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Immunomodulatory Activities of *Piper guineense* Leaves in Wistar Rats

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Abstract

Piper guineense is composed largely of important phytochemicals which include alkaloids, flavonoids, tannins, saponins, resins and essential oil. In this research, the immunomodulatory activity of aqueous leaf extract of *Piper guineense* was determined by assessing the effect of the extract on cellular and humoral immune functions in seventy-two female Wistar rats which were fed orally with the extract for seven days. The humoral immune response was determined by assessing the effect of the extract on hemagglutination antibody (HA) titre. The cellular immune response was determined by assessing the effect of the extract on Delayed Type Hypersensitivity (DTH). Cyclophosphamide was used as an immunosuppressant to determine the effect of the extract in rats in an immunocompromised condition. In the humoral test, the plant showed a dose-dependent increase with all the tested doses with a higher significant effect in the 400 mg/kg. Hemagglutination titre which was decreased in the cyclophosphamide-treated group was observed to increase significantly with the administration of both doses of the extract. The Delayed Type Hypersensitivity response (DTH) revealed that the plant extract increased DTH significantly at both 200 mg/kg and 400 mg/kg doses when compared with the control ($p < 0.01$). The increase was significant when compared with the negative control ($p<0.01$). The overall trend obtained in the parameters studied for the assessment of immunomodulatory activity of aqueous leaf extract of *Piper guineense* indicated that the extract has ameliorative effects on cyclophosphamide-induced immunosuppression and is a good candidate as an imunostimulatory agent.

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Introduction

The immune system is a system of biological structures and processes within an organism that protects against disease. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions. The use of plant products as immunomodulators is still in a developing stage. A variety of plant-derived materials such as polysaccharides, lectins, peptides flavonoids and tannins have been reported to modulate the immune system (Bodhankar *et al*., 2001).

Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine (Pezzuto, 1997). Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents (Verma *et al*., 2013). The benefits of immunomodulators stem from their ability to stimulate natural and adaptive defense mechanisms, such as cytokines, which enable the body to help itself. The natural immunomodulators act to strengthen weak immune systems and to moderate immune systems that are overactive (Verma *et al*., 2013). Over the years, plant extracts and plant-derived medicines have made immense contributions to the overall health and wellbeing of human beings. The antimicrobial ability of plant extracts and oils has established a platform for the processing and transformation of these plant products into pharmaceuticals, preservatives and natural medicine. As a result of this, there is a growing interest in plant's usage for medicinal purposes due to the presence of several antibacterial compounds present in them. Hence, it is agreed that medicines developed from plants are comparably safer than their synthetic counterparts thus rendering enormous therapeutic

benefits at an economical treatment rate. The immune system comprises mostly blood components and majorly the white blood cells and thus, when suppressed may lead to reduction in blood counts and susceptibility to infection (Baumann and Preiss, 2001).

Cyclophosphamide, an anticancer agent targets tumour cells but at a high dose acts on the hematopoietic cells thereby reducing the number of blood cells. Cyclophosphamide induces myelo-suppression in experimental animals (Saroj *et al*., 2012). It belongs to nitrogen mustard subclass of alkylating agents and acts as an immunosuppressive agent by causing alkylation of DNA, in turn by interfering in DNA synthesis and function. It is also used extensively as an immunosuppressant (Saroj *et al*., 2012). Disease conditions like AIDS, diabetes, cancer etc. also result in immunosuppression.

Piper guineense (African black pepper) commonly known as Uziza in Ibo language, Iyere in Yoruba, is a climbing black pepper of the plant family *Piperaceae* and contains over 700 species all over the world. They are erect herbaceous climbing vines that are cultivated in the tropical regions of Central and Western Africa and even in Nigeria. The plant known to provide medicinal, insecticidal, culinary and dietary benefits to man

Materials and Methods

Plant Material and Preparation of Extract

Plants of *P. guineense* were collected from Evbuotubu market Benin City, Nigeria. It was identified and authenticated by the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Fresh leaves of *P. guineense* were shade-dried, grounded and finely powdered. The powdered leaves (200 g) were soaked in distilled water (2 litres) and kept at room temperature (25^0C) for 48 hr with intermittent mixing. The mixture was allowed to stand for 48 hr before filtering. . The extract was then stored in a refrigerator till when needed.

Experimental Animals

Seventy-two (72) Wistar Albino rats weighing 130 g – 150 g of either sex were used for this study. The animals were housed and allowed to acclimatize for two weeks under standard laboratory conditions (12h light 12h dark) during the experimental period. The animals were fed with standard rodent pellet and clean water *ad libitum*. The animals were handled according to standard protocols for the use of laboratory animals (National Institute of Public Health Service policy on Humane Care and use of Laboratory Animals (USA, 2002).

Antigenic Challenge: Fresh sheep blood was collected in sterile Alsevar's solution (1: l proportion). Sheep red blood cells (SRBCs) were washed three times in pyrogens-free normal saline and centrifuged at 3000 rpm for 10 min. The supernatant was removed with a Pasteur pipette and suspended in normal saline. Ten percent (10 %) sheep red blood cells was prepared by adding 10 ml of sheep red blood cells in 90 ml of normal saline. The synthetic immunosuppressant drug used was Cyclophosphamide and it was of analytical grade (Khandelwal Laboratories Ltd. Mumbai).

Assessment of both Humoral (HT) and Cellular (DTH) Immune Functions

Thirty-six Albino rats were used for each of this study; the rats were divided into six groups with six rats in each group (for each of the assays)

Group I (control group): received normal saline

Group II (negative control): received normal saline and cyclophosphamide 50 mg/kg body weight

Group III: received *Piper guineense* 200 mg/kg body weight.

Group IV: received *Piper guineense* 400 mg/kg body

Group V : received *Piper guineense* 200 mg/kg + cyclophosphamide 50 mg/kg

Group VI : received *Piper guineense* 400 mg/kg + cyclophosphamide 50 mg/kg

All animals were sensitized with 0.5 ml of sheep red blood cells (SRBC), intraperitoneally on day 0. Groups III – VI received sample extract from day 1 – 7, according to the doses listed above. Cyclophosphamide was administered on day 5, two hours after administration of the extract. On day 8, the animals were sacrificed. Serum was obtained from the animals for the determination of hemagglutination antibody titre.

Hemagglutination Titre (HT) Assay

Hemagglutination titre assay was performed using the procedure of Bin-Hafeez *et al*.(2007), serum was diluted in 50µl PBS (pH 7.2) with two fold serial dilutions in 96–well microtitre plates and mixed with 50µl of 1.00 % SRBC suspension in PBS. Plates were incubated at 37^0C for 1 hr. The value of antibody titre was considered with the highest serum dilution showing visible hemagglutination.

Delayed Type Hypersensitivity (DTH)

For Delayed Type Hypersensitivity (DTH) response, the animals in the cell-mediated immune response group were challenged with sheep red blood cells (SRBC) on day 7, which was injected into the left hind paw. The same volume of normal saline was

injected into the right hind paw as trauma control for nonspecific swelling. The paw volume was measured after 72 hr using a venier caliper (Eze *et al*., 2014).

Statistical Analysis

Data was statistically analyzed using one way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison tests to determine significant differences in data of various groups. The values are expressed as means $\pm$ SEM. Levels of significance were considered at 95% and 99% statistical confidence levels. For $p < 0.05$ or $p < 0.01$, the differences were considered significant.

Results and Discussion

The results of the effects of the aqueous leaf extract of *P. guineense* on antibody production (hemag - glutination titre) revealed that there a significant increase while it was suppressed significantly in the negative control group (cyclophosphamide-treated group) when compared with the control ($p<0.01$). This cyclophosphamide-induced immunosuppression was relieved by administration of the extract in the 200 mg/kg and 400 mg/kg doses, both doses were observed to significantly increase hemagglutination when compared with the negative control group (Table 1).

The effect of the plant extract on cellular immune response (DTH) revealed a significant increase at different doses (Figure 1). It was observed that the groups of rats treated with doses 200 mg/kg (Group III) and 400 mg/kg (Group IV) showed increased response in foot pad edema which was found to be statistically significant ($p < 0.01$) when compared to other groups. Cyclophosphamide-induced immune - suppression was also reversed to a significant level by administration of 200mg/kg and 400 mg/kg of the extract to cyclophosphamide-treated rats, when compared with the negative control group which revealed a significant increase ($p<0.01$) of DTH at different doses.

The modulation of the body immune responses through suppression or stimulation is responsible for maintaining a disease-free state in an individual. Substances which are capable of activating the host defense mechanisms through the immune system have been used globally as a way to control diseases in humans (Yapo *et al*., 2011). Immunostimulation in a drug-induced immunosuppression and Immune-suppression in an experimental hyper- reactivity model by the same preparation can be said to be true immunomodulation (Verma *et al*., 2013).In this study, *P. guineense* leaves showed an overall stimulating effect on the immune function in rats. Stimulating effects were observed on both humoral and cellular immunity.

The reaction of an antibody and antigen can be easily detected by agglutination (clumping) of the antigen. If the antigen is an erythrocyte, the term hemagglutination is used. Agglutination tests can be used to measure the level of antibodies to particulate antigen. In the hemagglutination test, the aqueous extract of *Piper guineense* leaves showed an increase in hemagglutination titre with the different doses than that of the control group but the increase was most significant in the group that received a dose of 400 mg/kg ($p<0.01$). This activity could be due to the flavonoids compounds in the plant extract (Okoye and Ebelendike, 2013) which may interact with the B cells acting as antigen and hence activate subsequent proliferation and differentiation into antibody secreting (plasma) cells (Sumen *et al*., 2004). The antibody molecules are products of B lymphocytes that form the plasma cells leading to the formation of antibodies such as immunoglobulin (Ig) including Ig G, Ig A, Ig M, Ig E and Ig D which are central in immune response (Steven, 2003) .

Hemagglutination titre was suppressed significantly in the negative control group (cyclophosphamide-treated group) when compared with the control ($p<0.01$). This cyclophosphamide-induced immune-suppression was relieved by administration of the extract in the 200 mg/kg and 400 mg/kg doses, both doses were observed to significantly increase hemagglutination when compared with the negative control group (Table 1). This may be due to the enhanced responsiveness of macrophages as well as T and B lymphocyte subsets involved in antibody synthesis in the Wistar albino rats as observed in other studies (Sharififar *et al*., 2009).

The cellular immune response was determined by DTH response i.e. increase in foot pad thickness using vernier calipers. The observations in Figure-1 indicate that rats treated with doses 200 mg/kg (Group III) and 400 mg/kg (Group IV) showed increased response in foot pad edema which was found to be statistically significant ($p < 0.01$) when compared to other groups. Cyclophosphamide-induced immunosuppression was also reversed to a significant level by administration of 200mg/kg and 400 mg/kg of the extract to cyclophosphamide - treated rats, when compared with the negative control group which revealed a significant increase ($p<0.01$) of DTH at different doses as shown in Figure 1. The effect of *Piper guineense* extract on cellular immune response of rats was demonstrated by the effect of the extract on delayed type hypersensitivity (using foot pad thickness). DTH is a Type IV hypersensitivity which can be used as an indication of cell mediated

response (Scott *et al.*, 2005). It requires the specific recognition of a given antigen by activated T lymphocytes which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induce vasodilation, macrophage accumulation and activation, promoting increased phagocytic activity and increased concentration of lytic enzymes for more effective killing thus contributing to the cardinal signs of inflammation. General characteristics of DTH are an invasion of immune cells at the site of injection and induction became apparent within 24 to 72 hr in this study. The plant extract showed a significant difference in the DTH response which explains that the extract has stimulatory effect on T lymphocytes especially T_{DTH}-lymphocytes and therefore effect on cellular immunity as demonstrated by DTH. This activity could be due to the presence of flavonoids, alkaloids, peptides, essential oil and phenols which are major components of the plant (Achimewhu *et al*., 1995). These substances have been tested for their efficacy as both chemical and biological markers (Pattanaik *et al.*, 2002).

This increase in foot pad thickness could be due to sensitized lymphocytes which are converted to lymphoblasts and secreting a variety of pro-inflammatory lymphokines attracting more phagocytic cells to the site of reaction (Steven, 2003). The infiltrating cells are probably immobilized to promote the defensive inflammatory reaction. Whereas T–helper cells may have increased damage by secreting cytokines that activate cytotoxic T cells which recruit and activate monocyte and macrophages causing a bulk of tissue damage observable by increased thickness of the footpad (Sharififar *et al.*, 2009).

The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported. Such products, if well tolerated by the patient, may be developed into alternative coadjuvants in the treatment of disorders caused by an exaggerated or unwanted immune response, such as in autoimmune diseases, allergies, glomerulonephritis, chronic hepatitis, etc (Rossi-Bergmannet *et al*.,1994).

Conclusion

The overall trend obtained in this study indicates that *P. guineense* is a good candidate as an immunostimulating agent. *Piper guineense* extract stimulated both cellular and humoral immune responses. It was also observed to also reverse significantly cyclophosphamide-induced immune - suppression and so can be recommended as a treatment measure for convalescent and immunocompromised people. It is a good medicinal plant with several pharmacological properties to be explored. It can be consumed at high doses without side effects and so, it is strongly recommended for effective use in phytomedicine.

References

Achimewhu, S. C., Ogbonna C. C. and Hart, A. D. (1995). Chemical composition of indigenous wild herbs, spices, fruits, nuts, and leafy vegetables used as food. Plant Food for Human Nutrition 2: 378-379.

Baumann, F. and Preiss, R. (2001). Cyclophosphamide and related anticancer drugs. *Journal of Biomedical Science* 764(1): 173-192.

Bin Hafeez, B., Ahmad, I., Haque, R. and Raisuddin, S. (2001). Protective effect of *Cassia accidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *Journal of Ethnopharmacology* 75: 13-18.

Bodhankar, S., Makare, N. and Rangari, V. (2001). Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. *Journal of Ethnopharmacology* 78: 133-137.

Coombs, R.A. and Gell, P.G. (1975).Classification of allergic reaction responsible for clinical hypersensitivity and disease. *Clinical aspects of Immunology* (2nd edition).Edward Publishers 778pp.

Eze,C.O., Nworu, C.S., Esimone, C.O. and Okore, V.C. (2014). Immunomodulatory activities of methanolic extract of the whole aerial part of *Phyllantus niruri* L. *Journal of Pharmacognosy and Phytotherapy* 6(4): 41-46.

Okoye, E.I. and Ebelendike, A.O. (2013). Photochemical constituents of *Piper guineense* (Uziza) and their health implication on some microorganisms. *Global Research Journal of Science* 2(2): 42-46

Pattanaik, S., Hota, D., Prabhakar, S., Kharbanda, P. and Pandi, P. (2002). Effect of piperine on the steady-state pharmacokinetics of phenytoin in patients with epilepsy *Phytotherapy* 20: 683-686.

Pezzuto, J.M. (1997). Plant-derived anticancer agents. *Biochemical Pharmacology* 53: 121–133.

Rossi-Bergmann, B., Costa, S.S., Borges, M.R.S., Da Silva, V., Noleto, G.R. and Souza, M.L.M. (1994). Studies on *Kalanchoe pinnata. Phytotherapy Resources* 8: 399.

Saroj, P., Verna, M., Jha, K.K. and Manju, P, (2012). An overview of immunomodulation. *Journal of Advanced Scientific Research* 3(1): 7-12.

Scott, I.M., Gagnon, N., Lesage L., Philogene, B.J., and Anason, J.T. (2005). Efficacy of botanical insecticides from Piper species (Piperaceae) extracts for control of European chafer. (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* 98: 845-855

Sharififar, F, Ponou, M. S. and Arabnejad, M (2009). Immunodulatory effect of aqueous extract of *Acholea welmasi* in mice. *Indian Journal of Experimental Biology* 47: 669 – 671.

Steven, C.D. (2003). A laboratory perspective of cytokines. *Chemical Immunology and Serology* 6(2): 86 – 87

Sumen, C. Mampal, A.R., Mazo, I. B. and Andrian, U.H. (2004). Intraviral microscopy. *Immunity* 21: 315-329.

Verma, A., Sahu, R.A. and Sahu, M.S. (2013). Immunomodulatory activity of alcoholic extract of *Habenaria intermedia* in mice. *International Journal of Pharmacy and Pharmaceutical Sciences* 5(3): 403-409.

Yapo, F.A., Yapi, F.H., Ahiboh, H., Hauhouot, M.L., Guede, N.Z., Jjaman J.A., and Minnet, D. (2011). Immunomodulatory effect of the aqueous extract of *Erigeron floribundus* (Kunth) sch Beep (Asteraceae). *Tropical Journal of Pharmaceutical Research* 10(2): 187-193.

Table 1: Effect of Aqueous Extract of *Piper guineense* on Hemagglutination Antibody Titre on Rats

Group	Treatment	Mean hemagglutination antibody(HA) titer using reciprocal of dilution factor
I(Control group)	Normal saline	37.33±5.33
II(negative control)	Normal saline+ CP	14.67±1.33[b]
III	PG 200 mg/kg	106±13.49
IV	PG 400 mg/kg	405.3±69.46[c]
V	PG 200 mg/kg+ CP	61.33±15.69[d]
VI	PG 400 mg/kg+ CP	96.00±14.31[b;f]

Values are expressed as mean ± S.EM of 6 rats. [b]p<0.01; [c]p<0.01 statistically significant when compared to group I, [d]p<0.05; [f]p<0.01 Statistically significant when compared to group II.; CP (Cyclophosphamide), PG (*Piper guineense*).

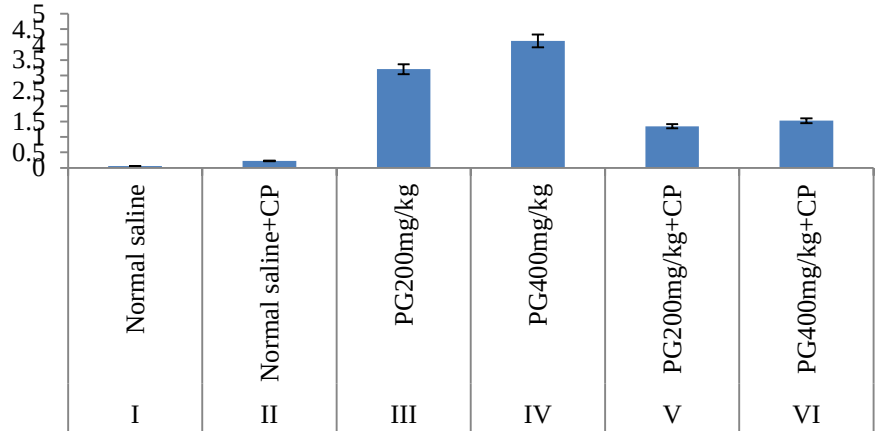

Figure 1: Effect of aqueous extract of *Piper guineense* on mean foot-paw edema (mm) in DTH model

I – Normal Saline (Control); II – Normal Saline + Cyclophosphamide (CP);III – *Piper guineense* (PG) 200 mg/kg
IV – PG 400 mg/kg; V – PG 200 mg/kg + CP; VI – PG 400 mg/kg + CP