T-helper cell production may be one of the mechanisms through which the extracts exert their immune-modulatory effects.

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