



Aqueous extracts of *Telfairia occidentalis* leaf reverses pyrogallol induced leucopenia and stimulates the immune system in wistar albino rats

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ABSTRACT

Studies have reported that plant extracts possess medicinal values including immunomodulatory activities. This study was carried out to evaluate the immunomodulatory effect of different concentrations of aqueous extracts of *Telfairia occidentalis* in male wistar albino rats. The extract was administered orally by gavage at doses of 100mg/kg and 300mg/kg daily to immune depressed rats divided into 4 groups for 21 days. The assessment of immunomodulatory effect of this plant was carried out by testing the changes in the humoral and cellular immune responses of the animals challenged by sheep red blood cells (SRBCs). The results obtained showed a significant ($p < 0.05$) increase in the TWBC at 300mg/kg dose and no significant difference ($p > 0.05$) at the 100mg/kg dose when compared to the control. The PCV test showed a significant ($p < 0.05$) increase at both doses (100mg/kg and 300mg/kg). The RBC count showed a significant ($p < 0.05$) increase at the 300mg/kg dose while there was no significant difference at the 100mg/kg dose. The DTH response also showed a significant increase at both doses compared to the control. The plant extract equally showed elevated humoral antibody response at dose of 100mg/kg and 300mg/kg and this was significant. In all, *Telfairia occidentalis* exhibited an immunostimulatory effect on the humoral immune function and anti-inflammatory activity on the cell mediated immune response in wistar albino rats.

Keywords: *Telfairia occidentalis*; Immunomodulatory; Humoral antibody; Delayed-type hypersensitivity; anti-inflammatory.

INTRODUCTION

Immunology is probably one of the most rapidly developing areas of biomedical research holding great promise with regard to prevention and treatment of wide range of disorders. Immune systems can be modulated in different ways with the use of a very wide variety of substances, behavioural changes, dietary modification etc (4). Immunomodulatory agents are intended to interfere with the functions of the immune system to produce a therapeutic result by shifting the homeostasis of the immune system to reduce or eliminate disease symptoms (24). The primary object in the past has been to suppress immune system to permit allotransplantation. Activation of the immune system by "non-self" antigen (alloantigen) or "self antigen" (autoantigen) is generally believed to require processing of the antigen by the phagocytic cells such as macrophages, monocytes or related cells (25). Immunomodulation is a procedure involving the adjustment of the immune system to a desired level as in immunopotentialiation, immunosuppression or induction of immunological tolerance (10). Plants are the essential and

integral part in complementary and alternative medicine and due to this, they develop the ability to form secondary metabolites like alkaloids, flavonoids, steroids and phenolic substances which are in turn used to restore health and heal diseases (24). Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against such pathology (2,5). Because of the concerns about side effect of conventional drugs, the use of natural products as an alternative to conventional treatment in healing and treatment of many diseases has been on the rise in the last few decades. Medicinal plants serve as therapeutic alternatives, safer choices or in some cases as the only effective treatment. It has been shown that most pharmacological activities are related to the immunostimulatory and antioxidant properties of plant secondary metabolites. A large number of these plants and their isolated constituents have shown beneficial effects such as antioxidant, anti-inflammatory, anticancer, antimicrobial and immunomodulatory effects (16). *Telfairia occidentalis* is well known for its medicinal properties, however there is no documented evidence of any investigation of its immunomodulatory actions (12). It has been reported to possess antioxidant and free radical scavenging effect, antidiabetic, antiplasmodial and antibacterial properties as well as able to boost blood level (12, 17). It is also known to be rich in nutrients such as amino acids (9)

EXPERIMENTAL SECTION

Plant Material

Fresh leaves of the plant *Telfairia occidentalis* were collected from a local farm at Umuariaga village in Umudike, Abia State, Nigeria. The leaves were shade-dried for 5 days and then ground to powder from which extracts used in the study were prepared.

Animals

Healthy male Wistar albino rats weighing 80-120 grams were used for the study. All animals were housed in an animal house under normal room conditions. A commercial pellet diet and water were fed the animals ad libitum.

Preparation of Plant Extract

The dried powdered plant material leaves (10 grams) was dissolved in 100ml of distilled water and left to stand for 24 hours then filtered. The filtrate was concentrated and used for the study.

Antigen Preparation

Fresh blood was collected from sheep sacrificed in a local slaughter house and preserved in EDTA bottles. It was washed three times with normal saline via centrifugation. The suspension was adjusted to 1×10^8 SRBC/ml for immunization and challenge.

Treatment Regimen

Animals were divided into four groups each having four rats. Group I (normal control) received oral administration of water via gavage. The dose volume for this group was 0.3ml. Group II (negative control) received gavage administration of 2% pyrogallol solution with dose volume of 0.3ml. Group III received the plant extract at a dose of 100mg/kg body weight (bw) and pyrogallol solution. This group was referred to as the low dose group. The group IV animals received also the same gavage administration of the plant extract of 300mg/kg bw and pyrogallol solution. This group was referred to as the high dose group. All administrations were done daily for 21 days.

Sheep Red Blood Cell (SRBC) – Induced Humoral Antibody (HA) Titer

To specifically assess effects on antibody formation, groups of four rats per treatment were immunized with 0.1ml of sheep Red Blood Cell suspension (1.0×10^8 SRBC/ml) intraperitoneally. The day of immunization was referred to as Day 0. Seven days later (Day +7), the rats were challenged by injecting 0.1ml of 1.0×10^8 SRBC suspension into the left hind foot pad of the rats. Blood samples were collected from all the animals separately by ocular puncture using glass capillary tubes on Day + 7 (after challenge) for measurement of primary antibody titer and on Day +14 for measurement of secondary antibody titer. Antibody levels were determined by the method described by Agrawal, *et al.*, (2010). After allowing the collected blood to clot, serum was isolated and 25microL was placed into one well of a 96-well micro titer plate. Serial two-fold dilutions of the serum were made using 25microL normal saline each time of transfer across the plate. To the 25 microL diluted serum in each well, 25 microL of 1% v/v SRBC suspension in normal saline was added. The micro titer plate was maintained at room temperature for 1 hour and the content then examined visually for haemagglutination. The value of the highest serum dilution showing haemagglutination was defined as the antibody titer for the given rat

Sheep Red Blood Cell – Induced Delayed Hypersensitivity (DTH) Response

A modified method of Gaikwad and Mohan (2011) was used to analyze effects on DTH responses in the treated rats. Daily treatment with *Telfairia occidentalis* leaf extract (by gavage) began 14 days prior to the challenge. The negative and normal control rats received water respectively each day. On Day 0 all rats were immunized. After 14 days of gavage treatment, 0.1ml of SRBC solution injected subcutaneously into their right hind footpad, the thickness of each rat's left footpad was measured just before the challenge using a Schneltaster caliper that could measure to a minimum unit of 0.01mm. The rats were then challenged by injecting 0.1ml of SRBC solution intraperitoneally into their left hind footpad (deemed time 0). Footpad thickness was the re-measured after 24hrs. The difference between the thickness of the left footpad just before and 24 hours after challenge (in mm) was taken as a measure of DTH.

Carbon Clearance

The method described by Dash *et al.*, (2006) was used to analyze phagocytic activity by the white blood cells in rats. For each treatment regimen, a total of 16 rats were utilized. Daily treatment with *Telfairia occidentalis* leaf extract (by gavage) occurred for 14 days prior to the assessment of in situ phagocytic activity. The negative and normal control groups received pyrogallol and water respectively, daily. A colloidal carbon ink suspension was injected via the tail vein into each rat 48 hours after the final treatment. From each rat, blood samples (25ml) were then withdrawn from the retro-orbital plexus under mild ether anesthesia, immediately after the injection and then 15 minutes thereafter. Each blood sample was lysed with 2ml of 0.1% acetic acid and the absorbance of the resulting solution evaluated at 675nm. The phagocytic index K, was calculated using the following equation:

$$K = \frac{(\text{Loge OD1} - \text{Loge OD2})}{t_1 - t_2}$$

Where OD1 and OD2 are the optical densities at time t_1 and t_2 respectively.

Total White Blood Cell (TWBC) Count, Red Blood Cell (RBC) Count, Haemoglobin Estimation, and Packed Cell Volume (PCV) was determined using standard haematological technique as described by Ochei and Kolharktar (2008)

Statistical Analysis

Statistical analysis of the data obtained from the experiment was performed/ carried out using the one way analysis of variance (ANOVA) followed by post HOC LSD test. The significance in the difference was accepted at $p > 0.05$. The results are expressed as mean \pm SD (standard deviation).

RESULTS

Table 1. Effect of aqueous extract of *Telfairia occidentalis* leaf on humoral antibody titre.

GROUPS	MEAN \pm SD(PRIMARY)	MEAN \pm SD (SECONDARY)
Negative Control	0.67 \pm 1.155	4.00 \pm 3.464
Normal Control	4.00 \pm 0.000	42.67 \pm 18.475
100mg/kg	*4.00 \pm 0.000	*24.00 \pm 11.314
300mg/kg	*6.00 \pm 2.828	*48.00 \pm 22.627

Table 1 shows a dose dependent significant increase in humoral antibody formation compared to the negative control.

Table 2. Effect of aqueous extract of *Telfairia occidentalis* on DTH response

GROUPS	MEAN \pm SD
Negative Control	46.667 \pm 0.207
Normal Control	95.333 \pm 0.438
100mg/kg	55.295 \pm 0.361
300mg/kg	112.125 \pm 0.601*

Table 2 shows a dose dependent significant increase in DTH response compared to the negative control.

Table 3. Effect of aqueous extract of *Telfairia occidentalis* on Carbon Clearance

CARBON CLEARANCE	MEAN ± SD
Negative Control	0.067 ± 0.058
Normal Control	0.133 ± 0.000
100mg/kg	0.113 ± 0.000*
300mg/kg	0.113 ± 0.000*

Table 3 shows a significantly ($p < 0.05$) increased phagocytic activity in Rats treated with different doses of the extract compared to negative control.

Table 4. Effect of aqueous extract of *Telfairia occidentalis* on Haematological parameters

GROUPS	TWBC	RBC	HB	PCV
Negative Control	2333.33 ± 0.416	143.33 ± 11.547	11.000 ± 0.400	32.00 ± 2.000
Normal Control	4833.33 ± 0.472	193.33 ± 5.774	12.933 ± 0.416	38.67 ± 1.155
100mg/kg	2250.00 ± 0.212	205.00 ± 7.07*	12.800 ± 0.283*	38.50 ± 2.121*
300mg/kg	4800.00 ± 0.707*	360.00 ± 0.565*	14.100 ± 0.990*	42.00 ± 2.828*

Values are presented as mean ± SD. $P < 0.05$ is considered to be significant difference compared to the negative control ($n=4$). *denotes significant result.

Table 4 shows a significant stimulation of the haematopoietic cells by the plant extract at low and high doses of 100mg/kg and 300mg/kg bw.

DISCUSSION

Telfairia occidentalis (TO) a medicinal plant that have been shown to possess antioxidant and anti-diabetic properties has also been reported to manage garlic induced haemolytic anaemia (17). In this study, the immunomodulatory effects of *Telfairia occidentalis* was studied on Wistar albino rats. The results seem to suggest that the extract was able to improve the response of the host immune system against a pathogen by activating the system.

Telfairia occidentalis leaf extract in this study showed significant increase in key haematological indices such as PCV, RBC, WBC and Haemoglobin concentration of the experimental rats treated with the extracts compared to the control especially at higher doses. The increase in these parameters suggests an increased production of majority of the cells involved in the immune system which are produced in the stem cells of the bone marrow. This 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 (25). Delayed-type hypersensitivity reaction (DTH) is characterized by influx of non-specific cells including macrophages. The DTH reaction is directly correlated with the host's cell-mediated immune function which involves the T-lymphocytes and their products (lymphokines). In this reaction, the T-lymphocytes get sensitized when they are challenged by an antigen and are converted to lymphoblasts which produce lymphokines. The lymphokines recruit scavenger cells to the target site promoting increased phagocytic activity and killing of the 'foreigners' (15). The infiltrating cells are probably immobilized to promote the defensive (inflammatory) reactions (10). This study showed an increase in DTH response in the rats that received the plant extract suggesting an increase in inflammatory reactions in the host's cell (13). Effect on inflammatory response is a clear indication of modulation of the immune system() Oluwole et al., (19) had earlier reported that *Telfairia occidentalis* possess anti-inflammatory activity. It is possible that the extract is able to activate different immunological pathways leading to either pro or anti-inflammation depending on the prevailing need of the immune system. The rate of carbon clearance is usually used as a measure of reticuloendothelial system (RES) competency so that a faster removal of particles is correlated with an enhanced phagocytic activity of the RES cellular component (1). This present study showed an increase in the rate of carbon clearance in the rats that received the plant extract.

The plant extracts equally showed an increased humoral antibody response at both concentrations of 100mg/kg and the 300mg/kg dose. These antibodies which are products of B-lymphocytes are central to the humoral responses (15). This study therefore suggests an enhancement of the humoral response in the rats treated with the plant extract.

CONCLUSION

In this study, the extracts of *Telfairia occidentalis* leaves generally showed immunostimulatory effect on the humoral immune function and cell mediated immunity in the wistar albino rats. It is possible that the presence of phytochemicals like flavonoids and tannins as reported by previous studies may be responsible for the observed immune stimulatory ability.

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