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Original Research Article

Modulatory Effects of Aqueous Leaf Extract of *Gongronema latifolium* (asclepiadaceae) on Some Natural and Adaptive Immune Responses in Rodents

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ABSTRACT

Gongronema latifolium (GL) is widely used by indigenous communities in southern Nigeria as a food spice and is known for its rich natural compounds with significant biological and pharmacological properties. This prompted the investigation into the effects of the aqueous *G. latifolium* leaf extract (AGL) on several specific and non-specific immune responses in rodent (mice) models and *in vitro* essay. The effects of AGL on delayed-type hypersensitivity (DTH) response, phagocytic activities of monocytic cells using carbon clearance assay, haemolytic complement activation test, antibody expression studies, and cyclophosphamide-induced neutropenia assay were examined. Administration of AGL (100, 200, or 400 mg/kg) significantly ($p<0.05$) increased footpad inflammation in a dose-related manner at 24 hours after antigen challenge in DTH studies. In a homologous prime-boost immunization strategy with tetanus toxoid (TT) or ovalbumin (OVA) antigens, there was a significant ($p<0.05$) elevation of total immunoglobulin, IgG, and IgG1. The mean phagocytic indices of carbon clearance were significantly ($p < 0.05$) greater after oral delivery in treated groups, even at the lowest dose of 100 mg AGL/kg. In immunocompromised mice, after cyclophosphamide-induced immunosuppression, short-term daily supplementation of AGL (100, 200, or 400 mg/kg) produced a significant increase in total neutrophil count and affected other leukocyte differential counts. At all concentrations greater than 125 g/ml in the in vitro assay, AGL inhibited the classical complement system. These findings indicate that the aqueous extract of *Gongronema latifolium* may enhance both cellular and humoral immune responses. Consequently, further research will be needed to learn more about the immune system's strengthening effects and mechanisms.

***Keywords*:** Delayed type hypersensitivity, *Gongronema latifolium*, humoral immunity, haemolytic activity of complement

Introduction

Gongronema latifolium (Asclepiadaceae) is a versatile medicinal climbing shrub found in various tropical African communities, where it is valued for its nutritional and ethnomedical uses.

This edible plant is primarily located in the rainforest zones of Nigeria and other tropical African countries, as well as certain regions in Southern Asia and America.[1,2] *G. latifolium*, commonly known as "Utazi" among the Ibos of Nigeria, "Arokeke" by the Yorubas, "Utasi" by the Efik/Ibibio tribes, and "Iteji" by the Ikales of Ondo in South-west Nigeria, is also called "Kurutu Nsurogya" by the Akan-Asantes of Ghana, "Gasub" by the Serers of Senegal, and "Ndodo-Polole" by the Kissis of Sierra Leone. [3]

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In traditional medicine across various ethnic groups, extracts and decoctions of *G. latifolium* leaves are utilized for diverse purposes. For instance, the Ikale community in Nigeria reportedly uses it to treat malaria, nausea, and anorexia. [4, 5] The Efik and Quas tribes of Cross River State in Nigeria also utilize the leaves for treating malaria, diabetes, hypertension, and constipation.[6] In Senegal and Ghana, *G. latifolium* leaves are applied on children's joints to aid walking, while the boiled fruits are consumed as a laxative. [7]

According to another report, it is use in some West African communities to treat cough, intestinal worms, dysentery, dyspepsia and malaria[7]. Traditionally, asthmatic patients have been reported to get relieved when they chew the fresh leaves of *G. latifolium* while a cold maceration of the roots of the plant can be consumed as treatment for the asthmatic conditions.[7] My other uses have also been reported, hepatitis, bilharzia infection, microbial infections and dyspepsia.[8-9]

They have also been scientific efforts to investigate the pharmacological properties responsible for the claimed benefits of *G. latifolium extract* and fractions. Antiinflammatory,[3,5,10] antimicrobial,[3,5,11] antidiabetic activity, [6,12] antioxidant, [13-14] anticancer, [15] hepato- and nephroprotective activities[16] are among the many reported pharmacological effects of various extracts.

Many medicinal plants have been utilized to treat infections and other diseases due to their different effects on animal immune systems. We have previously shown that various versatile medicinal plants, at least partly, owe their medicinal properties to the modulation of specific and non-specific immune responses to antigenic challenges and

infections.[17-19] We advanced a plausible explanation that the various physiological effects and pharmacological responses of many of these versatile medicinal plants may be explained by the modulatory effects of these herbal therapies on the immune system, acting through effects on several targets and pathways on many disease conditions with significant immune-inflammatory components.[18-21]

Immunomodulation is the change of immune responses which may either upregulate or downregulate the immune responsiveness.[17] Thus, immune system modulation refers to alterations in the immune response, including induction, expression, amplification, or inhibition of any component or stage of the immune reaction. The advancement of immunomodulatory medicines has gained prominence due to recent progress and the growing understanding of immune cells. Their complex and interconnected functions play a crucial role in the body's defense against infections and tumours.

Although ethanol fruit extract and fractions of *Gongronema latifolium* was investigated[22] for antioxidant and immunodulatory activities, there are, generally, insufficient studies on the possible effects of the leaf extracts of *G. latifolium* on immune responses. This study was, therefore, motivated and designed to examine the modulatory effects of the aqueous extracts *G. latifolium* on both innate and specific immune responses in immunocompetent and immunocompromised mice.

Materials and Methods

Collection and authentication

Fresh leaves of *Gongronema latifolium* plant (about 2 kg) were procured from a local market in Aguata, Anambra State, Nigeria in the month October, 2013. The material was identified and authenticated by Mr Ugwuozor, a plant taxonomist, Department of Botany, Nnamdi Azikiwe University Awka, Nigeria.

Extraction of plant leaves

The plant leaves were cleaned, washed, air-dried under a shade, and pulverized with a hammer mill. The fine powder (200 g) was extracted with hot distilled water, maintained at about 40 ^{0}C for 4 h and thereafter allowed to cool. The extract mixture was filtered through Whatman No. 1 filter paper and then-freeze dried to obtain the aqueous *Gongronema latifolium* extract (AGL) for use in the study. The AGL was stored in aliquots at -20 ^{0}C until use in the experiments. Routine yields were 13.25% (w/w) of the powdered leave sample. Preliminary phytochemical tests were carried out using previously described standard procedures.[23]

Animals

Adult Swiss albino mice (25±5 g) were obtained from the animal facility of Nnamdi Azikiwe University, Awka, Nigeria for the study. The mice were housed in the institutional animal facility under ambient temperature and natural lighting conditions. They were fed standard rodent chow pellets (Livestock Feed PLC, Lagos, Nigeria) and given free access to drinking water. The mice were grouped in six per polyacrylic cage and acclimatized for one week before being used in various investigations. The experiments were conducted in compliance with the Institute for Laboratory Animal Research Guidelines for the Care and Use of Laboratory Animals.[24] Additionally, local institutional ethics approval was obtained from the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (FPSRE/UNN/2/00051).

Acute toxicity studies and median lethal dose (LD_{50}) determination

The acute toxicity (LD_{50}) of AGL was estimated in mice by the oral route[25]. The tests involved two phases: in the first phase, the toxic dose range was determined. The mice were placed in groups (n=3) and AGL (10, 100, or 1000 mg/kg) was administered by oral gavage (*per os*). The treated mice were then monitored for 48 h for signs of acute intoxication and mortality. In the second phase of the study, four different doses of AGL (1600, 2900, 3600 and 5000 mg AGL/kg body weight) were administered *per os* as determined by the earlier lethality outcomes. The mice were then observed for lethality and signs of acute intoxication for 24 h.

Randomisation schedule of animals for the immunomodulatory investigations

Mice were randomised into five groups (n= 6) and six (6) animals as follows; Group 1: received 0.2 ml (*p.o*) of distilled water/mice and served as the negative control; Group 2: received 100 mg AGL/kg (*p.o*); Group 3: received 200 mg AGL/kg (*p.o*); Group 4: received 200 mg AGL/kg (*p.o*); Group 5: received 100 mg Noni/kg (*p.o*) and served as the positive control. Noni^{TM} (Good "N" Natural, Ronkonkoma, NY) is an extract of *Morinda citrifolia*, an established "immune booster"

Preparation of Antigen

Sheep red blood cells (SRBC) were collected and washed three times in large volumes of pyrogen-free 0.9% normal saline. The cells were adjusted to a concentration of 0.1 ml containing 1×10^9 cells for immunization and challenge.

The effect of oral AGL supplementation of AGL on delayed-type hypersensitivity (DTH) response

The cell mediated immune response in mice was determined using the delayed type hypersensitivity test. Briefly, mice were treated according to the experiment schedule described in the previous section. Either AGL (100, 200, or 400 mg/kg), Noni (100 mg/kg) or distilled water was administered (*p. o.*) once daily for 5 d. On day 5, the mice so treated were inoculated (i.p.) with 0.1 ml of 1×10^9 cells/ml of SRBC on the right footpad. The mice received a booster immunization of 0.1 ml SRBC on day 14. On day 27, the size of the left footpads of the mice were determined and then given a SRBC (0.1 ml) booster challenge immunization. The thickness of each footpad was thereafter measured again after 24 h later. The DTH response was determined from the increase footpad (oedema) relative to the initial size and expressed as mean percent oedema. [26]

The effect of oral AGL supplementation of AGL on phagocytic activity

The *in vivo* phagocytic activity of the reticuloendothelial system in mice using AGL was determined using the carbon clearance test[26]. Mice were randomized into groups and treated daily for 10 days. The treated mice received a single intravenous injection of carbon suspension (1:50 dilution of Indian ink; Hi-Media Laboratories Pvt. Ltd, Mumbai, India) at a dose of 5 ml/kg body weight 48 hours (h) after the last dose was given. Blood samples were drawn from the retro-orbital venous plexus before injection (0 min) and 15 min after carbon injection. Blood sample was lysed with 0.1% sodium carbonate (Na_2CO_3) solution and the optical density (OD) of the lysed sample was measured spectrophotometrically at 650 nm in a UV-2102 PC^{TM} spectrophotometer (Unico, Shanghai, China). The phagocytic index was then calculated as

$\boldsymbol{k} = \frac{Ln\ OD_{0\ min} - Ln\ Ln\ OD_{15\ min}}{t_{15\ min} - t_{0\ min}}$; where $OD_{15\ min}$ and $OD_{0\ min}$ are the optical densities at time $t_{15\ min}$ and $t_{0\ min}$, respectively. [27]

The effect of AGL supplementation on total leucocytes count (TLC) and differential leucocytes count in cyclophosphamide-induced leucopoenia

Mice received the schedule treatments, either AGL (100, 200, or 400 mg/kg), Noni (100 mg/kg) or distilled water was administered (*p. o.*) once daily for 10 days. On day 10, blood samples were collected from the retro orbital plexus and baseline total leukocytes (TLC), total neutrophils and total lymphocytes counts of the mice were determined. On days 11, 12, and 13, the mice were given a single bolus of cyclophosphamide (30 mg/kg; i. p.), 1 h after the various treatments in those days. Blood samples were collected once more, as described, on day 14 following cyclophosphamide administration. Total leucocytes (TLC) and differential leucocytes count (neutrophils and lymphocytes) were determined and compared to the control groups.[17,50]

The effect of oral AGL supplementation on antibody responses to tetanus toxoid and ovalbumin

In this model, the effect of AGL on humoral immune responses was determined in mice in two separate experiments using ovalbumin (OVA) or tetanus toxiod (TT) as the antigen in a homologous prime-boost strategy. Mice were randomized into groups as shown in the

treatment design, immunized and challenged respectively on day 5 and 20 with 100 µg OVA/mouse (50 µl/footpad) or 0.1 ml of tetanus toxiod/mouse injected into their hind footpads. All mice were then bled by retro-orbital venous puncture on day 19 for primary immune response and also sampled on day 26 (7-day post-booster). Sera samples recovered each time were used for the determination of host primary and secondary humoral responses using Enzyme Linked Immunosorbent Assay (ELISA) as described in the preceding section.[28]

Determination of antibody titre by ELISA

The antibody titres elicited against OVA in the treated and control groups were estimated by enzyme linked immunosorbent assay. Flat bottom polystyrene plates were coated with 2 µg of OVA or TT in 100 µl bicarbonate-coating buffer (pH 9.6) and incubated overnight at 4ºC. Unbound OVA or TT was then washed off with ELISA wash buffer (PBS-T; containing 0.05% Tween-20 in 0.15 M phosphate-buffered saline [PBS, pH 7.2]). Non-specific binding was blocked by the addition of 100µl of 5% fat free milk in phosphate buffer saline (BSA; Sigma, St. Louis, MO) in PBS to each well and the plate was incubated for 1 h at room temperature (RT). The wells were then washed again with PBS-T and the serum were diluted with 2% fat free milk in phosphate buffer saline (1:20) and 100µl of serum were coated and added to dedicated wells. The plates were incubated 1 h at 37ºC before unbound serum proteins and other constituents were washed off using PBS-T. To assess the antibody titre, 100 µl horseradish peroxidase (HRP)-conjugated goat anti-mouse total IgG1 or IgG2a secondary antibodies (BD Bioscience, Heidelberg, Germany) were added (each at 1:1000 dilution) to dedicated wells in the plates and the plates were then incubated another 1 h at RT. Thereafter, the unbound conjugates were washed off with PBS-T and 100 µl/well of freshly prepared 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulpuriic acid solution (ABTS) substrate was added. The plate was incubated at RT for 30 min before the reaction in each well was stopped by the addition of 100 µl of peroxidase stop solution (Kirkegaard & Perry Laboratories, Inc., KPL, Gaithersburg, MD). The color developed in each well was assessed at 405 nm using an automatic Thermomax® ELISA Plate Reader (Molecular Device, Sunnyvale, CA). The mean absorbance value of the sera samples for each treatment group (assayed in duplicate) was then calculated. Sera samples from naïve mice that received neither extract nor immunized with any antigens were also included in the ELISA to monitor for non-specific background signals.[27]

Haemolytic Complement Fixation Assay

The effect of AGL on the haemolytic activity of complement system through the classical pathway was investigated in vitro by a modified microtitre assay method.[17]

Sheep red blood cell was obtained from a female sheep and was washed 3 times using normal saline by centrifugation. A 0.1 ml of 1×10^9 cells solution of the washed SRBC was injected into the rat intraperitoneally (i. p.) and it was noted as day zero. On the 14th day, the rat was given the second immunization. Seven days after the last immunization, blood sample was collected through retro-orbital vein and it was centrifuged to obtain the serum, the serum was used as antisheep antiserum.

A 1:10 dilution of sheep red blood cells (SRBC) and veronal buffer was incubated at 37°C for 30 min with an equal volume of rat antisheep antiserum that was obtained from the immunized rat (1:1000 dilution). 1mg/ml solution of the aqueous extract was prepared in sterile distilled water while 1mg/ml suspension of methanol/ methylene chloride extract was prepared in 1% tween 80 and then serially diluted twofold in 0.5 ml of veronal buffer saline (VBS^{++}). Four different concentrations of each test sample were obtained in triplicate test tubes. Then, a 0.5 ml of 1:20 dilution of fresh guinea pig pooled serum in veronal buffered saline was added to each tube. After pre-incubation at 37 ^{0}C for 30 min, a 0.5 ml of the suspension of the sensitized SRBC (1×10^9) was added. Tubes were incubated at 37°C for 1 hour and the reaction was stopped by centrifugation at 1500 × g for five (5) minutes. A 0.5 ml aliquot was drawn from each tube and mixed with 2 ml of distilled water and the degree of haemolysis measured spectrophotometrically at 541 nm. Control in this assay consisted of similarly treated incubates in which samples (0 % stimulation) were omitted. The effect of the treatment on the haemolytic activity of complement was calculated.

G. latifolium extracts AGL were studied over 62.5 – 500 µg/ml concentrations. Controls in this assay consisted of similarly treated incubates in which samples (0% stimulation) were omitted. The effect of the treatments on the haemolytic activity of complement was then calculated.

Statistical analysis

All results were expressed as mean ± standard error of mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) using Graphpad® Prism Software (Version 7.0). Differences between mean observations were considered significant at p values$\leq$0.05.

Results and Discussion

Immunomodulatory agents from plant and animal sources can improve an organism's immune response against pathogens by stimulating the immune system. It is essential for these agents to undergo systematic studies to confirm their clinical effectiveness and therapeutic claims. In this study, the aqueous extract of *Gongronema latifolium* demonstrated a general stimulatory effect on immune responses in mice. Immuno-stimulatory activities were observed in both humoral and cellular immunity

While there are genuine risks of toxicity and adverse effects associated with some plant products, phytotherapeutic products are from natural sources and have also shown to be very useful globally, especially in developing countries where they constitute an essential portion of remedies for healthcare.[29-30]

In the acute toxicity study of aqueous extract of *G. latifolium* (AGL) in Swiss albino mice, a single dose oral administration up to 5000 mg/kg caused no lethality and did not induce overt signs of toxicity in the treated animals in the mice for up to 24 hours. Hence, it was determined that the LD_{50} was reasonably safe for a single dose of the test extracts up to 5000 mg/kg. Generally, substances with LD_{50} greater than 5000 mg/kg by the oral route are regarded as safe.[25] This result guided the choice of dose range of AGL (100, 200, and 400 mg/kg) used in the study. Furthermore, it highlighted the potential clinical symptoms evoked by AGL during the trial. The acute toxicity test results revealed that the extract has a very large safety margin and was well tolerated by the mice.

Treatment with AGL (100, 200 and 400 mg/kg) showed a dose-dependent increase in the delayed-type hypersensitivity (DTH) response to SRBCs antigen in mice. DTH response to cellular antigen corresponds to cell-mediated immune response. The increase in DTH due was significant at doses of 200 and 400 mg AGL/kg and was as high as 46.32% and 52.31% higher than untreated control, respectively (Figure 1). These values are comparable to the increase in DTH of 55.09% obtained in the group of mice treated with the standard, Noni® (Figure 1). The effect of AGL on cell mediated immunity (CMI) was investigated by the DTH response of in mice immunised with a T-cell dependent antigen, SRBC[31]. During CMI responses, sensitized T-lymphocytes are converted to lymphoblasts and secrete lymphokines when challenged by antigens, attracting more phagocytic cells to the infected site. In this study, administration of AGL significantly increased the DTH reaction, as indicated by greater foot-pad enlargement in response to T-cell dependent antigen-SRBC in treated mice. This result reveals the stimulatory effect of AGL extract on T lymphocytes, macrophages, and other accessory cell types involved in delayed-type hypersensitivity. These cells are required for the expression of inflammatory mediators at the inflammatory site[32]. Macrophages and other cells which are stimulated are mobilized and infiltrate the infected site to produce defensive inflammatory response.[32-33]

Short term oral supplementation with AGL significantly increased the clearance of colloidal carbon particles from blood, as evidenced by a higher phagocytic index ($p<0.05$) at doses above 100 mg AGL/kg dose (Figure 2). Similarly, the immunostimulatory effects of AGL on the elimination rate of exogenously administered colloidal carbon particles from blood circulation were determined using the carbon clearance test. This process involves enhanced phagocytic activity, potentially involving macrophages. Phagocytes, including neutrophils and monocytes, are immune cells that play a crucial role in both early and

late stages of immune responses. Their primary function is to circulate and migrate through tissues, ingesting and destroying pathogens and cellular debris.[34] The degradation of ingested material is facilitated by the production of reactive oxygen species within the phagolysosomes of phagocytes.[35]Phagocytes serve as the first line of defense in the body's immune response. Monocytes/macrophages, the most significant type of phagocytes, are essential components of nonspecific immunity. Monocytes/Macrophages and granulocytes play a significant role in nonspecific immune defence mechanisms.[36] Macrophages play a crucial role in the immune system, functioning as phagocytic, microbicidal, and tumoricidal effector cells. They contribute to the host's defense against infectious stress by secreting cytokines that stimulate other immunocytes.[37] They also play a role in innate immunity by attacking, destroying, and ingesting foreign substances through the production of cytokines and nitric oxide.[38] In this this study, treatment with AGL produced remarkable increase in the phagocytic clearance of colloidal carbon. This is indicated by the higher phagocytic indices of the treated group of mice, comparable to that of the standard drug, Noni. The extract's potential to increase the reticuloendothelial system (RES) activity may contribute to this effect. The RES plays a crucial role in host defense by removing foreign material, and the enhanced activity likely stimulates natural resistance.[39]

Daily oral administration of the extracts improved cyclophosphamide-induced immunosuppression in mice. Pre-treatment of animals with AGL (100, 200 and 400 mg/kg), before cyclophosphamide administration produced 54.0%, 50.4% and 32.2% reduction in total leukocytes count, respectively (Figure 3). Total leucocytes count in AGL-treated groups were comparable to the values in mice that received the standard drug, Noni®. These values are significantly ($P<0.05$) lower than the 61.34% reduction in the group of mice that received cyclophosphamide alone (Figures 3a).

Similarly, the differential leucocyte counts of lymphocytes in the group that received AGL (400 mg/kg) was higher by at least two-fold when compared to the mean value in the untreated control group (Figures 3b). Generally, the prior administration extracts produced remarkably amelioration of the cyclophosphamide-induced leucopenia as evaluated by the total leucocyte count (TLC) and differential counts of lymphocytes and neutrophils when compared to the negative control (Figures 3).

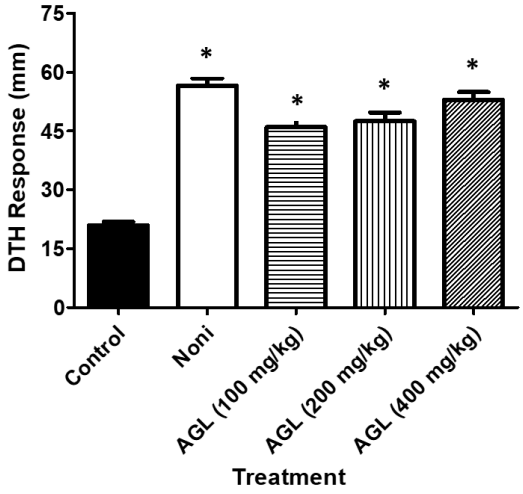

Figure 1: Delayed type hypersensitivity response I mice treated with aqueous extract of *G. latifolium* (AGL). Five groups (n =6) of mice were treated (daily, *per os*) with or without AGL (100, 200, 0r 400 mg/kg) or Noni (100 mg/kg). DTH response determined from the increase (swelling) rrelative to initial ootpad size and expressed as mean thickness (mm± SEM). **P*<0.05 versus untreated control.

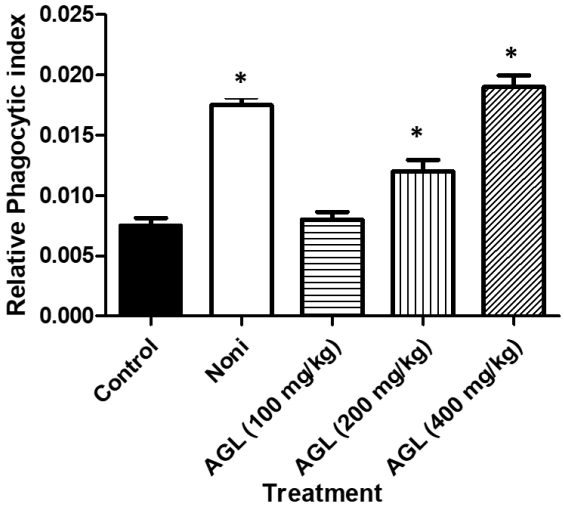

Figure 2: treatment with aqueous extract of *G. latifolium (*AGL) increases phagocytic index measured by clearance of colloidal carbon in mice. P<0.05 versus control

In addition to the carbon clearance assay, the immunomodulatory effect of AGL on cell-mediated immune response was investigated using the cyclophosphamide-induced neutropenia model. Cyclophosphamide, a nitrogen mustard alkylating agent, has its activated form, phosphoramide mustard, which induces cytotoxic effects primarily by alkylating DNA. This results in cross-linking of nucleic acid strands (DNA and RNA) and inhibition of protein synthesis. Consequently, cyclophosphamide causes myelosuppression, leading to stem cell loss and preventing bone marrow from regenerating new blood cells. This leads to thrombocytopenia and other cytotoxic manifestations. [50]

Remarkably, the aqueous extract of *G. latifolium* protected the treated mice against immunosuppresssion and leucopenia induced by cyclophosphamide treatment in a dose-dependent manner. This is shown by the prevention of the lower values haematological parameters as seen in untreated group that received cyclophosphamide alone. Immunosuppression caused by cyclophosphamide and other anticancer agents often complicates the course of cancer chemotherapy, worsening patients' recovery and treatment outcomes. This underscores the immunorestorative and chemoprotective benefits of AGL against bone marrow suppression (total leucocytes and differential leucocytes count) induced by cyclophosphamide, a chemotherapeutic cytotoxic agent. Therefore, aqueous extract of *Gongronema latifolium* (AGL) could be a candidate therapeutic agent with chemoprotective potentials for the management or treatment haematopoietic damages, especially damages to cells involved in the immune responses. These data could be complemented by further experiments.

Short-term daily oral administration of AGL (100, 200, and 400 mg/kg body weight), followed by a uniform prime-boost immunization with ovalbumin, led to significantly ($P<0.05$) higher levels of ovalbumin-specific IgG1 (Figure 4a) and IgG2a (Figure 4b) in the sera of immunized mice compared to the mean titre values in the negative control mice. The mean secondary antibody levels developed against ovalbumin (OVA) were at least 2-fold higher in the group of immunized mice that received daily oral supplementation of AGL. The increase in primary and secondary antibody expression was comparable to the levels observed in the group that received the standard Noni®, a commercial extract of *Morinda citrifolia* known for its potent immunostimulant properties, including enhancing antibody responses[49]. In the same manner, daily oral supplementation of AGL significantly increased the mean tetanus toxoid (TT)-specific IgG1 (Figure 4c) and IgG2a (Figure 4d) in the sera of mice immunised with TT using a similar homologous prime-boost plan. Antibodies, also known as immunoglobulins, are proteins produced by B lymphocytes in response to specific antigens. These expressed antibodies neutralise the antigens directly or opsonize them for easier phagocytosis. Therefore, measuring the level (titre) of antigen-specific antibody production is an effective

method for evaluating humoral immunity. In this study, the impact of AGL on humoral (antibody) immune response was evaluated in response to tetanus toxoid or ovalbumin. The primary and secondary titres of immunoglobulin G (IgG1 and IgG2a) in mice treated with AGL increased in a dose-dependent manner compared to the control group, suggesting a stimulatory effect of AGL on antibody production.

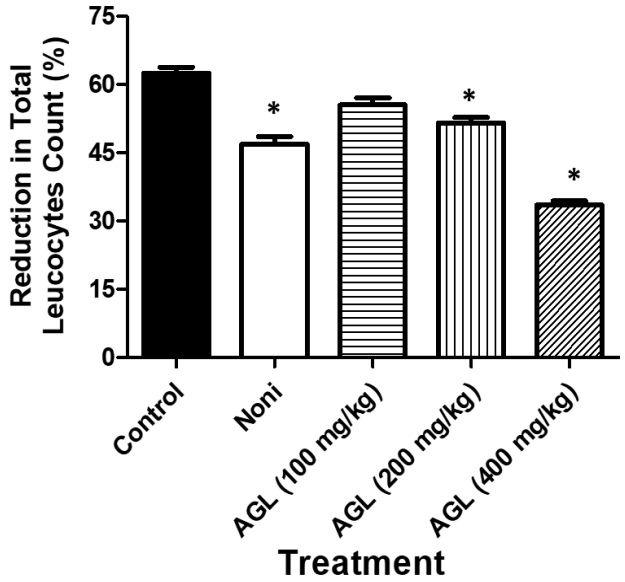

Figure 3a: Effect of aqueous extract of *G. latifolium* (AGL) on cyclophosphamide-induced myelosuppression measure by total leucocytes count in mice. **P*<0.05 versus control

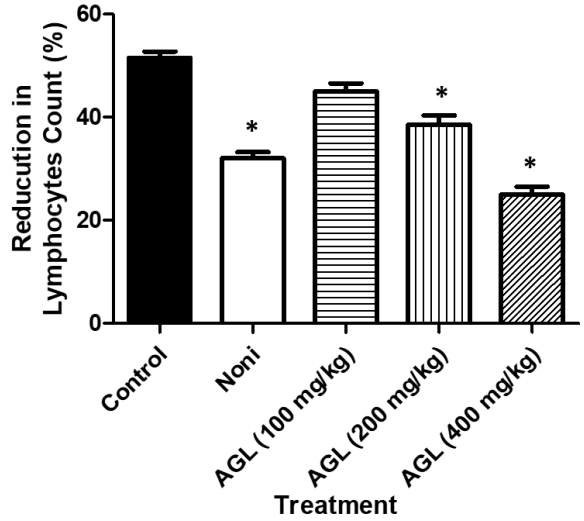

Figure 3b: Effect of aqueous extract of *G. latifolium* (AGL) on cyclophosphamide-induced myelosuppression measure by lymphocytes count in mice. **P*<0.05 versus control

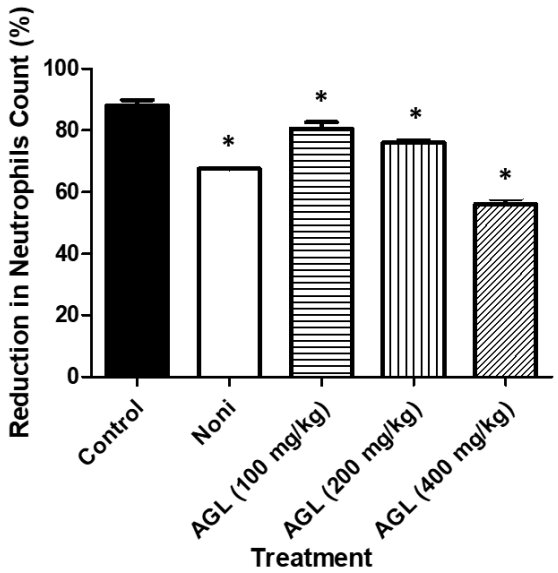

Figure 3c: Effect of aqueous extract of *G. latifolium* (AGL) on cyclophosphamide-induced myelosuppression measure by nrutrophils count in mice. **P*<0.05 versus control

A high optical density (OD) was recorded, indicating strong haemolytic activity of complement proteins on sensitized sheep red blood cells. The addition of AGL (62.5-500 µg/ml) significantly ($P < 0.05$) reduced the mean optical density of the medium in a concentration-dependent manner, relative to the negative control. Therefore, AGL inhibited the haemolytic activity of complement proteins on sensitized sheep red blood cells (Figure 5). The complement system is an essential component of the innate immune system and collaborates with the adaptive immune system in various ways. The complement system plays a role in immune responses and is associated with various infectious and non-infectious inflammatory diseases.[41] In the in vitro assay, AGL effectively inhibited haemolytic complement activity, thereby modulating innate and adaptive complement-dependent immune responses.

Pharmacologically, *G. latifolium* has been reported as hypoglycemic,[42-43] hypolipidemic,[43] nephroprotective,[44] antifertility;[45] anti-inflammatory,[10] antiulcer,[46] anticancer[15] and antimicrobial.[11, 47-48] The pharmacological activities of *G. latifolium* have been attributed to some of the chemical constituents that have shown immunomodulatory potentials in other investigations, such as alkaloids, flavones, sterols, saponins, tannins, flavonoids.[9, 14, 47]

Conclusion

These results suggest that the aqueous extract of a diabetic folklore recipe did not exhibit any acute toxicity signs or symptoms. Further, isolation, identification, chemical compositions, and the major active compounds of the recipe responsible for the hypoglycemic effect should be undertaken in order to confirm and clarify the mechanism behind this activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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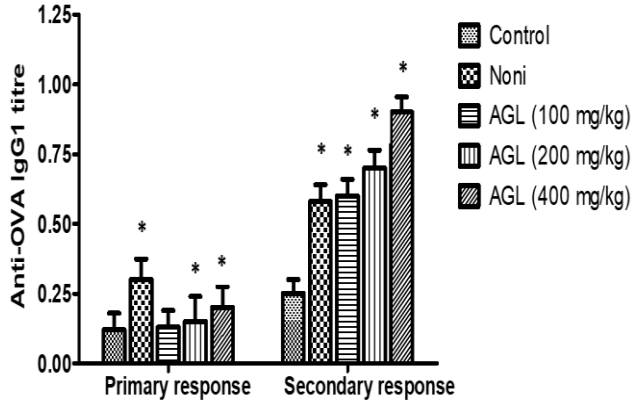

Figure 4a: Ovalbumin-specific IgG1 responses in mice treated with aqueous extract of *G. latifolium* (AGL). *P*<0.05

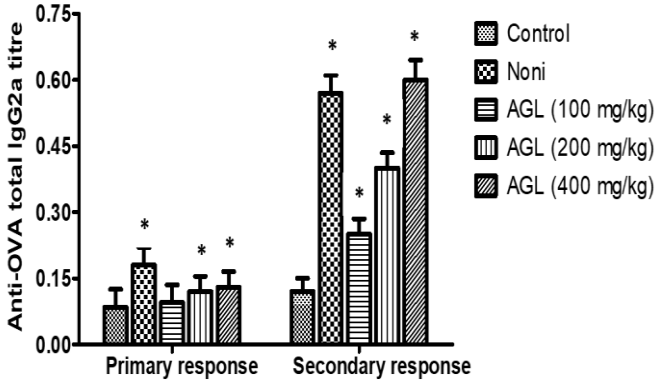

Figure 4b: Ovalbumin-specific IgG2a responses in mice treated with aqueous extract of *G. latifolium* (AGL). *P*<0.05

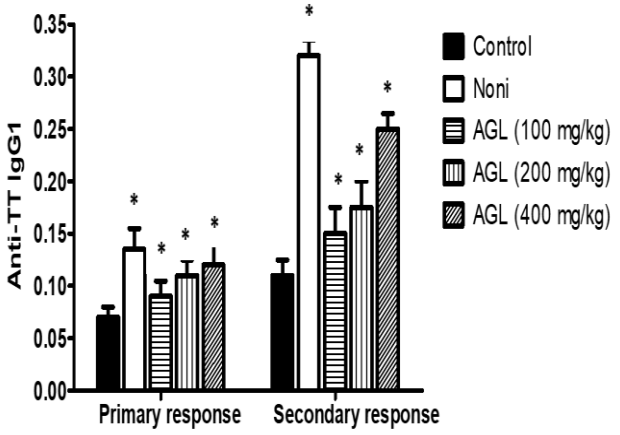

Figure 4c: Tetanus toxoid- specific IgG1 responses in mice treated with aqueous extract of *G. latifolium* (AGL). *P*<0.05

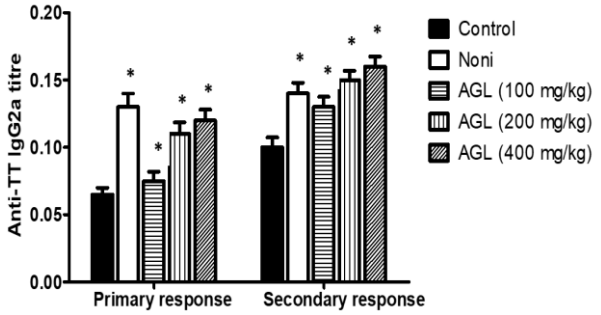

Figure 4d: Tetanus toxoid- specific IgG2a responses in mice treated with aqueous extract of *G. latifolium* (AGL). *P*<0.05

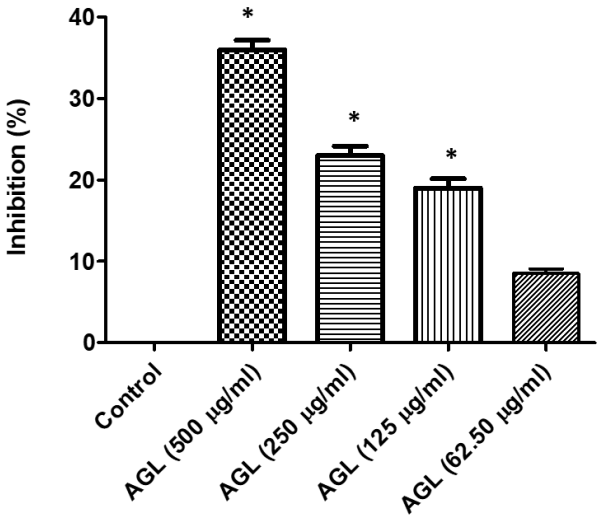

Figure 5: Effect of aqueous extract of *G. latifolium* (AGL) on haemolytic complement fixation. **P*<0.05 versus control

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