Effect of aqueous extract of whole plant of *Phyllantus niruri* on the immune system of healthy albino rats.

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ABSTRACT

Background: The effect of aqueous extract of whole plant parts of *Phyllantus niruri* in some specific and non-specific immune response was investigated.

Methods: A total of 25 Albino rats weighting (250=300g) were used in this study. The rats were divided into five groups with 5 rats in each group. Group1 received normal saline, 2 and 3 were administered a single dose of the aqueous extract at 200 and 400 mg/kg body weight respectively, 4 received of vitamins E (100 IU) and received vitamin C (100mg/kg) per oral respectively while group 5 received cyclophosphomide (30mg/kg body weight vial oral administration).

Results: The immunomodulation and the effect of the extract on the body weight, relative lymphoid organ weight, splenic cellularity and peripheral blood hematologic parameters were evaluated. The ingestion of the extract caused a significant increase ($P< 0.01$) in the body weight, weight and number of cells of spleen, lymph nodes, RBCs, WBCs and platelets. The extract of Phyllantus niruri increased ($P< 0.01$) and hemoglobin concentration in a non dose-dependent manner.

Conclusion: This plant extract also exhibited increased hypersentivity whilst enhancing the proliferative responses of splenic lymphocytes for both T cell and B-cell mitogens. Phyllantus niruri extract demonstrated significant potential as an immunomodulatory agent in a non-dose dependent manner and it may be important to carry out warrant further investigations to determine the phytochemical components attributable to the observed responses.

Keywords Albino rats, aqueous extract, Haematological, Immune system, *Phyllantus nuriri*.

1. INTRODUCTION

Phyllanthus niruri has been reported to exhibit marked anti-hepatitis B virus surface antigen activity in in-vivo and in-vitro studies. Infectious hepatitis is due to the inability of the body immune system to eliminate the virus from the liver cells: hence the "carrier state". An infection with the virus is documented by detectable levels of various viral antigens in the blood, including HBsAg (the surface antigen of the virus) as well as antibodies to the core of virus (HBC antibodies). Some authors postulated that *Phyllanthus niruri* might inhibit proliferation of the virus by inhibiting replication of the genetic material of the virus [1, 2]. The body's immune system deals with pathogens through humoral immunity (involving the synthesis of B lymphocytes to produce immunoglobulins) and cell mediated immunity (the synthesis of antibodies and effectors cells which destroy pathogens and also responsible for the inflammatory response of delayed type hypersensitivity) [3]. Plant extracts have curative properties against immune deficiency conditions or syndrome by boosting humoral and cell mediated body immunities [4]. Some components of plants extracts work in synergy with the body immune system in inhibiting microbial growth, supporting organs and boosting immune system [5]. The tendency of man to live long is largely dependent on the functionality of his immune system. As a primitive animal, man is perhaps the most contemporary predecessor of mammals that has survived and lived a long life. If attacked by microorganisms or parasites, the body defense system it releases chemicals to kill the organisms by either producing a protein molecules or phagocytic cells [6, 7]. Our bodies have an amazing range of defenses against the invasion of pathogens. Some of these are very general; while others are specific to individual types of pathogen. The more we understand about the complex interactions between our bodies and the pathogenic organisms that cause

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disease, the better the opportunity of reducing the immense toll of human suffering and loss of life that results from these infections every year. Hence, this study was designed to evaluate the effect of aqueous extract of whole plant of *Phyllantus niruri* on the immune system of albino rats of Wistar strain.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Reagents
Cyclophosphamide was used as a negative control. While the combination of Vitamin E and C was used as a positive control. All chemicals and reagents5used were both of analytical grades and not expired.

2.1.2 Equipmen
Centrifuge,Rotary evaporator, weigh balance, incubator, extractor, blender, refrigerator,

2.1.3 Biological materials and Preparations
The whole plant (stem, root and leaves) of *Phyllanthus niruri was* collected from the premises of university of Benin. The whole plant of was washed with water. They were chopped into small pieces, air dried in a shade and further dried in an incubator for 3 days at 450C, blended into powder and stored in an air tight container. The powder was extracted with ethanol: water (3:1) mixture on a reflex water bath for 3 hr. The cycle was repeated three times. The extract was concentrated on the rotary evaporator and air dried to get the semisolid extract [9]. Twenty four (24) healthy Wistar albino rats aged between 6 and 8 weeks weighing 250-300g were randomized into five experimental groups. They were maintained under standard laboratory conditions and temperature (250C ± 10C) and light and dark cycle (12h light and 12h dark cycle) in polyvinyl cages and thereafter fed with pelleted growers mash feed and given clean water ad libitum. Male and female rats were kept in separate cages and were acclimatized for 2 weeks before the commencement of the research.

2.1.4 Antigen Preparation
Fresh blood was collected from a healthy sheep at a sheep abattoir at Aduwawa market, Benin City and mixed with sterile Alsevers solution at a 1:1 ratio. The blood was centrifuged at 6400 revolution per min for 10min to enable red blood cells to settle at the bottom of the test tube, The supernatant was decanted and the sheep red blood cell (SRBC) were washed three times with same alsever solution, which was then re-suspended to get a concentration of 0.1ml containing 1×108 cells/ml. They were then kept in a refrigerator at 8oc for later use.

2.2 Methods

2.2.1 Grouping and Administration
Animals were classified into five groups and were administered based on their groupings. Group1: Control group received normal saline,

Group 2: Received 200 mg body weight of plant extract,

Group 3: Received 400 mg/kg body weight of plant extract ,

Group 4: Received 100 IU of vitamins C and vitamin E

Group 5: Received cyclophosphamide at 30 mg/kg body weight

Animals received the drug and plant extract three times in a week for a period of 14 days before they were immunized with sheep red blood cell.

2.2.2 T cell Proliferation assay
Wistar albino rats (males and females, body weight 250-3000gm, n=6 per group) were sensitized with 10% SRBC (1 x 108 cells /ml), at day 0 and day 7 subcutaneously. The immunized groups were given methanol extracts of *P. niruri* daily body weight and cyclophosphonamide (30mg/kg) orally at days 0, 3, 6, 9 and 12, while immunized control group received normal saline. The animals were fed standard rodent pellets and water ad libitum. On day 14, the animals were sacrificed and blood was collected from the, thymus weight was determined immediately after the animals were euthanized. The weight was measured in milligrams and expressed as relative weight using the formula: relative weight= weight of thymus in milligrams/weight of the animals in grams×100.

2.2.3 CD4 T Cell Count: Procedures
Animal blood was collected into EDTA bottles. About 20 µl of the sample was added to 20 µl of phycoerythrin (PE) antibody in a tube. This was adequately mixed and incubated in the dark for 15 minutes at room temperature, and 800 ul of the CD4 no lyse buffer was added to the tube and mixed adequately. The sample tube was plugged

into the sample port of the cyflow machine for counting of CD4+ T cells. The result of the counting is displayed on the monitor was recorded as the number of cell/µl of blood in the worksheet.

2.3 Statistical Analysis

Phyllanthus niruri treatment groups was compared with the control group by employing one way ANOVA and Turkeys multiple comparison tests using GraphPad Prism 8 (GraphPad Software, Inc., CA, USA). Differences with a p-value of 0.05 or lower were considered to be statistically significant.

3.0 RESULTS

Table 1: Morphometric measures (g) of albino rats treated with aqueous extracts of *P.niruri*

Groups	Body weight (g)		Weight of organs (g)		
	Initials	Final	Liver	Spleen	Thymus
Control	198±3.31	217±2.10	3.6±0.17	1.28±0.09	0.388±0.02
200mg/kg	194±7.97	218±5.15	3.44±0.13	1.54±0.08	0.438±0.012
400mg/kg	191.6±5.25	230±3.536	3.42±0.07	0.44±0.10	0.428±0.012
Vit. C&E	203.6±3.80	223±2.731	3.2±0.07	0.48±0.11	0.44±0.017
Cyclophosp. Hamide	191.6±2.66	193±2.55	2.76±0.23	0.9±0.14	0.266±0.03

These values are expressed as mean ± SEM with P<0.05 a as level of significance compared to the control.

Table 2: Heamatological parameters (mg/dl) of albino rats treated with aqueous extract of whole plant of *P.niruri*

	White cell count	Lymphocyte	Monocyte	Neutrophil	Eosinophil
Control	1813±198.50	40.6±1.20	4.28±0.20	43.6±2.112	1.26±0.81
200mg/kg	2407±34.19	47.8±0.86	7.08±0.40	51.6±1.08	1.45±0.45
400 mg/kg	2313±79.24	45.2±1.28	5.18±0.23	47.0±0.84	1.42±0.09
Vit. C&E	2571±33.78	42.6±0.92	5.2±0.17	46.0±1.30	1.52±0.086
Cyclophosp.	1908±28.53	35.2±1.58	3.5±0.18	41.6±1.08	0.92±0.086

These values are expressed as Mean ± SEM with P<0.05 as level of significance compared to the control

Table 3: Serological parameters of albino rats treated with aqueous *P.niruri*

Groups	% glucose (mg/dl)	% hemoglobin (g/dl)	Serum protein (mg/100ml)	Albumin globulin2412
Control	80.2±1.66	5.84±0.27	7.3±0.14	7.44±0.19
200mg/kg	89.2±1.83	8.5±0.14	8.36±0.18	8.18±0.19
400 mg/kg	84.0±1.30	6.78±0.14**	7.34±0.19	7.48±0.15
Vit. C&E	88.4±1.36	7.54±0.81**	7.5±0.17	7.46±0.11
Cyclophosp	69.4±1.63	3.98±0.09	6.36±0.16	6.08±0.12

These results are expressed as mean ± SEM with P<0.05 as level of significance compared to the control

Table 4: Hemagglutination antibody (Ha) titer and delayed type hypersensitivity response of albino rats treated with aqueous *P.niruri*

Groups	Ha titer	Hypersensitivity rxn % increase in paw vol. Time (hours)			
		0	24	48	96
Control	1.40±0.07	11±0.35	13.22±2.7	11.46±0.19	10.6±0.27
200mg/kg	1.64±0.09	16.4±0.93**	15.6±0.93	10.6±0.80	4.36±0.11
400mg/kg	1.50±0.07	17.6±0.51**	13.7±0.66	8.52±0.24	4.28±0.12
Vit. C&E	1.48±0.11	17.5±0.5**	15.4±0.62	8.5±0.17	5.58±0.12
Cyclophosp.	1.04±0.09	17.7±0.54	12.5±0.71	11.0±0.35	10.6±0.19

These results are expressed as mean ± SEM with P<0.05 as level of significance compared to the control

Table 5: Effects of CD4, T Cells and B- Cell counts of albino rats treated with aqueous *P. niruri*

Groups	CD4 X10^{9}/L	T-CellsX10^{9}/L	B-Cells X10^{9}/L
Control	51.9±0.51	2.72±0.09	2.28±0.09
200mg/kg	62.3±0.66	3.70±0.09**	3.84±0.09
400mg/kg	54.3±0.50	3.18±0.07**	2.62±0.09
Vit. C&E	54.3±1.20	3.24±0.09**	2.34±0.05
Cyclophosp.	29±0.71	1.1±0.07	0.72±0.11

These results are expressed as mean ± SEM with P<0.05 as level of significance compared to the control

Table 1 shows Morphometric measures of albino rats treated with aqueous extracts of P.niruri. The extract caused a significant (P<0.01) body weight increased in the treatment groups as well as the control groups and the positive control groups (vit. C&E). The negative control group (group administered cyclophosphamide) rather experienced a decrease in weight compared to the control. The difference in weight of the liver, thymus and spleen in the treatment groups and the positive control groups when compared to with the control was not significant (P > 0.01). However, that of the negative control group experienced a significant (P<0.01) decrease in weight of the liver when compared to the control. Table 2 shows Heamatological parameters of albino rats treated with aqueous leaves P.niruri. The white blood cell, lymphocytes and monocytes counts in the treatment groups as well as the positive control groups increased when compared to the control groups at P<0.01. The neutrophil and the eosinophil counts were not significantly different from that of the control groups even at (P<0.05). However, there was a significant decrease (P <0.01) of the white blood cell, lymphocytes, monocytes, neutrophils, and eosinophil count in the negative control groups. Table 3 shows Serological parameters of albino rats treated with aqueous P.niruri. The serological parameters such as % glucose and %hemoglobin increased significantly (P <0.01), when compared to the control group. However, this was not the case for serum protein, Albumin and globulin, as there was no significant increase (P > 0.05) observed. The groups treated with cyclophosphamide experienced a decrease in %glucose, %hemoglobin, serum protein, and Albumin and globulin (P<0.05). Table 4 shows Hemagglutination antibody (Ha) titer and delayed type hypersensitivity response of albino rats treated with aqueous P.niruri. The hemagglutination antibody titer value of the treatment groups and the positive control groups was not significant prior to the induction of sheep red blood cell on the paw when compared to the control group. After induction, there was a significant increase (P <0.01) in hypersensitivity reaction at time 0 hours in the aforementioned groups, which decreased continuously when compared to the control groups. However, in the negative control groups that were treated with cyclophosphamide, the hemagglutination antibody titer prior to induction of sheep red blood cell decreased significantly (P <0.01). After induction of sheep red blood cell, the hypersensitivity reaction, however, remained unchanged throughout the experiment when compared to the control. Table 5 shows the Effects of CD4, T Cells and B- Cell counts of albino rats treated with aqueous P. niruri The CD4, T-cell and B-cell counts in the 200mg/kg body weight treatment groups increased significantly at P <0.01 when compared to the control, while that of the 400mg/kg treatment groups and the positive control groups when compared to control were not significant(P<0.05). Groups treated with cyclophosphamide experienced a decreased number of CD4, T-cell and B-cells when compared with the control (P<0.01).

4. DISCUSSION

The human immune system is a very important system that wages against infectious pathogens; it is comprised of many components that are in balance with one another, and whenever this balance is distorted, the body experiences issues or gets surmounted by pathogens. The numerous side effects and cost of synthetic drugs have necessitated the search for crude, less toxic, natural agents that are cheap, accessible and have immuno-stimulating properties. Increased level of total antibodies or of a particular antibody against non-pathogenic antigen such as sheep red blood cell can be used to measure increase in humoral immune response, the quantity of a particular antigen and the cell can also be used to measure the functional status of the humoral immune antigen recognition, activation and expression [10]. The capacity of the immune system is the function of the number of B lymphocytes that has the ability to develop into plasma cells [10]. The treatment with different doses of the *P. niruri extract* was well tolerated by all the animals, as there were no toxic effects observed by direct visual observation of the animals throughout the experiment. There were no death and apparent behavioural changes recorded during the course of the experiment in all treatment groups as compared to the control group. Moreover, the administration of aqueous extract of whole plant parts *of P. niruri* stimulated the increase ((P<0.01)) in the size of body weight of treated Wister albino rats. This increase in the rat weight may be due to the fact that *P. niruri* is a good source of nutrition. Further findings, revealed that the extract boosted the number or amounts of white blood cell and lymphocyte count, suggestive of the immunopotentials of P. niruri, since white blood cells are involved in fighting infections and cleaning of injured and dead cells in the body tissues according to Vasudevan et al [11]. However, overstimulation of white blood cells can cause trauma, uremia, leukemia, hemorrhage, myocardial infarction, etc [12]. The aqueous extract of *P. niruri* is safe with reduced tendency to cause over stimulation of the immune

system in a non-dose dependent concentration. Reduction of blood level of neutrophil as seen in the negative control group may suggest tissue damage, malignant disease, megaloblastic anemia and splenomegaly [13]. The white blood cells are very important components of the blood that affects the immune response. The increase in white blood cells is an important marker of immune response. Also, increase in lymphocytes, monocytes and slightly eosinophil compared to the control confirms the immunomodulatory properties of *P. niruri*. Although, the increased observed in neutrophils and eosinophils were not significant ((P>0.01)).Delayed type hypersensitivity is a cell mediated immune response and in this study the delay hypersensitvitivity was improved, indicative of this is the ability of *P. niruri* to boost cell mediated immune response. The delayed type hypersensitivity that usually occurs between 24 and 72 hours are usually estimated by the memory T-cells, macrophages, and CD4 and CD8 T-cells [14]. The decrease in cellular immune response observed in the treatment groups using extract of *P. niruri* could be associated with the ability to decrease immune burden and potentiate cell-mediated immune response [15]. Cyclophosphamide is an immunosuppressant and acts by cross-linking DNA to prevent cell replication and thus its function. It affects particularly the proliferating lymphocytes. Cyclophosphamide pretreatment has been reported to sharply decrease the activity of all lymphoid cells, especially the CD4+ T lymphocyte [16]. It was reported also that both destruction of donor antigen stimulated T cells in the periphery and elimination of donor reactive clones of T cells in the thymus were essential mechanisms of cyclophosphamide-induced tolerance. In line with previous reports, cyclophosphamide group in this study exhibited a significant increase (P<0.01) in CD4 cells aids in fighting infectious diseases [17]. CD4+ T cells contribute a myriad of activities in protective immunity against viruses that are initiated by infection or by vaccination. These activities can be broadly separated into distinct categories that include recruitment of key lymphoid cell populations into secondary lymphoid tissue or sites of pathogen infection, provision of help for expansion or function of other effector cells, or offering direct effector function through production of cytokines or cell-mediated cytotoxicity [18]. One key activity of CD4+ T cells is recruitment of other lymphoid cells: CD4+ T cells can promote engagement of CD8+ T cells with dendritic cells (DCs) in secondary lymphoid tissue [18-20], cause influx of lymphoid cells into draining lymph node [20], and recruit innate or antigen-specific effectors to the site of viral replication [21-23]. The significant increase ((P<0.01)) in the CD4 cell count in this study, is an indication of the immunoprotective ability of the aqueous whole plant extract of *P. niruri*.

5. CONCLUSION

Ethanolic plant extract of various ayurvedic plants have been known for their potentials as immunomodulators, thus explaining their use traditionally in the treatment of autoimmune diseases. From the study, it is logical to make the scientific submission that the aqueous leaf extract of *P. niruri* modulated the immune system of rats, thereby establishing scientifically the folklore use of the aqueous leaf extract of *Phyllanthus niruri* for the prevention and/ or cure of infective and degenerative diseases

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Conflict of interest

Authors declares no conflict of interest

Contributions of Authors

The research work was conceptualized by Osasere E. Obaro-Onozeyi, the practical and writing of the article was collectively done by all authors

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