



Effects of Zidovudine, Aqueous Leaf Extract of *Gongronema latifolia* and their Combination on some Haematological Indices of Wistar Rats

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Abstract: In this study, the effects of the ethanolic leaf extract of *Gongronema latifolium* (Utazi) and Zidovudine as well as their combination on some haematological parameters was evaluated. Sixteen (16) Wistar rats were divided into four (4) groups of 4 rats each. The control group (Group A) were given food and water only while the test groups (B, C and D) received 0.05 mg/kg body weight of leaf extract and 0.04 mg/kg body weight of zidovudine, 0.05 mg/kg body weight of extract only, and 0.04 mg/kg body weight of zidovudine only, respectively. The result showed significant ($P < 0.05$) reduction in Red blood cell (RBC) count from 9.98 ± 1.41 in group A to 8.02 ± 0.58 , 7.88 ± 0.26 and 7.45 ± 2.74 in groups B, C and D respectively. The white blood cell count (WBC) increased significantly ($P < 0.05$) from 8.03 ± 2.19 in Group A to 10.55 ± 0.78 , 16.68 ± 1.74 , and 11.06 ± 2.38 in Groups B, C and D respectively. There was a non-significant ($P > 0.05$) decrease in Packed cell volume (PCV) from 39.00 ± 1.15 , in Group A to: 38.50 ± 8.54 , 38.00 ± 4.51 and 37.25 ± 2.34 in Groups, B, C and D respectively. For haemoglobin concentration (Hb) there was no significant ($P > 0.05$) difference between groups A and D, however there was significant ($P < 0.05$) increase in Groups B and C when compared to the control as can be seen from the result; 12.97 ± 0.90 , 14.20 ± 1.41 , 14.03 ± 0.52 and 12.50 ± 0.92 for A, B, C and D respectively. For neutrophil count, we observed a significant ($P < 0.05$) increase in all the treated groups when compared with the control; 5.67 ± 2.32 , 18.95 ± 6.13 , 23.43 ± 5.83 and 13.50 ± 7.47 , for groups A, B, C and D respectively. For lymphocyte count, there was a significant ($P < 0.05$) decrease in all the groups as compared to the control; 92.00 ± 1.93 , 72.45 ± 8.97 , 70.85 ± 5.83 and 83.93 ± 5.78 for Groups A, B, C and D respectively. The result showed that *Gongronema latifolium* leaf ameliorated the hazardous impact of zidovudine on white blood cells, haemoglobin and neutrophil count but did not improve the PCV and RBC of treated animals. It's inclusion in meals may also compromise the immune system.

KEYWORDS: Zidovudine, combination, effects, haematological indices, *Gongronema latifolium*

1.0 Introduction

Haematological profile provides important information about the type, number and appearance of cells in the blood, especially; red blood cells, white blood cells and clotting cells. All blood cells are made in the bone marrow; some medications or disease can damage the bone marrow, causing a reduction in the numbers of different types of red or white blood cells. Red blood cells are one of the most vital components of the blood. A single drop of blood contains millions of red blood cells which travel constantly through the body delivering oxygen and removing waste. Reduction in the number of

red blood cells (anaemia) exposes the body to lack of oxygen, and waste accumulation, whereas too much increase may make them clump together and block tiny blood vessels. White blood cells are the keys to the body's immune or defense system. They fight infection and protect the body from foreign particles such as harmful germs or bacteria. They are formed from the stem cell of the bone marrow and have a life span of a few days (<http://www.medichecks.com>).

Zidovudine, is a nucleoside analog, reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV and AIDS. It is an analogue of thymidine (Izzedine *et al.*, 2001). It was the first approved treatment for HIV sold under the names Retrovir and Retrovis (Broder, 2009). It slows down HIV

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spread significantly but does not stop it entirely. It is usually used in conjunction with other NRTIs anti-viral drugs (Jayasekara *et al.*, 1999).

Zidovudine is known to suppress the ability of the body to produce red blood cells (anaemia) and white blood cells e.g neutropenia. Reversible pure red cell aplasia is a recognized complication of zidovudine, typically occurring in the first 3 months of therapy (Weinkove, 2005). Haemoglobin levels fall suddenly in patients receiving zidovudine and red-cell transfusions are required to continue therapy in many of the zidovudine recipients (Chariot *et al.*, 1999).

The use of herbs to treat diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals (Edgar *et al.*, 2002). These natural alternatives also present with fewer side effects.

Gongronema latifolium Benth Hook, (Asclepiadaceae) is known as Amaranth globe in English, “Utasi” by the Efiks, Ibibios and Quas, “Utazi,” by the Ibos and “madumaro” by the Yorubas in Nigeria (Ugochukwu and Babady, 2002). It is commonly grown in gardens in the South-eastern, South-southern and western parts of Nigeria.

Gongronema latifolium is a climbing shrub that grows up to 5m long with hollow stems. The leaves are opposite, simple with petioles of 2.5-3cm long while the blade is broadly ovate to almost circular. The flowers are bisexual, small, regular, yellow-green and fragrant while the pedicel is 2-4 mm long. The calyx lobes are elliptical to round while the fruit is a pair of pendent follicles, each one narrowly cylindrical 7-10 cm x 1-1.5cm. It is many seeded. It grows in rain forest, deciduous and secondary forests can be propagated by seed or softwood, semi-hardwood and hardwood cuttings.

The Efiks and Quas use *Gongronema latifolium* crude leaf extract in the treatment of malaria, diabetes, and hypertension and as laxative. Also, it is used as a spice and vegetable (Morebise, 2002). A range of pharmacological activities including anti-bacterial, antioxidant anti-inflammatory, hepatoprotective, anti-plasmodial, anti-asthmatic, anti-sickling, anti-ulcer, analgesic and antipyretic activities have been associated with it (Atawodi, 2005;

Sonibare and Gbile, 2008; Akuodor *et al.*, 2010). Scientific studies have established the hypoglycemic, hypolipidemic and antioxidative effects of aqueous and ethanolic extracts of *Gongronema latifolium* leaf (Ogundipe *et al.*, 2003; Ugochukwu *et al.*, 2003).

Gongronema latifolium is claimed by many from the South Eastern part of Nigeria to boost haemoglobin levels in human. Zidovudine on the other hand has been reported to cause anaemia in patients within the first three months of treatment. Thus, there is the need to investigate the extent to which this information is correct and educate the general public on the research findings. Keeping this in view, the present study was undertaken to evaluate the effects of the leaf extract of *Gongronema latifolium* and zidovudine on some haematological parameters of Wistar rats with that of zidovudine administered and to compare with zidovudine. It was also aimed at investigating the effects of consuming both chemical compounds.

2.0 Materials and Methods

2.1 Equipment and Reagents

The equipments and reagents used in the present study included Grinding machine (Landers and Cissa, England), Weighing balance (Ohaus, U.S), UV-Visible spectrophotometer (Unicom Ltd, UK), Oven (Tenor and Empress Registered, Italy, PCV haematocrit reader, PCV haematocrit centrifuge and Water bath- model HH-1042-0, (Germany).

2.2 Experimental animals

Sixteen Wistar rats weighing 181.00 ± 13.00 g were used. The animals were fed with standard rat chow (Bendel Feeds and Flour Mills Ltd., Ewu, Esan, Edo State, Nigeria.) and water *ad libitum*. The animals were kept in a well ventilated housing under the following conditions: 12 hour light, 12 hour dark cycle; temperature $28 \pm 2^\circ\text{C}$, and relative humidity between $50 \pm 10\%$

2.3 Plant material and identification

Fresh leaves of *Gongronema latifolium* were purchased from Ahia Ohuru Market, Aba in Abia South Local Government Area of Abia State, Nigeria, and identified by Mrs. Ann Osuagwu of the Department of Botany, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.4 Preparation of ethanolic leaf extract of *G. latifolium*

The leaves were washed, air-dried for 18 days and blended using a mechanical blender. The powdered leaves (200 g) of *Gongronema latifolium* were macerated with 98% ethanol (1000 ml) for 48 hours. At the end of this duration, it was filtered and the filtrate was placed in a water bath at 80°C for the ethanol to evaporate. After solvent evaporation, 10 g of greenish jelly-like extract corresponding to an extraction yield of 5% was obtained. The rats were given 0.05 mg /kg body weight; this dosage was extrapolated from the average amount consumed by man per diet with respect to body weight.

2.5 Phytochemical screening

The phytochemical evaluation of the plant extract was done using the method described by Trease and Evans (1989) and Sofowora (1993) to detect the presence of phytochemicals in the plant. The following phytochemicals were tested for: phenolics (ferric chloride test), alkaloids (Dragendorff test), anthocyanin and phytosterol (Liebermann Burchard Test), saponins, flavonoids (Shinoda Test), tannins, cyanogenic glycosides, cardiac glycosides and steroids.

2.6 Animal grouping and administration of chemical compounds

The rats were divided into four groups: A, B, C and D. Group A (control) received normal rat pellet and water. Group B received 0.05 mg/kg body weight of the extract and 0.04 mg/kg body weight of Zidovudine, Group C received 0.05 mg/kg body weight of the leaf extract while Group D received 0.04 mg/kg body weight of

Zidovudine. All administrations were done orally and on daily basis. The experiment lasted for 28 days.

2.7 Blood collection and analysis of haematological parameters

The animals were minimally exposed to chloroform vapour; blood sample were collected into heparinized bottles via cardiac puncture. The white blood cell count was determined following the standard procedures as described by Ramnik (2003). The total red blood cell, lymphocyte and neutrophil counts were determined by standard methods described by Cheestrough (2000) while total haemoglobin and packed cell volume were determined by the standard haematology method described by Ochei and Kolhaktal (2008).

2.8 Statistical analysis

Statistical analysis of the data obtained from the experiment was carried out using one way analysis of variance (ANOVA) followed by post hoc LSD test (Fisher 1935). The significance in the difference was accepted at $p < 0.05$. The results were expressed as mean \pm SD (standard deviation).

3.0 Results

The phytochemical screening revealed the presence of anthocyanins, phenolics, phytosterol, saponins, flavonoids, glycosides, tannins and cyanogenic glycosides while alkaloid and cardiac glycosides were absent.

The separate administration of the plant extract and zidovudine increased the white blood cell count, haemoglobin concentration and neutrophil count whereas the red blood cell count and leukocytes were significantly decreased (Table 1). The PCV was not significantly altered by both the extract and zidovudine. The trends of alterations in these parameters were similar to what was obtained with the combined administration of the extract and zidovudine.

Table 1: Effects of ethanolic leaf extract of *Gongronema latifolium*, zidovudine and their combination on some haematological parameters

Groups	White Blood Cell count	Packed Cell Volume	Red Blood Cell count	Haemoglobin concentration	Leukocyte Count	Neutrophil Count
Control	8.02± 2.19 ^b	39.00± 1.52 ^a	9.98 ± 1.41 ^a	12.97± 0.90 ^b	92.00± 1.93 ^a	5.67± 2.32 ^d
Zidovudine + Extract	10.55 ± 0.78 ^{ab}	38.50± 8.54 ^a	8.02 ± 0.59 ^b	14.20± 1.41 ^a	72.45± 8.97 ^c	18.95 ± 6.13 ^b
Extract only	16.68 ± 1.74 ^a	38.00± 4.51 ^a	7.88 ± 0.26 ^b	14.03±0.52 ^a	70.85 ± 5.83 ^c	23.43±5.83 ^a
Zidovudine only	11.06 ± 2.39 ^a	37.25± 2.34 ^a	7.45 ± 2.74 ^b	12.50± 0.92 ^b	83.93 ± 5.78 ^b	13.50± 7.47 ^c

The significance in the difference was accepted at $p < 0.05$. The results are expressed as mean of triplicate determinations ± SD.

Discussion

Zidovudine, the ethanolic leaf extract of *Gongronema latifolium*, and a combination of the two obviously had similar pattern of effects on the haematological indices evaluated in the rats as can be seen from the results. In this study, while zidovudine significantly increased white blood cell and neutrophil count when compared to the control is indicative of the fact that zidovudine plays an important role in the immune system. Increased neutrophil count could be attributed to stress or trauma, this means the drug may have also induced some stress on the animal. The drug also significantly reduced red blood cell and leukocyte count. Reduction in red blood cell is an indication of anaemia. Surprisingly, the drug had no significant effect on the PCV and haemoglobin concentration, this calls for further research to confirm the anti-anaemic property of the drug as PCV, haemoglobin and red blood cells go hand in hand in testing for anaemia.

Gongronema latifolium showed significant increase in white blood cell, haemoglobin concentration and neutrophil count. Again this portrays the plant as having immunological tendencies, and improved oxygen transportation within cells; however the reduction in red blood cell and leucocyte count resulting from its administration also calls for more research as red blood cell and haemoglobin go together.

Decrease in red blood cell count may be due to the presence of saponin in the extract which has been reported to reduce haematological parameters probably due to lysis of blood cells or suppression of blood cell synthesis (Scheider *et al.*, 1993.) The extract also exhibited no significant change on the PCV of the animals; this agrees with the report of Antai *et al* (2009) that the leaf extract of *Gongronema latifolium* has little or no effect on PCV and RBCs.

The root extract of the same plant have also been found to display similar effects on these haematological parameters as we observed in the leaf. Antai *et al* (2009) reported that the root extract of *Gongronema latifolium* significantly increased total white blood cell and had little or no effect on RBC count. According to them, low doses of the extract increased haemoglobin count while high doses increased white blood cell counts. The similarities in the effects of the leaves and root extracts of *Gongronema latifolium* on the haematological parameters are likely due to similarity in their phytochemical constituents.

Glycosides have been reported to cause increase in total white blood cell count (Antai *et al.*, 2009) and as such confer anti-inflammatory property and vital effect on inflammation, infection and malaria on the plant (Ugochukwu *et al.*, 2003). The presence of this metabolite in *Gongronema latifolium* might be responsible for

the increase in white blood cell in the extract treated animals.

The animals administered a combination of the plant extract and zidovudine seemed to create a balance in the effects of the independent treatments. For example for white blood cell, red blood cell and neutrophil count, the combination effect of the two treatments seemed to be an intermediate between the effects of the individual treatments. This shows that combining both treatments may help to cushion any side effect resulting from the independent treatments. When compared to the control, this group of animals had their white blood cell count, haemoglobin concentration and neutrophil count significantly increased while the red blood cell count and leukocyte count decreased significantly. The combination however had no significant effect on the PCV. The increase in haemoglobin in this group is an indication that haemoglobin synthesis may be increased by the consumption of *Gongronema latifolium* as opposed to zidovudine. Also the animals treated with zidovudine alone, exhibited the lowest haemoglobin level; this further points to zidovudine as an inducer of anaemia.

The animals treated with the ethanolic leaf extract of *Gongronema latifolium*, had the highest white blood cell count, as compared to the other groups. This is indicative of immunological tendencies which may be as a result of the phytosterols and flavonoids contained in the leaf which may have possibly influenced the production of white blood cells. The animals treated with the leaf extract had the lowest lymphocyte count. This could mean that *Gongronema latifolium* interferes with lymphocyte production in the body.

In conclusion, combining zidovudine and *Gongronema latifolium* leaf extract appears not to have exaggerated the individual effects of the drug and extract. The plant extract exhibited more positive effects on the haematological parameters more than the zidovudine. The plant may help to increase immunity and oxygen transport in patients receiving zidovudine treatment. There is need for further research to check the concentration dependent effect of administering different doses of the plant extract. Quantitative analysis, isolation and

characterization of the active ingredients from the plant also call for further studies.

References

- Akuodor, G. C., Idris-Usman, M. S., Mbahce-Megwas, U. A., Akpan, J. L., Ugwu, T. C., Okoroafor, D. O., Osunkwo, U. A. (2010). Studies on anti-ulcer analgesic and anti-pyretic properties of the ethanolic leaf extract of *Gongronema latifolium* in rodents. *African Journal of Biotechnology* 9(5):2316- 2321.
- Antai, A. B., Ofem, O. E., Ikpi, D. E., Ukafia, S. and Agiang, E. A. (2009): Phytochemistry and some haematological changes following oral administration of ethanolic root extract of *Gongronema latifolium*. *Nigerian Journal of Physiological Science* 24(1): 79-83.
- Atawodi (2005): Antioxidant potential of African medical plants. *African Journal of Biotechnology* 4(2): 128-133.
- Broder, S. (2009). The development of antiretroviral therapy and its impact on the HIV/AIDS pandemic. *Antiviral Research* 85(1): 1-2.
- Chariot, P., Droqou, I., de Lacroix-Szmania, I., Eliezer-Vanerot, M. C., Chazaud, B., Lombes, A., Schaeffer, A. and Zafrani, E. S. (1999). Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion. *Journal of Hepatology* 30(1):156-160.
- Cheesbrough, M. (2000). *District laboratory practice in Tropical countries Part 2* Cambridge University Press, Cambridge, UK. pp. 434.
- Edgar, J. A., Erhard, R., Russel, J. and Molyneux, (2002). Honey from plants containing pyrrolizidine alkaloids: a potential threat to health. *J. Agric. Food Chemistry* 50(10):2719-2730.
- Fisher, R. A. (1935). *The design of experiments* 9th edition. Macmillan, pp. 244.
- [Http://www.medichecks.com](http://www.medichecks.com). Accessed September 2014.
- Izzedine, H., Launay-Vacher, V., Amyrad, G., Legrand, M. and Deray, G. (2001). Pharmacokinetics of nevirapine in haemodialysis. *Nephrology Dialysis Transplantation* 16: 192-193.
- Morebise, O., Fatunso, M. A., Makinde, J. M., Olajide, O. A. and Awe, E. O. (2002). Anti-inflammatory property of the leaves of *Gongronema latifolium*. *Phytotherapy Research* 16(1):75-77.
- Ochei, J. and Kolhatkar, A. (2008). *Medical laboratory Sciences. Theory and Practice*. Tata Mc Graw Hill, New York, pp. 663-665.
- Ogundipe, O. O., Moody, J. O., Akinyemi, T. O., Raman, A. (2003). Hypoglycemic potentials of methanolic extracts of selected plant foods in

- alloxandized mice. *Plant Foods for Human Nutrition* 58(3): 1-7.
- Ramnik, S. (2003). *Medical Laboratory Technology: Method and Interpretations*. Jaypee Brothers Medical Publishers Ltd., New Delhi.
- Scheider, C., Rotscheidt, K., Breitmaler, E. (1993). 4 new pregnane glycosides from *Gongronema latifolium* (Ascepiadaceae). *Liebigs Annalen Der chemie*. **10**:1057-1062.
- Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. Sunshine House, Ibadan, Nigeria Spectrum Books Ltd; Screening plants for Bioactive Agents. pp. 134-156.
- Sonibare, M. A. and Gbile, R. O. (2008): Ethnomedical survey of anti- asthmatic plants in South Eastern Nigeria. *African Journal of Traditional, Complementary and Alternative Medicine* 5(4): 340-345.
- Trease, G. E. and Evans, W. E. (1989). *Pharmacognosy; A physician's Guide to Herbal Medicine*, 13th edition Balhere, Tindall, London.
- Ugochukwu, N. H., Babady, N. E., Cobourne, M., Gasset, S. R. (2003). The effect of *Gongronema latifolium* extract on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *Journal of Bioscience* 28(1): 1-5.
- Ugochukwu, N. H. and Babady, N. E. (2003). Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sciences* 73(15): 1925-1938.
- Weinkove, R. (2005). Zidovudine-induced pure red cell aplasia presenting after 4 years of therapy. *AIDS* 19 (17):2046-2047.