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Oral Administration of Methanol Extract of *Gongronema latifolium* (Utazi) Up-Regulates Cytokine Expression and Influences the Immune System in wistar Albino Rats

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Abstract: The aim of the present study was to evaluate the immunomodulatory potential of methanolic extract of *Gongronema latifolium* (utazi) on wistar albino rats. A total of sixteen (16) Wistar albino rats were used for the study and divided into four (4) groups of four (4) rats each. The normal control was administered normal saline while the negative control was administered pyrogallol in order to suppress the immune system. The treated rats were administered 200 mg/kg body weight and 400 mg/kg body weight of the plant extract and 2% pyrogallol to represent the low and high doses respectively. These administrations lasted 21 days after which blood was drawn through ocular puncture and serum collected for the assay. The immunomodulatory activity was evaluated on haematological indices (packed cell volume, haemoglobin and total white blood cell count), cytokine expressions (interleukine-2, interferon-1 and tumour necrosis factor), haemaglutination titre (primary and secondary humoral antibody) and the delayed type hypersensitivity (DTH). No significant ($p>0.05$) differences were observed in the haematological indices. Significant ($p<0.05$) increase was seen in the interferon-γ, while a non-significant ($p<0.05$) increase was obtained in tumour necrosis factor-α and interleukine-2. The primary and secondary antibody titre showed significant ($p<0.05$) increases and the delayed type hypersensitivity also indicated a significant ($p<0.05$) increase. These comparisons were made against the negative control. This study suggests that the extract of *G. latifolium* possess immunostimulatory effect.

Key words: Immuno-Stimulatory · Interleukine · Tumour Necrosis Factor · Haematological · *Gongronema Latifolium*

INTRODUCTION

The immune system can be defined as a network of lymphoid organs, tissues and cells and also the products of these cells. The main function of immune system is to protect the body against infectious microbes or foreign substances [1,2]. The success of this system in defending the body relies on an incredibly elaborate and dynamic regulatory communication networks, that involves multiple and functionally differing cell types which provide a large variety of defend mechanisms. The outcome is a sensitive system of checks and balances that produce an immune response that is prompt, appropriate, effective and self-limiting [3,4]. Immunomodulation refers to the action undertaken by the medication on autoregulating processes that steer the immunological defense System [5,6]. Immunomodulator are substances that are capable of interacting with the immune system to upregulate or down-regulate specific aspect of the host response [7, 8]. It is also known as biologic response modifier or immunoregulator which is function as drug leading predominantly to a non-specific stimulation of immunological defense mechanisms [9]. These may include some bacterial product, lymphokines and plant derived substances.

Plants are rich sources of phytoconstituents which are reported to incite para-immunity and also affects the non-specific immunomodulation especially of

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granulocytes macrophages, natural killer and complement system [10]. Several research works have been done on *Gongronema latifolium* in other to ascertain its medicinal use. [3], in his review on the antioxidant potential of African medicinal plants agreed with Liu *et al.* [11] on the antioxidant property of *Gongronema latifolium* and it is advised that this plant be consumed to increase the antioxidant ability of the immune system. The earlier studies of the researchers have shown that ethanolic leaf extract of *Gongronema latifolium* possess analgesic effects. The stem bark extract of *Gongronema latifolium* has equally been reported to have anti-ulcerative property. The anti-inflammatory property of the leaves of *Gongronema latifolium* was confirmed by Mitchison [12] which was also confirmed by Mitra *et al.* [13] and Morebise *et al*.[14].The present study was undertaken to find out the possible interaction of *G. latifolium* leaf on immune response.

MATERIALS AND METHODS

Plant Material: Fresh leaves of the plant *Gongronema latifolium* were obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria and authenticated by Dr Omosun, G of Department of PSB, Michael Okpara University of Agriculture, Umudike; Abia State, Nigeria.

Preparation of Methanol Extract: The dried powdered plant material leaves (80 grams) was dissolved in 600 ml of methanol and left to stand for 48 hours with intermitent shaking, then filtered using filter paper. The filterate was concentrated by heating under regulated temperature of 50°C using a water bath and allowed to cool. The concentrated extract (stock) used for the study.

Preparation of 2% Pyrogallol: Pyrogallol reagent was prepared by dissolving 2g of pyrogallol in 100ml of distilled water. It was shaken vigorously for uniform mixing and this solution was used for the study.

Preparation of Antigen: Fresh blood was collected from sheep sacrificed in a local slaughter house and preserved in EDTA bottles. It was washed three times with normal saline via centrifugation and the suspension was adjusted to 1×10^8 SRBC/ml for immunization and challenge.

Experimental Design: A total of 16 healthy male Wistar albino rats weighing 100-150 grams were used for the study. All animals were housed in an animal house under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were fed to the animals *ad libitum*. Animals were divided into four equal groups. Group I (normal control) received normal saline via gavage. Group II (negative control) received 100 ml/kg body weight pyrogallol solution. Group III received 200 mg/kg body weight of extract and pyrogallol and group IV received 400 mg/kg body weight of extract and pyrogallol solution. All administrations were done daily for 21days.

SRBC- Induced Humoral Antibody (HA) Titer: To specifically assess effects on antibody formation, groups of four rats per treatment were immunized with 0.1ml of sheep red blood cell suspension (1.1×10^8 SRBC/ml) intra peritoneal. The day of immunization was referred to as d ay 0. Seven days later (Day 7) the rats were challenged by injecting 0.1ml of SRBC suspension into the left hind foot pad of the rats. Blood samples were collected from all the animals separately by ocular puncture using glass capillary tubes on day 7 (After challenge) for measurement of primary antibody titer and on day 14 for measurement of secondary antibody titer. Antibody levels were determined by the method described by Shinde *et al*. [19]. Collected blood was allowed to clot then centrifuged to get serum. 25 microL as placed into one well of a 96-well micro titer plate. Serial two-fold dilutions of the serum were made using 25 microL normal saline each time of transfer across the plate. To the 25 microL diluted serum in each well, 25 microL of 1% v/v SRBC suspension in normal saline was added. The micro titer plate was maintained at room temperature for 1 hour and the content of then examined for haemagglutination. The value of the highest serum dilution showing haemagglutination was defined as the antibody titer for the given rat.

SRBC - Induced Delayed Hypersensitivity (DTH) Response: The method of Doherty [3] was used to assess the effect on DTH responses in the treated rats. Daily treatment with *Gongronema latifolium* leaves extract began 14 days prior to the challenge (i.e. starting on the same day as immunization with SRBC). The negative and normal control rats received pyrogallol and normal saline respectively each day. On day 0 all rats were immunized. After 14 days of treatment, 0.1ml of SRBC solution was injected subcutaneously into their right hind footpad, the thickness of each rat's left footpad was measured just before the challenge using a Schneltaster caliper that could measure to a minimum unit of 0.01mm. The rats were then challenged by injecting 0.1ml of SRBC solution intra

peritoneal into their left hind footpad (deemed time 0). Footpad thickness was the re-measured after. The difference between the thickness of the left footpad just before and 48 hours after challenge (in mm) was taken as a measure of DTH.

Hematological Profile: After 21 days of repeated gavage treatment, blood was collected from each rat via ocular puncture under light anesthesia. Various parameters such as total white blood cell (TWBC), haemoglobin (HB) levels and packed cell volume (PCV) were estimated using standard haematological techniques as described by Ochei and Kolhaktar (25)

Determination Of Cytokines-TNF, IL-2 and IFN: The sera obtained from the animal's blood sample were labeled and analyzed. Serum TNF- α, IL-2 and IFNγ were assayed by the enzyme linked immunoassay (ELISA) method using Biovendor assay kits. Absorbance was read to determine the optical density of each well immediately, using a microplate reader set at 450 as the primary wave length to measure color intensity. The intensity of this colored product is directly proportional to the concentration of the cytokines (IFNγ, IL-2 or TNF-α) present in the samples.

RESULTS

In Fig 3 above, it would be seen that there were slight mean value difference across the different groups; though there was no significant difference (p>0.05) when the treated groups of mean values (13.49±1.03) and (13.34±0.09) for the low and high dose respectively, were compared against the negative control (14.08±0.87).

From the chart as seen in Fig. 4 the mean value of the low dose group (0.54±0.05) showed no significant increase (P>0.05) when compared against the positive and negative control groups. However, comparing the mean valueof the high (1.57±0.32) dose group shows a significant increase (P<0.05) when compared against the low dose and negative (0.36±0.25) control group.

From the diagram above (Fig.5) there was no significant difference (P>0.05) when the low dose (1.34±0.18) and high dose (1.39±0.16) groups were compared against the negative control group (1.11±0.18).

From Fig 6 above, the mean values of the low dose group (0.76±0.19)and high dose group (0.95±0.20)shows no significant difference (P>0.05) when compared against the mean values of the negative control group (0.80±0.15).

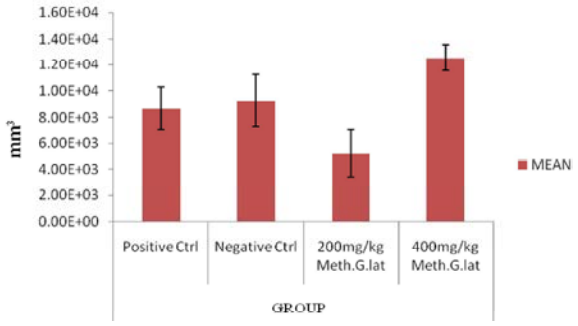

Fig. 1: Effect of Methanolic Extract of *Gongronema latifolium* OnTotal WBC Count Total WBC counts in the treated groups were significantly different (p>0.05) when compared with the negative control group.

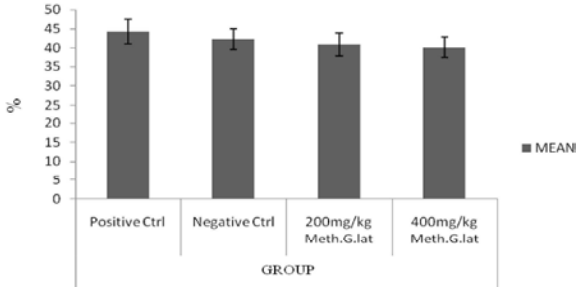

Fig. 2: Effect of Methanolic Extract of *Gongronema latifolium* On Packed Cell Volume The mean value of blood PCV showed no significant difference (p>0.05) when the treated groups were compared with the control groups (positive control and negative control groups).

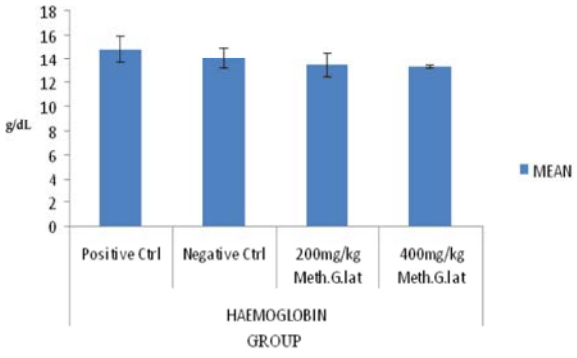

Fig. 3: Effect of Methanol Extract of Gongronema latifolium on Haemoglobin concentration

From the chart above (Fig 7) the low dose (3.5±0.42) and high dose (5.0±0.5) groups show a significant (P < 0.05) increase in the titre values when compared against the negative control group (1.50±0.58).

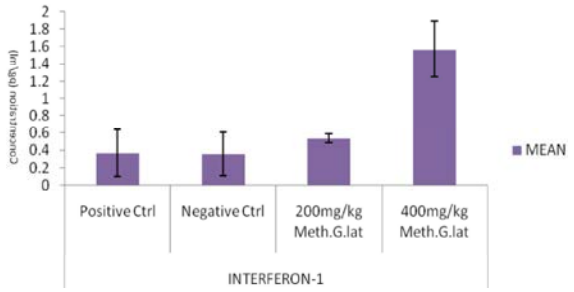

Fig. 4: Effect of Methanolic Extract of *Gongronema latifolium* On Interferon-γ

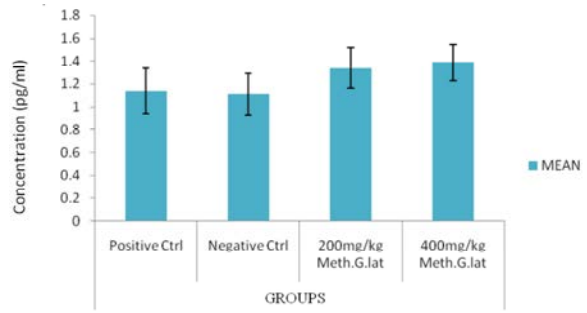

Fig. 5: Effect of Methanolic Extract of *Gongronema latifolium* On Tumor Necrosis Factor-α

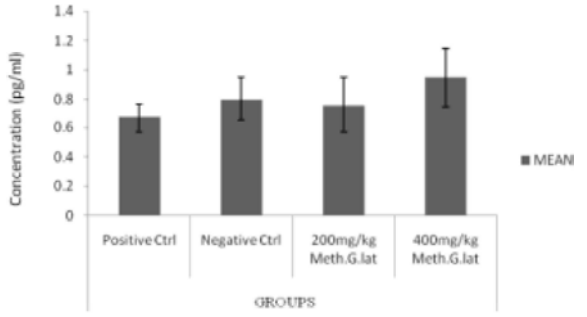

Fig. 6: Effect of Methanolic Extract of *Gongronema latifolium* On Interleukin-2

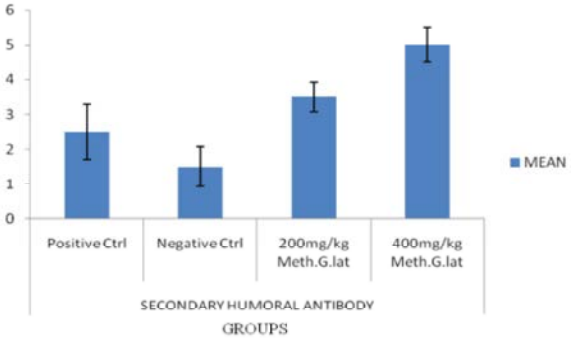

Fig. 7: Effect of Methanolic Extract of *Gongronema latifolium* On Primary Antibody Titre

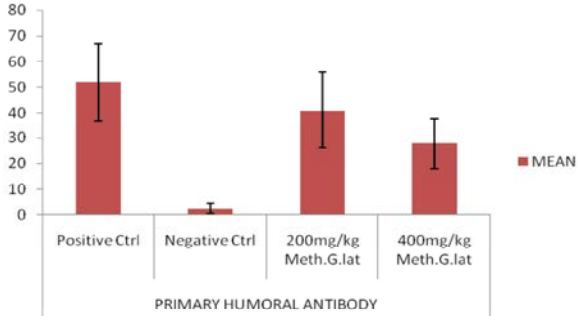

Fig. 8: Effect of Methanolic Extract of *Gongronema latifolium* On Secondary Antibody Titre

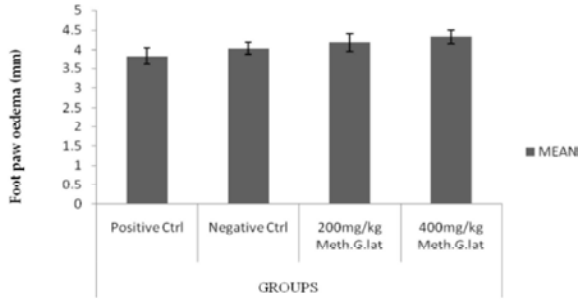

Fig. 9: Effect of Methanolic Extract of *Gongronema latifolium* On DTH

From the chart above, (Fig 8) low dose (41.00±15) and high dose (28.00±10) groups show a significant ($P < 0.05$) increase in the titre values when compared to the negative control group (2.5±1.91)

Figure 9 showed 200 mg/kg (4.18±0.22) dose showed a significant ($p>0.05$) difference in the DTH (delayed type hypersensitivity) when compared against the negative control (4.03±0.15), while the mean value of the high dose (4.33±0.17) displayed a significant ($p<0.05$) increase when compared against the negative control group.

DISCUSSION

Gongronema latifolium has been shown to have antioxidant effect [5, 14] and has thus been advised to be consumed in order to increase the body's antioxidant activity of the immune system. Phytochemicals have equally been attributed to be among the constituents of this plant as [15- 17] in their report showed that *Gongronema latifolium* contains flavonoids, terpenes, tannins, saponinsand alkaloids.

Some plants modulate both humoral and cellular immunity, while others activate just the cellular components of the immune system. Thus, the evaluation

of plants for their ability to promote or inhibit immunocyte proliferation is essential to the study of immunomodulation and drug discovery [18- 20].

In this present study, there was an indication of a non-significant effect of the leaf extract of *Gongronema latifolium* on the measured haematological parameters: total white blood cells (TWBC), packed cell volume (PCV) and haemoglobin (HB). This might be due to the presence of saponin, which has been reported to reduce haematological parameters probably due to lysis of blood cells or suppression of blood cells synthesis [20]. Haemolysis is brought about by saposis as it affected the interaction between transmembrane proteins and the cytoskeleton leading to aggregate unbound dimer fractions [21].

Results of cytokines expression, showed a significant ($P<0.05$) increase in interferon gamma (IFN-γ) expression observed in the high dose group when compared against the other groups. This suggests an antiviral effect and stimulatory activity of *Gongronema latifolium* against intracellular pathogens [22, 23]. Interferons are responsible for inducing transcription of a large group of genes which play a role in host resistance to viral infections, as well as activating key components of the innate and adaptive immune systems including antigen presentation and production of cytokines involved in activation of T cells, B cells and natural killer cells [16]. The increase in the expression of tumor necrosis factor on both low and high dosage groups when compared against the control groups also supports the observed influence of *Gongronema latifolium* on cellular immunity) TNF is produced by the T cells in response to antigen stimulation. The Tumor Necrosis Factor (TNF) superfamily of cytokines represents a multifunctional group proinflammatory cytokines which activate signaling pathways for cell survival, apoptosis, inflammatory responses and cellular differentiation [18,19].

Furthermore the results showed an up regulation in serum interleukin 2 (IL-2) expression in the rats given higher dose of the extract. This is not far from the fact that IL-2 has an immune enhancing function, it plays roles in supporting proliferation [2, 5, 9, 10] and survival [6] of T cells and differentiation of naïve T cells into effector and memory cells [7]. The proliferation and survival could be as a result of the ability of IL-2 to induce protein synthesis in antigen-activated T-cells; while its absence abrogates amino acids uptake and subsequent incorporation into proteins [13].

The results showed the extract has influence on humoral immune response. Primary and Secondary antibody titres were significantly ($P<0.05$) elevated in the test groups compared to the untreated control. Delayed type hypersensitivity (DTH) is characterized by the activation of phagocytic cells and inflammation induced by cellular mediated immunity. DTH response is an indicator that the plant extract and fraction have a stimulatory effect on lymphocytes and accessary cells types required for the expression of reaction response and B cell activation [9]. In this study, the extract showed a non significant difference on DTH compared to the controls suggesting a pro-inflammatory effect. This finding disagreed with the work of Mitchison [12] who earlier reported an anti-inflammatory effect. The mechanism behind this elevated DTH during the cell mediated immunity responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lymphokines, attracting more scavenger cells to the site of reaction [24]. An increase in DTH response indicates a stimulatory effect of the plant which has occurred on the lymphocytes and accessory cell types required for the expression of this reaction [20]. The main chemical constituents of *G. latifolium* are flavonoids tannins, saponins, polyphenol and alkaloids. Recent reports indicate that several types of flavonols stimulate human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T cells, cytokines, interleukin 2, g-interferon and macrophages and are thereby useful in the treatment of several diseases caused by immune dysfunction [23]. Result of this work is in agreement with the above assertion. In conclusion In this study, the extracts of *Gongronema latifolium* leaves generally showed immunostimulatory effect on both the innate immune function (humoral and cell mediated immunity) and the adaptive immune function while exhibiting pro-inflammatory activity as shown by the increase in DTH response and interleukine-2 expression in the wistar albino rats.

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