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Evaluation of Effect of Hydromethanol Seed Extract of *Azanza Garckeana* on TNFα and Immunogluobulins Levels in Cyclophosphamide Immune-Depressed Male Wistar Rats

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Abstract— *Azanza garckeana as well as other important plants like ginger, garlic, turmeric, etc., have been identified to possess enhancing properties on various physiologic mechanisms including improvement of immune functions. Thus, considering the complex nature of the immune system and its need for an effective regulatory mechanism, the present study evaluated the possible stimulatory effects of hydromethanol seed extract of Azanza garckeana (HSEAG) on tumor necrosis factor-alpha (TNFα) and immunogluobulins levels in cyclophosphamide immune-depressed male Wistar rats. Exactly 50 male Wistar rats were used for the study and distributed into 10 different groups of 5 individuals each. After 30 consecutive days of treatments with various doses of the extract (500mg/kg, 1500 mg/kg & 300mg/kg amongst others), blood was collected via cardiac puncture from the individual rats. Antibody in agar plate method was used in the determination of immunoglobulins level. The outcome of treatments with HSEAG and levamisole with no experimental immune depression, showed that the 1500mg/kg HSEAG exerted non-significantly ($p>0.05$) raised levels of IgA, IgG and IgM when compared to the rest group. The finding on the effect of HSEAG administration on TNFα levels in cyclophosphamide co-treated study models, there were non-significant reductions on this maker in the different doses of the extract-alone treated groups as against those co-treated with CMP + HSEA. Particularly, the 1500mg/kg HSEA treated group had substantial reductions in the TNFα level when compared to that of 1500mg/kg HSEAG + CPM treated. In conclusion, the present study has found that different doses of the HSEAG extract alone may possess the potential of down-regulating inflammation vis-a-vis anti-inflammation but with stimulatory effect in immune depressed scenarios.*

Keywords— *Azanza garckeana; hydromethanol seed extract of Azanza garckeana (HSEAG), immunogluobulins levels, inflammation.*

I. INTRODUCTION

The immune system is the main biological defensive mechanism that protects the body from various invading pathogens and molecules, including bacteria, viruses, fungi, and parasites as well as complex foreign chemicals (1), (2). The two main portions of this complex defense system are innate and adaptive, each of which serves a different but complimentary role (3), (4). Further, it is understood that, in preclinical research and clinical interventions, immune system monitoring is crucial as it assists in determining immunological activation or suppression, revealing mechanisms of action, and assessing the effectiveness of treatments (Hartmann et al., (5), (6). A focus in clinical practice, includes monitoring how patients' immune systems react to illnesses or treatments over time in order to inform treatment choices and translational research (6).

Significantly, a number of plants have been discovered to boost immune function, including but not limited to fresh citrus juice, ginger, garlic, turmeric, Azanza garckeana fruits, and more, (7), (8). The aforementioned properties of such plants are attributed to their content of bioactive molecules such vitamins, terpenoids, polyphenols, and β-glucans (7) (9). Interestingly, *Azanza garckeana* has been recorded as an important plant that serves dual purposes as food and medicine in tropical Africa (10). This plant is of the Malvaceae family and its generic name "Azanza" is extracted from the word "Azania", referring to black and surviving in Zanzibar. (10). Amongst various locations, the plant is also native to Northern Nigeria (mainly in Gombe State) and have been noted for its possible several biological/therapeutic potentials in the management of several diseases and ailments (e.g. hypertension, infertility, liver diseases, etc) by locals (11), (12).

Now, considering the complex nature of the immune system, an effective regulatory mechanism is needed; as its under or over regulated states may prevent its timely response or result in autoimmune conditions respectively (13), (14). In the light of the foregoing, more often than not, focus has been on resolving over-stimulating immune conditions (like autoimmune conditions and allergic reactions), with less concern on depressed immune conditions (primary and acquired immunodeficiencies).

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Consequently, the present study set out to evaluate the possible stimulatory effects of hydromethanol seed extract of Azanza garckeana (HSEAG) on tumor necrosis factor-alpha (TNFα) and immunogluobulins levels in cyclophosphamide immune-depressed male Wistar rats.

II. Materials and Methods

Research Design

The present evaluation was an experimental study that used the male Wistar rat model. Normal or routine laboratory procedures were followed to determine the serum immunoglobulins (Igs) and tumor necrotic factor alpha (TNFα) levels in the study models.

Handling of Plant Materials

A. Collection and Identification of Plant Materials

Fresh and mature fruits of *A. garckeana* were obtained from a standard farm in Gombe State, Nigeria. Thereafter, it was identified and authenticated by a plant taxonomist (Dr. M. Suleiman) of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, University of Port Harcourt, Nigeria. A sample of the plant was deposited as voucher at the herbarium domiciled in the Department of Pharmacognosy and Phytotherapy of same institution; the plant sample was numbered: UPHM0596.

B. Preparation of the Extract

Using the method of Ojeka et al., (15), the fine powder of the plant was subjected to crude maceration extraction using a combination of water (20 per cent) and methanol (80 per cent) as the solvent. The resulting crude extract was filtered after 72 hours by passing through a Whatmann number 4 filter paper. The filtrate was further concentrated using a rotary evaporator in vacuum at 400C and was later freeze dried. The yielded extract was then weighed out, put into a clean sample container and stored in a refrigerator (40^{O}C) until when ready for use.

Handling of Experimental Animal Models

The study animals, 50 male Wistar rats, were procured from and as well housed in the animal house of Faculty of Basic Medical Sciences (FBMS), University of Port Harcourt, and were maintained under the 12-hour light/dark cycle at room temperature and humidity. The study models were allowed access to acclimatize (for fourteen days) to their new accommodation and protocol prior to the beginning of experimentation on them. The study models had access to normal rat chow and water *ad libitum*. The animals were treated following the guidelines for the care and use of experimental animals (16).

Induction of immune depression with cyclophosphamide and evaluation of TNFα and serum immunoglobulin levels

Twenty-five (25) male Wistar rats were randomly separated into 5 groups of 5 rats each and subsequently treated as follows for 30days:

Protocol for the Experimental Study

i. For assessment of the serum immunoglobulins effects of HSEAG alone

Group 1: Negative Control: 1ml of distilled water
Group 2: Low dose *Azanza garckeana*: 500 mg/kg bw of HSEAG
Group 3: Medium dose *Azanza garckeana*: 1500mg/kg bw of HSEAG
Group 4: High dose *Azanza garckeana*: 3000mg/kg bw HSEAG
Group 5: Positive control: 30mg/kg bw of Levamisole

Blood was collected via cardiac puncture. The antibody in agar plate method was used (17) in the determination of immunoglobulins levels (using the SurgiField Micro plate reader, SM-300A).

ii. For effects of HSEAG and CPM co-treatment on TNFα and serum immunoglobulin levels in the study models

Group 1: Negative Control: 1ml of distilled water
Group 2: Low dose *Azanza garckeana*: 500 mg/kg bw HSEAG
Group 3: Medium dose *Azanza garckeana*: 1500mg/kg bw HSEAG
Group 4: High dose *Azanza garckeana*: 3000mg/kg bw HSEAG
Group 5: Low dose *Azanza garckeana* + *CPM*: 500 mg/kg bw of HSEAG + 30mg/kg bw cyclophosphamide
(CPM)
Group 6: Medium dose *Azanza garckeana* + *CPM*: 1500mg/kg bw HSEAG + 30mg/kg bw CPM daily for the period of treatment
Group 7: High dose *Azanza garckeana* + *CPM*: 3000mg/kg bw HSEAG + 30mg/kg bw CPM
Group 8: Positive Control: 30mg/kg bw CPM

Note that, blood was collected via cardiac puncture. Antibody in agar plate method was used (17) in the determination of immunoglobulins levels (using the SurgiField Micro plate reader, SM-300A).

Method of Data Analysis

The Numerical data obtained from the present study were subjected to statistical analysis using version 21.0 of the IBM Statistical Product and Service Solutions (SPSS) programme. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by post-Hoc LSD multiple comparison test. And P-value less than 0.05 was considered statistically significant. All numerical values were presented as mean ± standard error of mean (SEM).

Ethical Approval

The ethical approval was sought and obtained from the Ethics Committee of Centre for Research Management and Development, University of Port Harcourt, Nigeria vides reference number: UPH/R&D/REC/EXEC/085.

III. Results

Table 1 indicates the outcome on the effect of hydromethanol seed extract of *Azanza garckeana* (HSEAG) administration on immunogluobulins levels in male Wistar rats.

Looking at the changes in the levels of immunoglobulins (Ig) A, G and M, the different doses of HSEAG treated rats did not indicate any significant ($P>0.05$) variations when respectively compared to those of the control group.

Similarly, there were no significant ($P>0.05$) changes in the IgA and IgG levels of the levamisole treated group (group 5) when compared to both the control group and HSEAG treated groups. Meanwhile, the mean IgM level of the levamisole treated group (group 5) showed significant elevation when

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compared to those of the control group and group 2 (treated with 500mg/kg bw HSEAG).

Noteworthy also, is the fact that the levels of IgA, IgG and IGM of group 3 (treated with 1500mg/kg bw HSEAG) were non-significantly (P>0.05) higher than those of groups 2 and 4 (treated with 500mg/kg bw HSEAG and 3000mg/kg bw HSEAG respectively).

Table 2 shows the outcome on the effect of hydromethanol seed extract of *Azanza garckeana* (HSEAG) administration on immunogluobulins levels in cyclophosphamide (CPM) co-treated male Wistar rats. Recall that the CPM co-treatment was intended to induce immune depression.

The mean TNFα levels of all treated groups did not significantly vary when compared to those of the control group and CPM-only treated group respectively (p>0.05). Only group 6 (treated with 1500mg/kg bw HSEAG + 30mg/kg CPM) indicated significant (p<0.05) elevation when compared to that of group 3 (treated with 1500mg/kg bw HSEAG) in their TNFα levels.

In view of the changes in the levels of IgA and IgM, group 6 showed significant (p<0.05) elevations when compared to all other groups including the control group.

The IgM, group levels of all other groups did not vary significantly (p>0.05) when compared to the control group and amongst themselves.

In respect of the changes of IgG, only group 5 (treated with 500mg/kg bw HSEAG + 30mg/kg CPM) had significant (P>0.05) depressions when compared to those of groups 1 and 8. Every other group did not change in their IgG level when compared to that the control group and amongst themselves.

It is important to note that the level IgA was lowest in group 8 (treated with 30mg/kg CPM only) when compared to those of all study groups

IV. Discussion

The present study evaluated an uncommon portion of the *Azanza garckeana* plant (the seed) and its possible influence on the immunoglobulins and TNFα levels in a mammalian model and the main findings are so discussed in the following paragraphs.

The result of the sub-section of the study involving treatments with HSEAG and levamisole with no experimental immune depression, showed that no marked changes occurred in IgA, IgG and IgM levels when compared to that of the control group. Although, the 1500mg/kg HSEAG indicated marginally raised levels of the aforementioned immunoglobulins when compared to others.

Notably, the levamisole treated group of rats had marked elevated level of IgM when compared to that of the 500mg/kg HSEAG.

The above outcome is a revelation of a possible mild stimulation of humoral immunity by the 1500mg/kg dose of the HSEAG. This finding is in line with an earlier report of Babich et al., (18) who noted that garlic, Panax L. (*Araliaceae*), curcumin, ginger, and ginger volatile oils possess the ability to boost immunity. Indeed, this is an indication that a low dose of HSEAG could be potent in lowering the risk of inflammatory dysfunction like that in the respiratory disorders.

Again, the finding on the IgM elevation effect of levamisole with respect to the lowest dose of HSEAG is an indication of the fact that, beyond antihelminthic (antiparasitic) effect, levamisole may possess appreciable immunomodulatory properties. Considering the specific elevation of IgM, it can be said that the agent may be able to stimulate initial immunological response (19).

Now, considering the result of the effect of HSEAG administration on TNFα levels in cyclophosphamide co-treated study models, there were non-significantly lower levels of the maker in the different doses of the extract-alone treated groups as against those co-treated with CMP + HSEA. In fact, the 1500mg/kg HSEA treated group had significantly lower level of TNFα when compared to that of 1500mg/kg HSEA + CPM. This finding is unique to the present study in that, prior to it, no earlier study within reach has specifically evaluated the effect of the HSEA on TNFα level (aside from (12), it is however similar to the previous submissions of Yusuf et al., (20) and Lawal et al., (21). As the scholars both acknowledged that the pulp of *Azanza garckeana* extract indicated preclinical evidence of antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. Specifically, it can be submitted here that, mild doses of the HSEAG could exert an immunostimulatory impact in an experimental immune-depressed model.

This anti-inflammatory potential as seen in the marginal depression of TNFα levels by the HSEA alone treated rats in the present study, is suggestive of the fact that the active ingredients in the HSEA may have some form of depressive effects in the production of TNFα proteins in a natural scenario.

Drawing from the above finding, it can be said that the different doses of the extract alone may possess the potential of down-regulation inflammation vis-a-vis anti-inflammation. This attribute of the HSEA may be likable to the effect of cyclophosphamide (CMP) alone treatment as seen in the TNFα level between the CPM-only and 1500mg/kg HSEA-only treated groups. Such natural effect of the plant on the TNFα protein may be helpful in the resistance of infection and perhaps cancer.

Surprisingly, the outcome of the co-treatment of the different doses of the HSEA and CMP yielded rather elevated levels of the TNFα. This finding validates the fact the notion of Posadzki et al., (22), that stated that, medicament interactions between herbs and other medications have the potential to alter medicament levels and activities, which could result in toxicities or therapeutic failure. Thus, the above finding of the present study reveals that the co-treatment of HSEA and CMP in the study models may elicit counteractive effects on each other or better still may have pro-inflammatory influence.

The present study also evaluated the effect of the plant extract (HSEA) on immunoglobulin (Ig) levels.

It was noted that the different doses of the HSEA-only treatments, although mildly, showed the tendency of elevating IgA levels and this was sustained in the HSEA co-treatment with CPM. In fact, the 1500mg/kg HSEA significantly raised the IgA level when compared to all other groups.

On the result of the changes in the level of IgG, it was observed that, the values are similar for both HSEA-only and CPM-only

Egbejimi, M. A.; Bekinbo, M. T.; Egbejimi, K. D.; and Dapper, D. V., "Evaluation of Effect of Hydromethanol Seed Extract of Azanza Garckeana on TNFα and Immunogluobulins Levels in Cyclophosphamide Immune-Depressed Male Wistar Rats," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 9, Issue 4, pp. 51-56, 2026.

treated groups; only that, the 1500mg/kg HSEA exerted mild increase in the parameter.

In another finding of the present study, it was seen that higher doses of the HSEA resulted in raised level of IgM and this was significant in the 1500mg/kg HSEA treated group when compared to other groups.

Considering the above findings of the current study it may be said to be consistent with the earlier report of Yamada *et al.,*(23) who stated that the herbal remedies derived from some herbs (like Echinacea) can boost immunity by modulating immunoglobulins levels. Further, Kattan et al., (24) noted that the components of certain herbs may work synergistically or additively to produce the biological or curative effects and that such potential therapeutic property may be endowed with beneficial active compounds.

It is important to state here that, the different doses of the HSEA possess varying effects on Ig A, G and M and it is such that both IgA and Ig M were raised by moderate doses of the HSEAG.

As a vital component of the immune response, immunoglobulins recognise and bind to specific antigens, like bacteria or viruses, and facilitate the immune's ability to destroy them. Thus, the mild elevation of the IgA and IgM portends a beneficial attribute of the HSEA as it may be able to enhance immune response. Recall that one of the identified active ingredients of the HSEA is propanoic acid (or propionic acid) which has been reported to possess numerous biological activities, including but not limited to antioxidant properties. On the other hand, antioxidant enrichments have been shown in recent clinical trials to greatly enhance specific immune responses (25). It is therefore suggestive to submit that this mild elevation of IgA and IgM attribute by the HSEA may be due to it rich antioxidant constituents.

There were no significant differences in TNFα levels of groups 2 to 7 when respectively compared to that of the control group and amongst themselves. Only the TNFα level of group 8 was found to be significantly lower when compared to that of the control group.

V. Conclusion

The outcome of treatments with HSEAG and levamisole with no experimental immune depression, revealed that the 1500mg/kg HSEAG exerted marginally raised levels of IgA, IgG and IgM immunoglobulins when compared to others. Remarkably, it was also seen that the levamisole treated group of rats had a raised level of IgM when compared to that of the 500mg/kg HSEAG treated models.

Further, a view on the outcome of the effect of HSEAG administration on TNFα levels in cyclophosphamide co-treated study models, there were non-significant reductions on this maker in the different doses of the extract-alone treated groups as against those co-treated with CMP + HSEA. Particularly, the 1500mg/kg HSEA treated group had substantial reductions in the TNFα level when compared to that of 1500mg/kg HSEA + CPM treated. This finding is thus, indicative that different doses of the extract alone may possess the potential of down-regulating inflammation vis-a-vis anti-inflammation. Such attribute of the plant on the TNFα level could enhance the resistance of infection and possibly cancer. On the other hand, the outcome of the co-treatment of the various doses of the HSEAG and CPM yielded raised levels of the TNFα.

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Results

TABLE 1: Effect of hydromethanol seed extract of *Azanza garckeana* (HSEAG) administration on Immunogluobulins concentration.

Immunoglobulin Levels	Group and Treatment				
	Group 1: Control	Group 2: 500 mg/kg bw HSEAG	Group 3: 1500mg/kgbw HSEAG	Group 4: 3000mg/kg HSEAG	Group 5: 30mg/kg bw levamasole
IgA (g/l)	0.79 ± 0.15	0.88 ± 0.21	1.29 ± 0.27	0.78 ± 0.10	1.02 ± 0.18
IgG (g/l)	13.65 ± 0.58	10.50 ± 0.44	13.25 ± 1.41	10.92 ± 0.15	9.17 ± 3.42
IgM (g/l)	0.88 ± 0.12	0.64 ± 0.05	1.04 ± 0.19	1.02 ± 0.19	1.53 ± 0.34 [a, b]

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4.

TABLE 2: Effect of hydromethanol seed extract of *Azanza garckeana* (HSEAG) administration on TNFα and immunogluobulins levels in cyclophosphamide co-treated male Wistar rats.

Immunological Parameters	Group and Treatment							
	Group 1: Negative Control	Group 2: 500mg/kg bw HSEAG	Group 3: 1500mg/kg bw HSEAG	Group 4: 3000mg/kg bw HSEAG	Group 5: 500mg/kg bw HSEAG + 30mg/kg CPM	Group 6: 1500mg/kg bw HSEAG + 30mg/kg CPM	Group 7: 3000mg/kg bw HSEAG + 30mg/kg CPM	Group 8: Positive Control (30mg/kg CPM)
TNFα (pgl/l)	76.25 ± 12.15	60.25 ± 10.30	51.00 ± 1.96	71.75 ± 7.72	80.25 ± 9.49	80.75 ± 12.92 [c]	71.00 ± 8.55	55.75 ± 7.68
IgA (g/l)	0.79 ± 0.15	0.88 ± 0.21	1.29 ± 0.27 [b]	0.78 ± 0.10	0.80 ± 0.12	2.19 ± 0.28 [a, b, c, d, e]	0.97 ± 0.20 [f]	0.61 ± 0.03 [f]
IgG (g/l)	13.65 ± 0.58	10.50 ± 0.44	13.25 ± 1.41	10.92 ± 0.15	8.95 ± 2.56 [a]	10.90 ± 1.12	9.02 ± 1.34 [a, c]	10.85 ± 0.33 [e]
IgM (g/l)	0.88 ± 0.12	0.64 ± 0.05	1.04 ± 0.19	1.02 ± 0.19	0.68 ± 0.16	1.81 ± 0.11 [a, b, c, d, e]	0.75 ± 0.13 [f]	0.69 ± 0.02 [f]

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4. [e] Significant at p<0.05 when compared to group 5; [f] Significant at p<0.05 when compared to group 6; [g] =Significant at p<0.05 when compared to group 7.

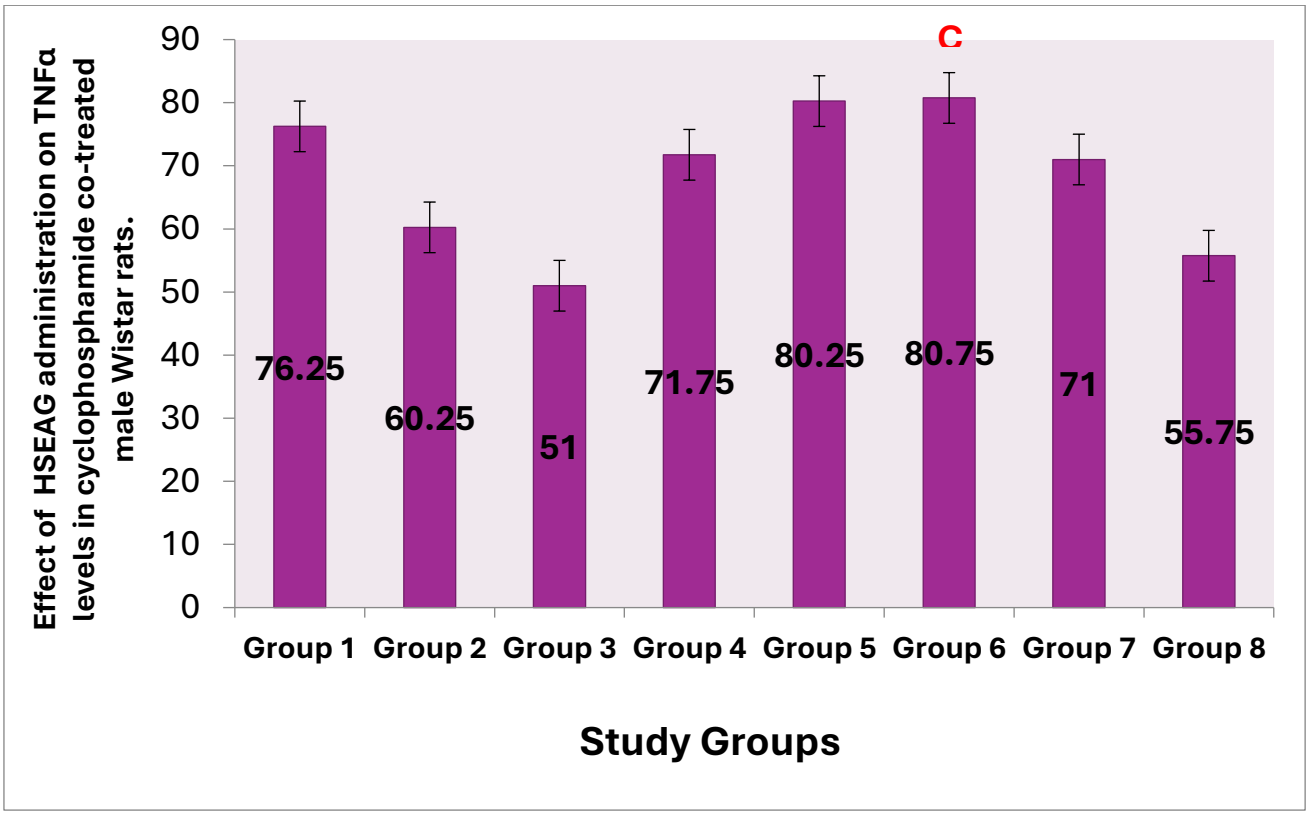

Fig. 1: Effect of hydromethanol seed extract of *Azanza garckeana* (HSEAG) administration on TNFα levels in cyclophosphamide co-treated male Wistar rats.

Values represent mean ± SEM, n=5; [a] Significant at $p<0.05$ when compared to control; [b] Significant at $p<0.05$ when compared to group 2; [c] Significant at $p<0.05$ when compared to group 3; [d] Significant at $p<0.05$ when compared to group 4. [e] Significant at $p<0.05$ when compared to group 5; [f] Significant at $p<0.05$ when compared to group 6; [g] =Significant at $p<0.05$ when compared to group 7.

Key:
Group 1: Negative Control
Group 2: 500mg/kg bw HSEAG
Group 3: 1500mg/kg bw HSEAG
Group 4: 3000mg/kg bw HSEAG
Group 5: 500mg/kg bw HSEAG + 30mg/kg CPM
Group 6: 1500mg/kg bw HSEAG + 30mg/kg CPM
Group 7: 3000mg/kg bw HSEAG + 30mg/kg CPM
Group 8: Positive Control (30mg/kg CPM)

Egbejimi, M. A.; Bekinbo, M. T.; Egbejimi, K. D.; and Dapper, D. V., "Evaluation of Effect of Hydromethanol Seed Extract of Azanza Garckeana on TNFα and Immunogluobulins Levels in Cyclophosphamide Immune-Depressed Male Wistar Rats," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 9, Issue 4, pp. 51-56, 2026.