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IMMUNOMODULATORY ACTIVITY OF ETHANOL EXTRACT OF TELFAIRIA OCCIDENTALIS IN WISTAR ALBINO RATS

ABSTRACT

The immune system is a biological structure and processes within an organism that protect against diseases. To function properly ,an immune system must detect a wide variety of agents, from viruses to parasite worms, and distinguish them from the organism's own health tissues. The effects of ethanol extract of *Telfairia occidentalis* leaf on the immune system of the wistar albino rats were evaluated. In this study, the experimental rats were divided into four groups made up of four animals each. Group 1 received 100mg/kg and pyrogallol, group 2 received 300mg/kg of the extract and and pyrogallol, group 3 received only pyrogallol (negative control) and group 4 received normal feed for 21days. The result showed a significant increase in hematological parameters of TWBC, RBC and haemoglobin in both dose groups compared to the negative control. Similarly there was a significant increase ($p<0.05$) in the humoral antibody titer response and delayed type hypersensitivity of rats treated with the extract compared to negative control. There was no significant difference ($p>0.05$) in the result of the carbon clearance test. The overall results indicated immunostimulatory properties of the plant extract.

Keywords: *Telfairia occidentalis,* Humoral antibody, Immune system, hematological parameters and pyrogallol.

INTRODUCTION

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. Pathogens can rapidly evolve and adapt to avoid detection and neutralization by the immune system. As a result, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system, in the form of enzymes that protect against bacteriophage infections. Mechanisms of immune function in very simple organisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms (Beck *et al.,* 1996). Adaptive (or acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer (Lisa *et al.,* 2001; O'Byrne and Dalglish, 2001). Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections. In humans, immunodeficiency can either be the result of a genetic disease such as severe combined immunodeficiency, acquired conditions such as HIV/AIDS, or the use of immunosuppressive medication. In contrast, autoimmunity results from a hyperactive immune system attacking normal tissues as if they were foreign organisms. Common autoimmune diseases include Hashimoto's thyroiditis, rheumatoid arthritis, diabetes mellitus type 1, and systemic lupus erythematosus.

Telfairia occidentalis is a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds, common names for the plant includes: fluted gourd, fluted pumpkin and Ugu in igbo, Iroko in yoruba and Ubong in efik. The plant is drought tolerant, diaecious perennial that is usually grown trellised. The young shoots and leaves of the female plants are the main ingredients of a Nigerian soup, edikangikong. The large dark-red seed is rich in fat and protein and can be eaten whole, ground into powder for another kind of soup or made into a fermented porridge. The fruit of the plant is large, weighing up to 13kg and in edible. The seed of *Telfairia occidentalis* is a rich source of minerals such as calcium, phosphorus, iron, zinc and copper, the oil from the seed contains 61% unsaturated fatty acids which offers protective role against atherosclerosis and cardiovascular disease (Odoemena and Onyeneke, 1998). The

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phospholipids, glycolipids and neutral lipid content of the seeds are 58, 26 and 15% respectively (Anosike, 1994) The study was carried out to determine the effect of ethanol extract of *Telfairia occidentalis* on the immune system of wistar albino rats.

MATERIALS AND METHODS

Materials

Telfairia occidentalis, Animal cages, Feeding racks, Water vessel, Feed (mash), Tissue paper and Sheep red blood cell.

Equipment Used

Weighing balance, Grinding machine, Oven (Uniscope SM9053 Surge-friends Medicals, England), Stringe and needles, Cornicalflasks, beakers,Test-tube racks, Pipette (Pyrase England), Plain specimen bottles, Centrifuge (800B Mufined Medical Manchester England), Micropipette, Measuring cylinder (Pyrase-England), Refrigerator, Timing devices, Capillary tube, Hand gloves, Hot plate (Surge-friends Medical England).

Reagent Used

Normal saline (Nacl), Distilled water, Pyrogallol, 96% Ethanol.

Methods

Collection of the Plant Material.

Leaves of *Telfairia occidentalis* were purchased from a farm at Umuariaga, Ikwuano L.G.A of Abia State and was properly identified by appropriate authority.

Preparation of the Ethanol Extract

The leaves were collected and air dried. They were ground to powder using grinder. The ethanol extracts were prepared continuously according to the demand. 10g of plant powder were dissolved in 200ml of ethanol and macerated for 48 hours. It was filtered using filter paper and concentrated by evaporation with water bath. The extract was re-dissolved in normal saline before administration.

Preparation of Pyrogallol

In this process, 5g of pyrogallol was dissolved in 50ml of distilled water and filtered.

Animal

Wistar albino rats aged 6-8 weeks were purchased from Department of Zoology University of Nigeria Nsukka. The rats were fed with normal feed. They were housed in a clean cage at room temperature. The cages were frequently cleaned and provided with clean water and vital feeds. The rats were allowed one week acclimatization on the guinea feed in a well ventilated room before the experiment. The rats were randomly divided into 4 groups of 4 animals each and housed in ventilated plastic cages.

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Group	No of animals	Diet
I	4	100mg/kg) of ethanol extract on *Telfairia occidentalis* + pyrogallol
2	4	300mg/kg)of ethanol extract on *Telfairia occidentalis* + pyrogallol
3	4	Pyrogallol (no extract)
4	4	Normal feed

Table 2: Experimental design

Preparation of Normal Saline Solution

4.5g of Nacl salt was added into 500ml of distilled water. The sheep red blood cell was washed with normal saline before use.

Plasma Analysis

Carbon Clearance Test

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:

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

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

SRBC- induced humoral antibody (HA) titer

The method described by Atal *et al*. (1986) was utilized to examine the rats after being fed with the extract of *Telfairia occidentalis* 7 days prior to sensitization and continuing up to the second time of challenge (i.e., day-7 up to and through day +14; for a total of 21days)

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To specifically assess the effect on antibody formation, the rats (both control and tests) were immunized with 0.1ml of SRBC suspension (5×10^8) SRBC/ml injected intra-peritoneally. The day of immunization was referred to as day 0. Seven days later (day + 7), blood samples were collected from all the rats through ocular puncture under light ether anesthesia on day +7 (after challenges) for assessment of primary antibody titer and on day 14. (after challenge) for measurement of secondary antibody titre.

Antibody levels were determined by the method described by shinde *et al* (1999). After allowing the collected blood to clot, serum was isolated and 25µl was placed into one well of a 96 well micro-titer plate. Serial two-fold dilutions of the serum were made using 25µl of normal saline each time of transfer across the plate. To the 25µl of diluted serum in each well was then added 25µl of 1% v/v SRBC suspension in normal saline.

The microtiter plate was maintained in room temperature for one hour and the content was well examined for haemagglutination (ie until control is well showed un-equivocally negative patterns). The value of the serum dilution showing haemagglutination was defined as the antibody titre for the given rat.

SRBC- induced delayed- type hypersensitivity (DTH) Response

The method of language *et al*. (1974) was used to analyze effects of DTH responses in the treated rats. Daily treatments with *Telfaria occidentalis* extract (by gavage) began 14 days prior to the challenge i.e., starting on the same day as immunization with SRBC. Controls received vehicle in parallel each day.

On Day 0, all rats were immunized with 0.1mL SRBC solution (1×10^8SRBC/mL) injected intra peritoneally into their right hind foot pad. After 14 days of gavage treatment, the thickness of each rat's left footpad was measured just before the challenge using a schnelltaster caliper that could measure to a minimum unit of 0.01 mm. The rats were then challenged by injecting 0.1mL SRBC solution (5×10^8 SRBC/mL) intra peritoneally into their left hind footpad. Foot thickness was then re-measured after 24hours. The difference between the thickness of the left foot just before and 48hours after challenge in (mm) was taken as a measure of DTH (Doherty,1981).

Hematological Profile

After 21 days of the repeated gavage treatment, blood samples was collected from each rat via ocular puncture under light either anesthesia, various parameters such as total white blood cell (WBC),packed cell volume(PCV), red blood cell (RBC), as well as hemoglobin (HB) levels were then evaluated using a standard hematology technique.

Red Blood Cell (RBC) Count

This was done using standard method as described by cheesebrough (2000). The blood sample was diluted 1:20 with 10% $NaCO_3$. The diluted sample was loaded into the Neubaer counting chamber with the aid of a Pasteur pipette. The RBC was counted from appropriate squares on the chamber under an electronic microscope.

Total White Blood Cell (WBC) Count

The white blood cell count was determined following the standard technique as described by Ramnik (2003). The blood sample was diluted 1:20 with Turks solution, which is 2% glacial acetic acid. The dilute sample was loaded into a Neubaer counting chamber with

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the aid of Pasteur pipette. The WBC was calculated by counting the required number of squares on the counting chamber under a microscope.

Packed Cell Volume (PCV)

This was done using standard technique as described by Ochei and Kolhartar (2008). Blood samples were collected into PCV tubes (heparinized) using capillary action. One end of the tube was sealed with plasticine and then configured using the haematocit centrifuges for 5 minutes at 2500g rpm. The test was read using a PCV haematocrit reader.

RESULTS

GROUP	TWBC
GROUP 1	4050.00 ±.35
GROUP 2	9050.00 ±.35
GROUP 3 (Negative control)	2333.33 ± .41
GROUP 4 (Normal control)	4833.33 ± .47

Significant increase in the total white blood cell count was observed in group 1 and 2 compared to the negative control.

Table 3: EFFECT OF THE PLANT EXTRACTS ON THE TOTAL WHITE BLOOD CELL COUNT.

GROUP	PCV
GROUP 1	39.50 ± .35
GROUP 2	43.00 ± .14
GROUP 3 (Negative control)	32.0 ± .20
GROUP 4(Normal control)	38.60 ± .11

PCV of group 1 and group 2 increased significantly in packed cell volume compared to the negative control..

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Table 4: EFFECT OF THE PLANT EXTRACTS ON THE PACKED CELL VOLUME.(PCV).

GROUP	RBC
GROUP 1	195.00 ± .70
GROUP 2	375.00 ± .35
GROUP 3(Negative control)	143.33 ± .11
GROUP 4(Normal control)	193.33 ± .57

Showing significant increase in red blood cell count in group 1 and 2 when compared with group 3

Table 5: EFFECT OF THE PLANT EXTRACTS ON THE RED BLOOD CELL COUNT (RBC)

GROUP	HB
GROUP 1	13.40 ± .11
GROUP 2	14.90 ± .70
GROUP 3(Negative control)	11.0 ± 0.40
GROUP 4 (Normal control)	12.93 ± 0.41

Showing significant increase in heamoglobin concentration in group 1 and 2 when compared with group 3.

Table 6: EFFECT OF THE PLANT EXTRACTS ON THE HEAMOGLOBIN ESTIMATION

GROUP	DTH
GROUP 1	0.30 ± .01
GROUP 2	0.78 ± 0.13
GROUP 3(Negative control)	46.66 ± 20.22
GROUP 4(Normal control)	95.33 ± 43.88

Significant increase in the delayed type hypersensitivity in group 1 and 2 compared with group 3.

Table 7: EFFECT OF THE PLANT EXTRACTS ON THE DELAYED TYPE HYPERSENSITIVITY (DTH) VALUE OF THE RAT REPRESENTED AS MEAN AND STANDARD DEVIATION.

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GROUP	CARBON CLEARANCE VALUE
GROUP 1	0.05 ± .07
GROUP 2	0.11 ± 0.00
GROUP 3(Negative control)	0.07 ± 0.06
GROUP 4(Normal control)	0.13 ± 0.00

Group 1 decreased significantly in the carbon clearance when compared with group 3. Group 2 increased significantly in the carbon clearance when compared with group 3.

Table 8: EFFECT OF THE PLANT EXTRACTS ON THE CARBON CLEARANCE VALUE OF THE RATS REPRESENTED AS MEAN AND STANDARD DEVIATION.

GROUP	PRIMARY TRITE
GROUP 1	6.00 ± 2.83
GROUP 2	10.00 ± 8.49
GROUP 3(Negative control)	2.67 ± 1.16
GROUP 4(Normal control)	4.00 ± 0.00

In primary trite, group 1 and group 2 increased significantly in the SRBC- induced humoral antibody when compared in group 3

Table 9: EFFECT OF THE PLANT EXTRACTS ON THE SRBC-INDUCED HUMORAL ANTIBODY (HA) TITER OF THE RATS REPRESENTED AS MEANS AND STANDARD DEVIATION.

GROUP	SECONDARY TRITE
GROUP 1	24.00 ± 11.31
GROUP 2	40.00 ± 33.94
GROUP 3(Negative control)	4.00 ± 3.46
GROUP 4(Normal control)	42.67 ± 18.48

In secondary trite, group 1 and group 2 increased significantly in the SRBC-induced humoral antibody when compared with group 3.

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DISCUSSION

Telfaria occidentalis has been shown to posses haematinic abilities as it stimulate the activity of the bone marrow. (Salman *et al*., 2008). This study investigated the possible effect of the ethanol leaf extract on immunological indices on rats. The results obtained showed a consistent significant increase in haematological indices of total white blood cell count (TWBC), Red blood cell (RBC) count, packed cell value (PCV) and haemoglobin concentration in test rat compared with the negative control and the normal control in some cases. The results equally showed a dose dependent effect with the higher dose of the extract giving a more significant increase than the lower dose. The result is consistent with the report of Alada (2000) who had earlier observed increase in haematological indices of rat fed with leaf extract of *Telfairia occidentalis.* The plant is shown to be rich in chemical constituents like proteins, vitamin A, C, Zinc, iron and magnesium (Fasuyi 2006). These could be responsible for the result observed in the study.

The result of Delayed Type Hypersensitivity (DTH) and Humoral Antibody titer showed a significant increase in the rat treated with the extract compared to negative control. There was however no significant difference in carbon clearance result obtained from treated rats compared to control. DTH is characterized by large influxes of non specific inflammatory cells in which the macrophage is a major participant. (Dunnet, 1964). Activation of DTH cell results in the secretion of various cytokines including interleukin-2, interferon-Y, macrophage migration factor and tumor necrosis factor-β (Askenase and Von loveren 1983). The overall effects of these cytokines are to recruit macrophages into the area and activate them, promoting increased phagocytic activity for more effective killing. DTH is agreed to be very important in host defense against parasites and bacteria that live and proliferate intracellularly. The extract enhanced DTH activity as seen in the increase in mean paw oedema of sensitized Rats.

CONCLUSSION

The present study has shown the immunostimulatory activity of the plant T*elfairia occidentalis* by potentiating humoral as well as cellular immunity. This study investigated the possible effect of the ethanol leaf extract on immunological indices on Rats. The result obtained showed a significant increase in haematological indices of Total white blood cell count (TWBC), Red blood cell count(RBC), packed cell value (pcv), and haemoglobin concentration in test Rats compared to negative control. The result of Delayed Type Hypersensitivity (DTH) and Humora l activity titer showed a significant increase in the rats treated with extract when compared to negative control. There was no significant difference in carbon clearance result obtained from treated Rats when compared to negative control. DTH is agreed tobe very important in host defense against parasite and bacteria that live and proliterate intracellularly

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***[1]Egba Simeon I.[1]Ikeh Emmanuel I., [3]Ugwu Okechukwu P.C. and [2]Odo Mathew C.**

[1]department Of Biochemistry, College Of Natural And Applied Sciences Micheal Okpara University Of Agriculture, Umudike Abia State, Nigeria.

[2]health Services Department, Micheal Okpara University Of Agriculture, Umudike Abia State, Nigeria.

[3]department Of Biochemistry, University Of Nigeria Nsukka Enugu State.

Email simeonegba@yahoo.com