Haematopoiesis-stimulating activity of *Tapinanthus globiferus* on cyclophosphamide-induced immunosuppression in albino rats

Olusegun S. Ajala, Sefunmi P. Akinseye

Department of Pharmaceutical Chemistry, University of Lagos, Nigeria

Corresponding author: Sefunmi P. Akinseye
Email: spakunic@gmail.com Telephone : +2348137992001

ABSTRACT

Background: Immunostimulation is a valuable but under-utilised alternative to conventional antimicrobial and *anticancer chemotherapies*. Optimum immunostimulation is a function of adequate haematopoiesis as against mere leucocyte proliferation. The immunostimulatory potentials of the African mistletoe, *Tapinanthus globiferus*, could be conjectured based on morphological and chemotaxonomic association with its European counterpart *Viscum album*, an established immunostimulant.

Objective: This study investigated the phytochemicals in the methanol leaf extract of *Tapinanthus globiferus*, its acute toxicity, and *stimulatory* effects on White Blood Cell (WBC), Red Blood Cell (RBC), and Platelet (PLT) counts.

Methods: Crude methanol leaf extract of *T. globiferus* was screened for phytochemical constituents according to established procedures. Immunosuppression was induced (using 30mg/Kg cyclophosphamide) in twenty-five albino male rats randomly and equally distributed into five groups (1-5). Extract was administered for 14 days at 50 mg/Kg, 100 mg/Kg, and 150 mg/Kg doses to groups 3, 4 and 5 respectively, groups 1 and 2 receiving 5% tween 80 and levamisole respectively instead. Blood sample was collected from each rat before and after immunosuppression, and after the 14-day treatment regimen, followed by analysis for total and differential white WBC, RBC, and PLT counts.

Results: Saponins, reducing sugars, cardiac glycosides, phenolic compounds, tannins, flavonoids, steroids, anthraquinones, and terpenoids were confirmed present. There was a statistically significant increase in each of the total and differential WBC, RBC, and PLT counts compared to the negative control ($p < 0.05$).

Conclusion: *Tapinanthus globiferus* possesses immunostimulatory, erythropoietic, and thrombopoietic activities.

Keywords: Immunosuppression, immunostimulatory activity, cyclophosphamide, mistletoe, *Tapinanthus globiferus*, haematological evaluation

Activité stimulante de l'hématopoïèse du Tapinanthus globifère sur l'immunosuppression induite par le cyclophosphamide chez les rats albinos

Olusegun S. Ajala, Sefunmi P. Akinseye

Département de chimie pharmaceutique, Université de Lagos, Nigeria

Auteur correspondant : Sefunmi P. Akinseye
Courriel : spakunic@gmail.com , Téléphone : +2348137992001

RÉSUMÉ

Contexte : L' immunostimulation est une alternative précieuse mais sous-utilisée aux chimiothérapies antimicrobiennes et anticancéreuses conventionnelles. L' immunostimulation optimale est fonction d'une hématopoïèse adéquate par opposition à la simple prolifération des leucocytes. Les potentiels immunostimulateurs du gui africain, *Tapinanthus globiferus*, pourrait être conjecturé sur la base de son association morphologique et chimiotaxonomique avec son homologue européen *Viscum album*, un immunostimulant reconnu.

Objectif : Cette étude a examiné les composés phytochimiques de l'extrait méthanol de la feuille de *Tapinanthus globiferus* , sa toxicité aiguë et ses effets stimulants sur le nombre de globules blancs (GB), de globules rouges (GR) et de plaquettes (PLT).

Méthodes : L'extrait brut de feuilleS de méthanol de *T. globiferus* a été analysé pour ses constituants phytochimiques selon les procédures établies. Une immunosuppression a été induite (à l'aide de 30 mg/kg de cyclophosphamide) chez vingt-cinq rats mâles albinos répartis de manière aléatoire et égale en cinq groupes (1-5). L'extrait a été administré pendant 14 jours à des doses de 50 mg/Kg, 100 mg/Kg et 150 mg/Kg aux groupes 3, 4 et 5 respectivement, tandis que les groupes 1 et 2 recevant 5% de tween 80 et de lévamisole respectivement. Un échantillon de sang a été prélevé sur chaque rat avant et après l'immunosuppression et après le régime de traitement de 14 jours, suivi d'une analyse des numérations totales et différentielles des GB, des GR et des PLT.

Résultats : La présence de saponines, de sucres réducteurs, d'hétérosides cardiaques, de composés phénoliques, de tanins, de flavonoïdes, de stéroïdes, d'anthraquinones et de terpénoïdes a été confirmée. Il y avait une augmentation statistiquement significative de chacun des nombres total et différentiel de globules blancs, de globules rouges et de PLT par rapport au témoin négatif ($p < 0,05$).

Conclusion : *Tapinanthus globiferus* possède des activités immunostimulatrices , érythropoïétiques et thrombopoïétiques.

Mots clés : Immunosuppression, activité immunostimulatrice, cyclophosphamide, gui, *Tapinanthus globiferus*, évaluation hématologique

INTRODUCTION

There have been tremendous advancements in antimicrobial chemotherapy since the discovery of penicillin. However, these advancements are hardly noticed when viewed against the increase in the emergence of new and deadly infectious diseases and the resistance of many old ones to standard treatments.[1] This paradox would remain as long as antimicrobial chemotherapy relies on small molecules that would selectively destroy microbial genome/genome products that are rapidly changing.[2] On the other hand, anticancer chemotherapy has not been able to remove cancer from its number one killer position worldwide[3] because its principle of selective toxicity fails to distinguish between cancer and normal cell genomes.[4] For example, Cyclophosphamide, a synthetic anticancer drug, is an immune suppressor because of its bone marrow suppression effect.[5]

A functional alternative approach to chemotherapy with a very high potential for handling the drawbacks mentioned above is immunostimulation which involves qualitative and quantitative instigation of the somewhat naturally discriminatory immune surveillance (i.e., the white blood cells) to recognise and destroy microbial invaders and malignancies.[6] The potential of this approach becomes manifest if one considers that dangerous infections like tuberculosis are almost indicative of an underlying immunosuppressive condition such as HIV/AIDS.[7,8] Moreover, organ transplant patients under immunosuppressant cover almost always end up with cancer.[9] Nevertheless, uncontrolled immunostimulation is undesirable and is naturally modulated by other haematopoietic products especially RBCs and PLTs which, in addition to their respective oxygen transport and coagulation primary roles, modulate and regulate WBC synthesis via chemokines and other inflammatory mediators.[10,11] Optimum immunostimulation, therefore, is a function of adequate haematopoiesis as against mere WBC synthesis stimulation.

Despite its aforementioned prospects, immunostimulation is grossly underutilised in therapeutics as evidenced by the scarcity of standard immunostimulatory medications in clinical medicine. Moreover, the few available ones like the recently-introduced current drugs such as granulocyte colony-stimulating factors used in chemotherapy-induced neutropenia are limited because of side effects such as pain in the bone and muscle and dysfunctional bone marrow. Therefore, there is a high need to discover new immunostimulatory agents.[12]

Many flowering plants have been reported as possessing immunomodulatory activities,[13] and many mistletoes belong to this category. Mistletoes are important in curative medicine as they are highly potent in curing circulatory problems and as anticancer agents. The mistletoe *Tapinanthus globiferus*, belonging to the Loranthaceae family, grows on shrubs and trees such as cocoa, mango, guava, and kola nut.[14] It is commonly consumed in West Africa to treat a cocktail of infectious and metabolic diseases, including diabetics, ulcers, hypertension, weakness of vision, bacterial infections, trypanosomiasis, epilepsy, heart failure, arthritis and other inflammatory diseases.[15,16,17,18] However, there are no reports on the ethnomedicinal use of the plant as an immune booster. On the other hand, *Viscum album* (family Loranthaceae) is a mistletoe with established immunostimulatory properties.[19] However, found mainly in Europe and not in this part of the world. It shares many chemotaxonomic and morphological properties with Tapinanthus globiferus to the extent that many authors often refer to *Tapinanthus globiferus* as *Viscum album*.[20]

Given the aforementioned close association between Viscum album and *Tapinanthus globiferus*, we conjectured the immunostimulatory activity of the latter and evaluated the same in cyclophosphamide-induced rat models. In addition, we investigated its effects on the RBC and PLT.

METHODS

Standard drugs used

Cyclophosphamide injection (Zuviphos-500TM; Zuvius Lifesciences Pvt. Ltd., Mumbai, India) containing 500 mg of cyclophosphamide powder was used to induce immunosuppression. In addition, levamisole (Retrax® worm syrup; Reals Group, Lagos, Nigeria) containing 15ml was used as the reference immunostimulant, and every 5 ml contained 40 mg levamisole.[8]

Plant material

The leaves of *Tapinanthus globiferus* (A. Rich.) Van Tiegh was obtained from a cocoa farm at Idanre L.G.A, Ondo State, Nigeria, in August 2019 and authenticated at the Centre for Research and Development, Federal University of Technology, Akure, Nigeria with voucher number 0157.

Preparation of plant extract

For three days, air-dried leaves were powdered and macerated in 1.5 L of methanol. Cold maceration with methanol was repeated three times, and the extract was concentrated in a digital rotary evaporator (Heidolph Laborota 4010) which was further dried in an oven for 40-50^{O}C.

Preliminary phytochemical screening

Frothing test for saponins, Fehling's test for reducing sugars, Keller Killani for cardiac glycosides, Lead acetate test for phenolic compounds, Ferric chloride test for tannins, Shinoda's test for flavonoids, Liebermann-Burchard's test for steroids, Meyer's test, Wagner's test and Dragendroff's test for alkaloids, Burntrager's test for anthraquinones and Salkwoski's test for terpenoids were employed according to established procedures.[21]

Preparation of test formulations

A working concentration of 70mg/ml of the methanol extract was prepared in 5% tween-80 and refrigerated. Then, the cyclophosphamide powder was reconstituted with water for injection to make a concentration of 25 mg/ml.

Experimental animals

Twenty-five male albino rats weighing 90-150 g and twelve male albino mice weighing 16-21 g were purchased. The animals were kept in well-ventilated compartments at the animal house of the College of Medicine, University of Lagos, Nigeria. They were fed standard rodent feed (Hybrid feed) and housed under average temperature and humidity. This research was conducted under the internationally accepted laboratory animal use and care principles by the National Research Council.[22] and ethical approval granted by the Health Research Ethics committee of the College of Medicine of the University of Lagos with CMULHREC Number: CMUL/ACUREC/09/20/762.

Acute toxicity studies

According to Lorke's method,[23] nine mice divided into three equal groups were fasted overnight, administered 10, 100, and 1000 mg/kg of the extract, and observed for 24 hrs. After 24 hours, following no occurrence of death, three mice divided into three equal groups were administered 1600, 2900, and 5000 mg/kg of the extract and then observed for 24 hrs. After which, the number of deaths was recorded, and lethal dose (LD50) was calculated using the formula below:

$$LD_{50} = \sqrt{(D_{100} \times D_0)}$$

Where D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Evaluation of the haematopoiesis-stimulating activity of the methanol extract

Experimental design

Twenty-five rats were acclimatised and randomly divided into five groups (1-5). Each received 30 mg/Kg of cyclophosphamide intraperitoneally for three days to induce myelosuppression. Afterwards, group 1 (negative control) received 0.4 ml of 5% tween-80, and group 2 (positive control) received 5mg/kg of levamisole per oral for two weeks. Groups 3, 4, and 5 orally received 50 mg/kg, 100 mg/kg, and 150 mg/kg of the extract, respectively, for two weeks.

Haematological analysis

Blood samples via the retro-orbital plexus were collected from all groups into EDTA bottles before myelosuppression (day 0), after myelosuppression (day 4), and after two weeks of administration (day 18). BC-3200 auto haematology analyser was used to analyse blood samples for total white blood cells (WBC), lymphocytes (LYM), middle-sized cells (MID), and granulocytes (GRAN).

Statistical analysis

Data were analysed with GraphPad 9.0 and plotted as Mean ± SEM using Microsoft Excel. Differences between the means of each haematological parameter gotten on day 0 and day 4 in each group were determined by paired t-test. Similarly, paired t-test was used to analyse day 4 and day 18 data of each parameter in each group, and the significance level was considered at $p<0.05$.

RESULTS

Phytochemical profile

The phytochemical profile of the extract is illustrated below in Table 1.

Table 1: Phytochemical profile of the extract

TEST	OBSERVATION
Saponins	+
Reducing Sugars	+++
Cardiac Glycosides	++
Phenolic Compounds	+++
Tannins	+++
Flavonoids	++
Steroids	++
Anthraquinones	+++
Alkaloids	-
Terpenoids	++

+++= heavily detected; ++= detected; += slightly detected; -= absent

Acute toxicity

The D_{100} and D_0 were found to be 1,600 mg/Kg and 1,000 mg/Kg respectively and the calculated lethal dose = 1,265 mg/Kg.

Haematological analysis results

The myelosuppression effect of cyclophosphamide

Day 0 and Day 4 paired t-test comparisons of the investigated haematological parameters showed a clear suppression of the total WBC, LYM, MID and GRA counts in all the rat groups ($p < 0.05$; $p < 0.01$; $p < 0.001$) (Figures 1a-1d). In the same vein, there was a statistically significant suppression of RBC ($p < 0.05$) (Figure 1e). However, no significant platelet suppression was observed ($p > 0.05$) (Figure 1f).

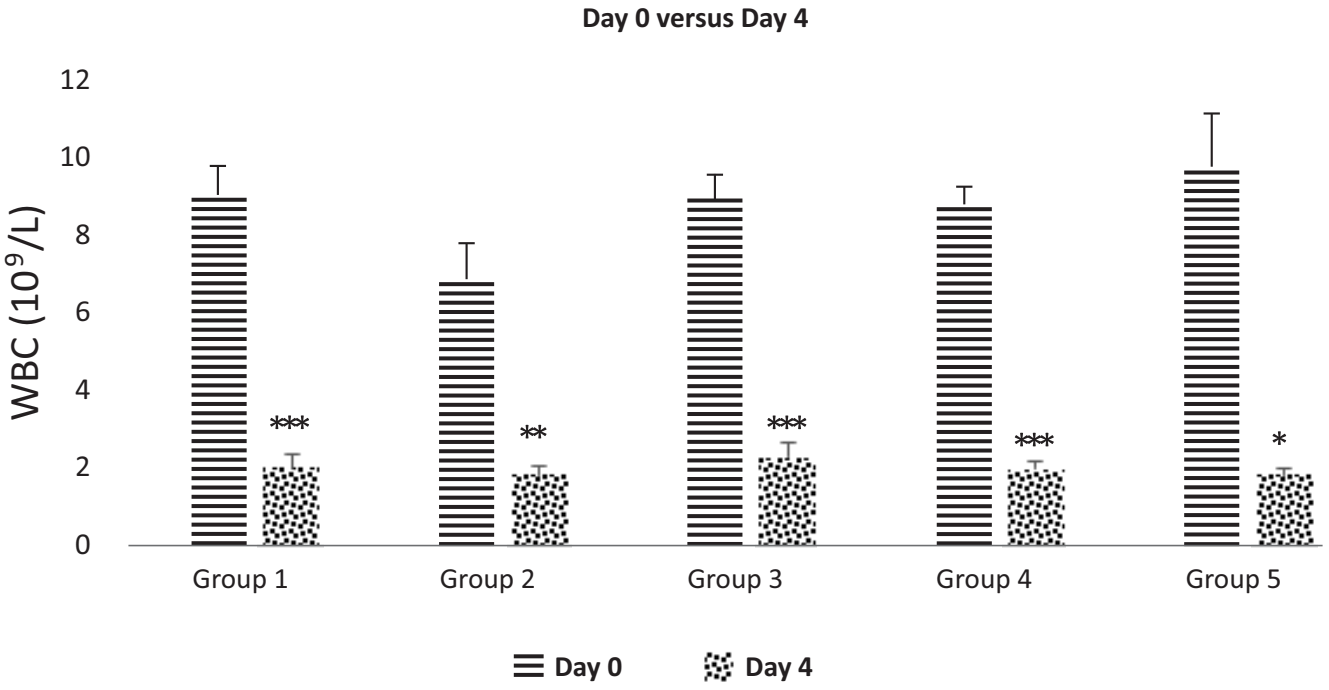

Figure 1a: Before and after CYP treatment (Day 0 and Day 4) Paired t-test comparison of white blood cell (WBC) counts showing WBC suppression effect of CYP. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

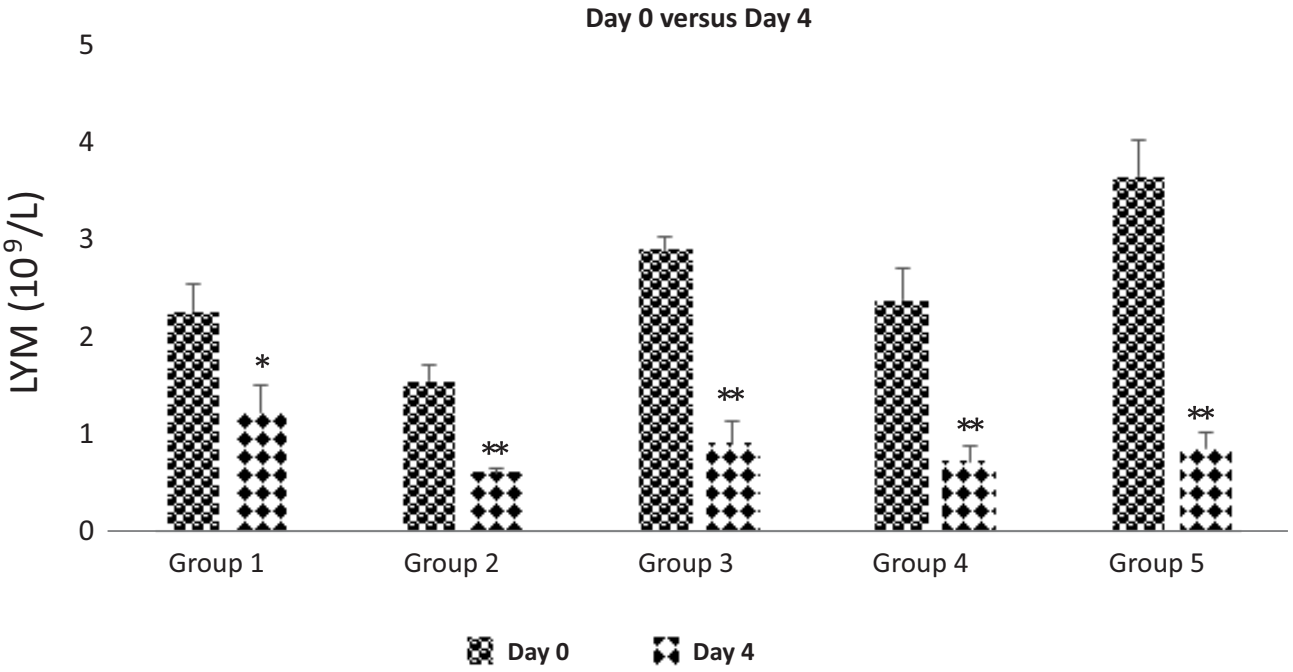

Figure 1 b: Before and after CYP treatment (Day 0 and Day 4) Paired t -test comparison o f lymphocyte (LYM) counts showing LYM suppression effect of CYP. * $p < 0.05$; ** $p < 0.01$.

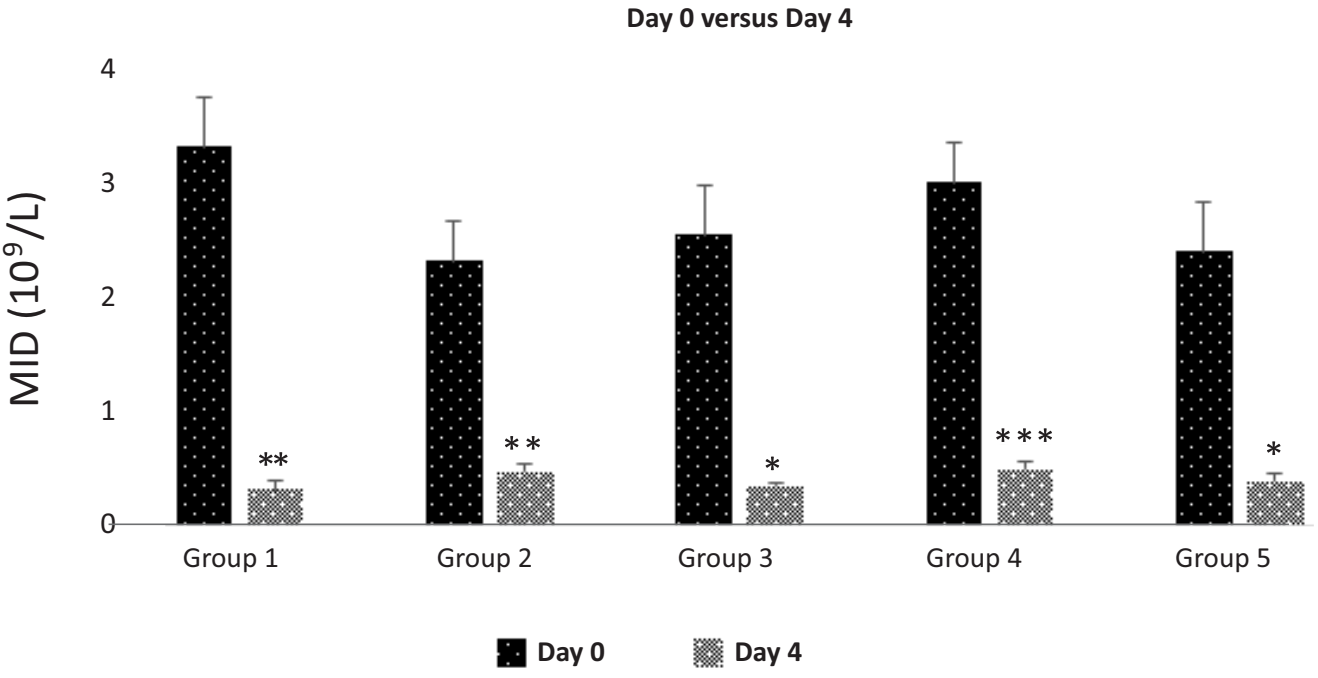

Figure 1c: Before and after CYP treatment (Day 0 and Day 4) Paired t-test comparison of middle-sized cell (MID) counts showing MID suppression effect of CYP. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

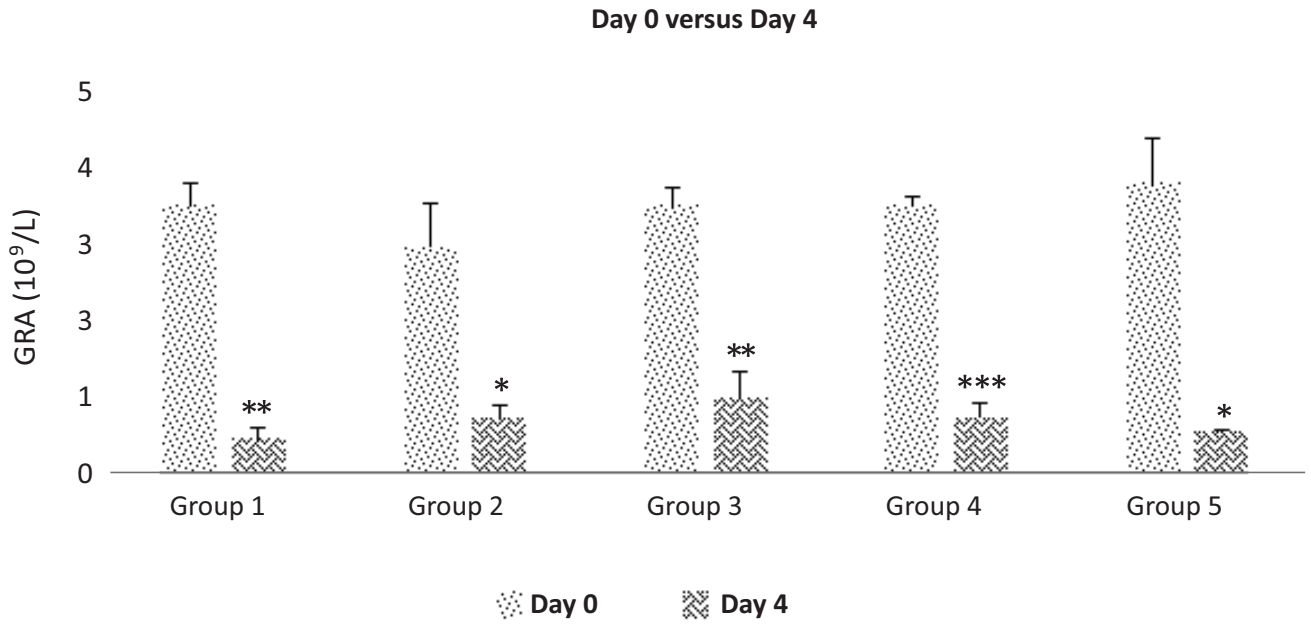

Figure 1d: Before and after CYP treatment (Day 0 and Day 4) Paired t-test comparison of granulocyte (GRA) counts showing GRA suppression effect of CYP. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

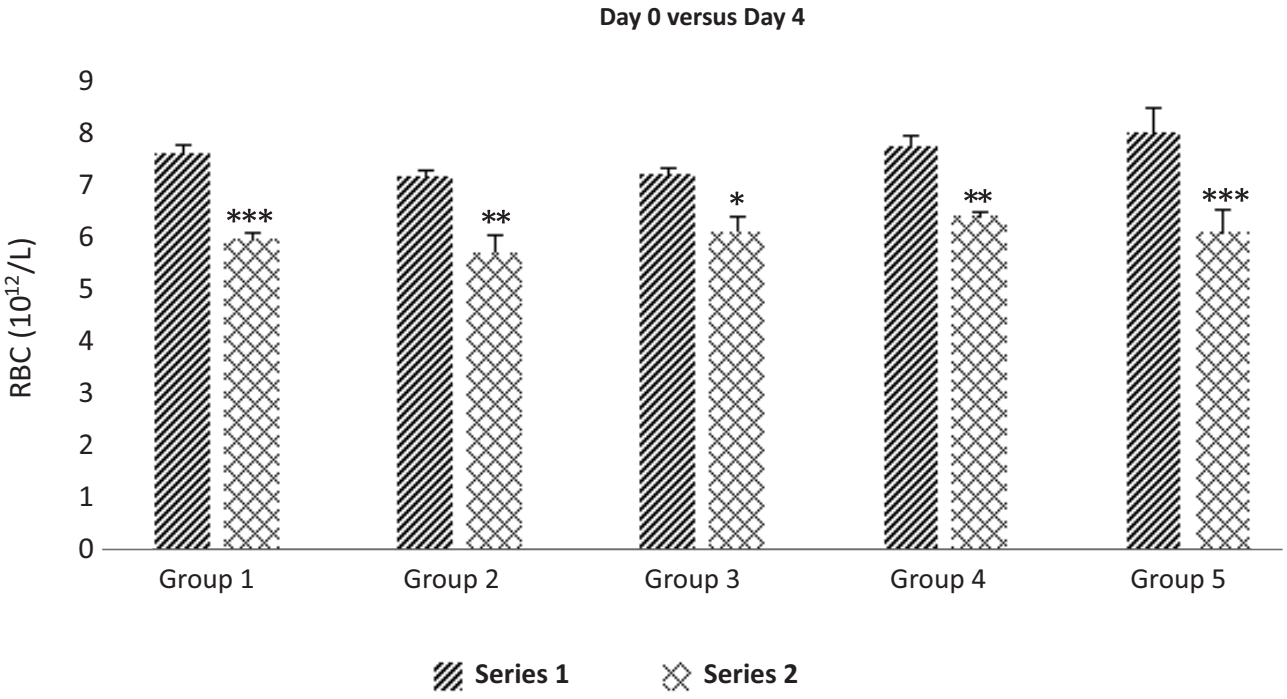

Figure 1e: Before and after CYP treatment (Day 0 and Day 4) Paired t-test comparison of red blood cell (RBC) counts showing RBC suppression effect of CYP. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

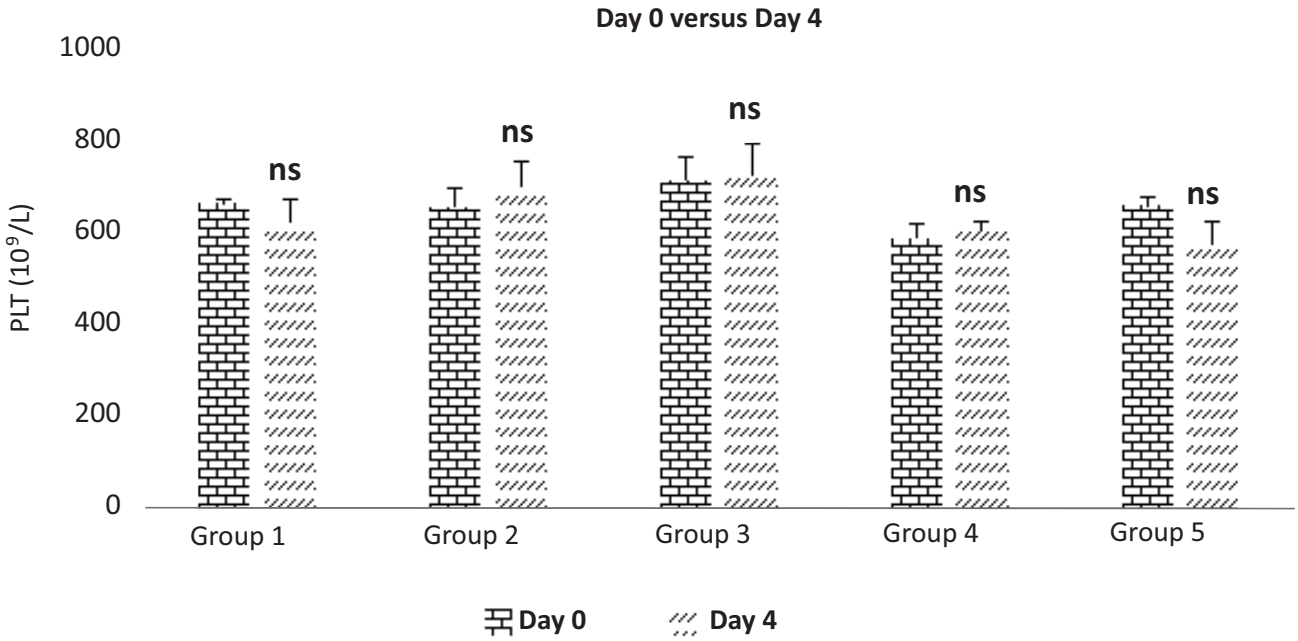

Figure 1f: Before and after CYP treatment (Day 0 and Day 4) Paired t-test comparison of platelet (PLT) counts. ^{ns} $p > 0.05$

The stimulatory effect of the extract on the haematological parameters

Similarly, Day 4 and Day 18 paired t-test comparisons of the investigated haematological parameters showed the stimulation of the total WBC and LYM in all the rat groups ($p < 0.05$; $p < 0.01$; $p < 0.001$) (Figure 2a & 2b). In addition, GRA and PLT was only stimulated in groups 4 and 5 ($p < 0.05$) (Figure 2d & 2f) and a statistically significant increase ($p < 0.05$) in RBC and MID was observed only in groups 3 and 4 respectively (Figure 2c & 2e).

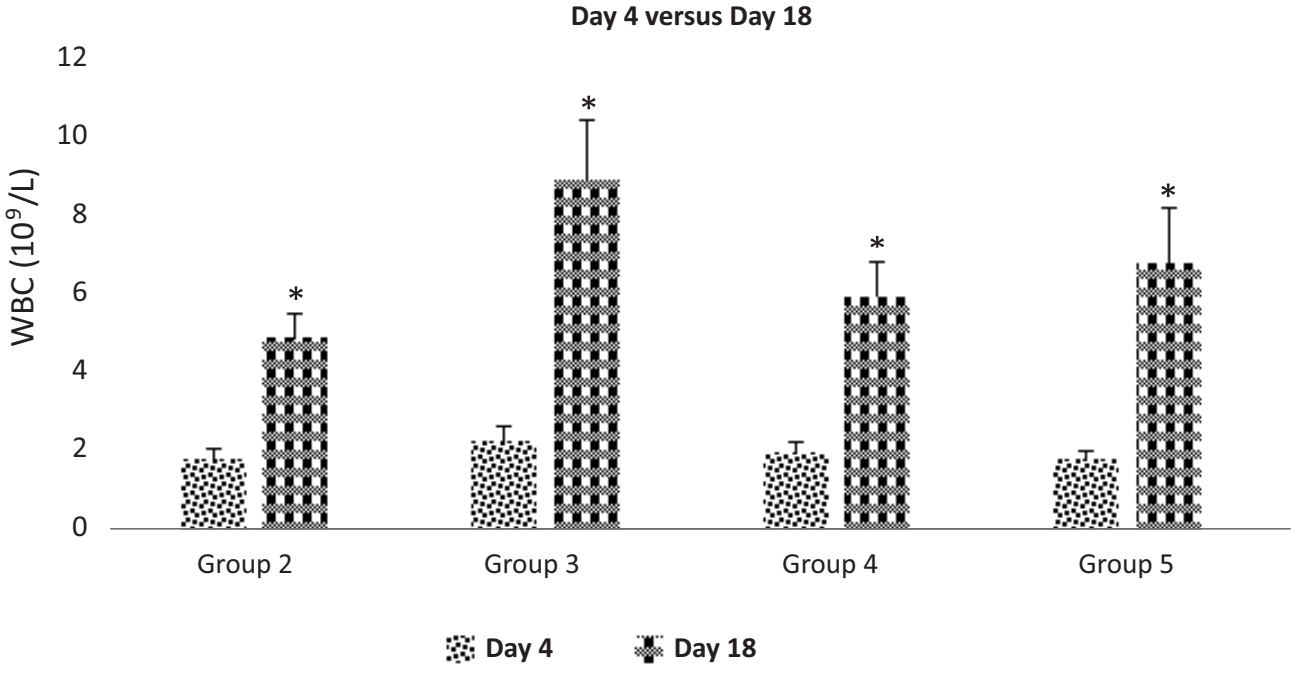

Figure 2a: After CYP treatment and 2 weeks administration (Day 4 and Day 18) Paired t-test comparison of white blood cell (WBC) counts showing WBC stimulation. * $p < 0.05$.

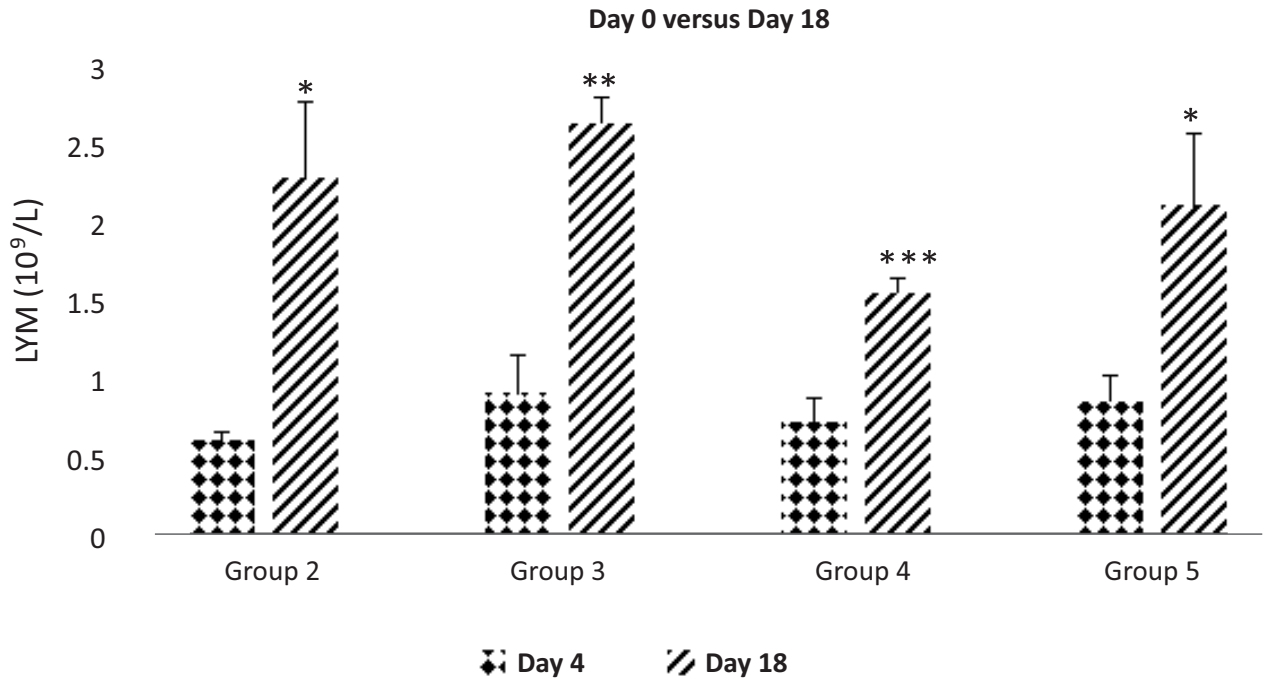

Figure 2b: After CYP treatment and 2 weeks administration (Day 4 and Day 18) Paired t-test comparison of lymphocyte (LYM) counts showing LYM stimulation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

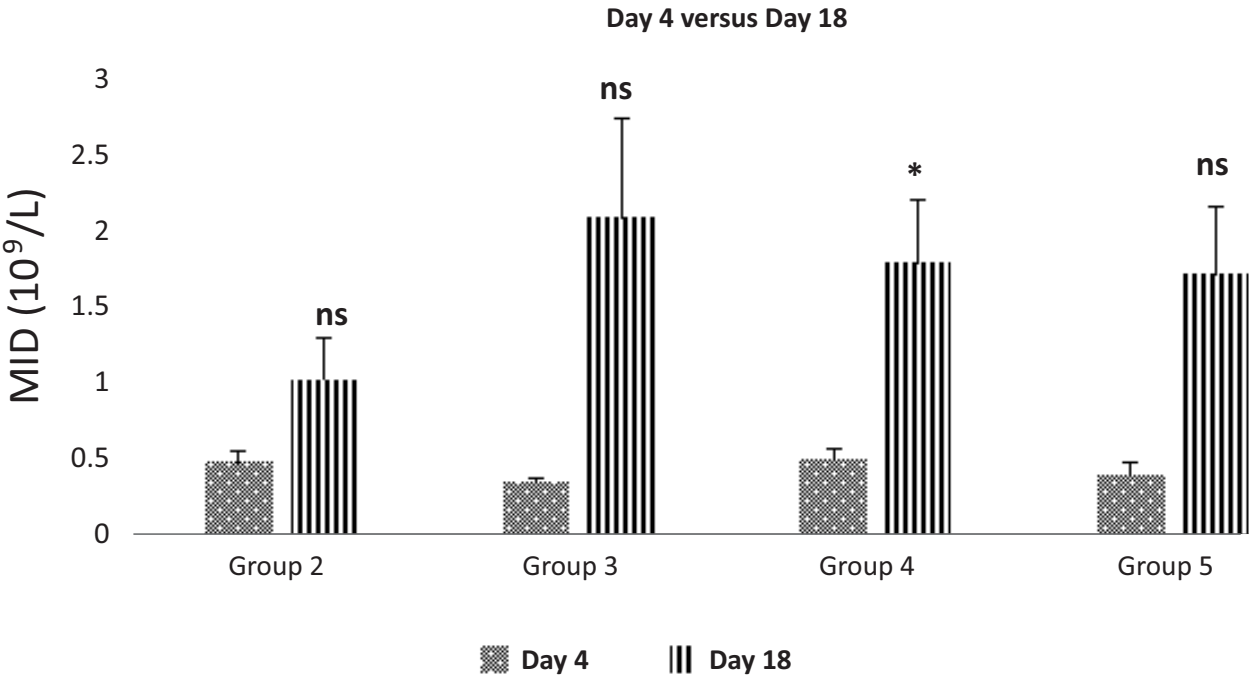

Figure 2c: After CYP treatment and 2 weeks administration (Day 4 and Day 18) Paired t-test comparison of middle-sized cell (MID) counts showing MID stimulation. * $p < 0.05$; ^{ns} $p > 0.05$

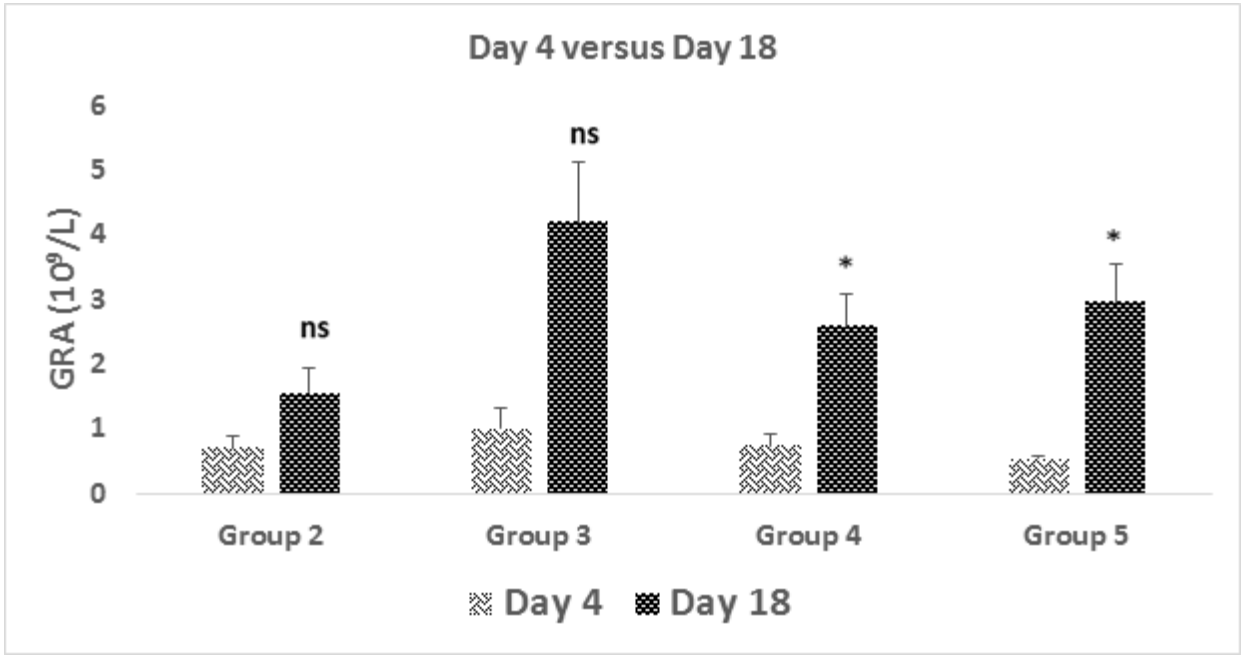

Figure 2d: After CYP treatment and 2 weeks administration (Day 4 and Day 18) Paired t-test comparison of granulocyte (GRA) counts showing GRA stimulation. * $p < 0.05$; ^{ns} $p > 0.05$

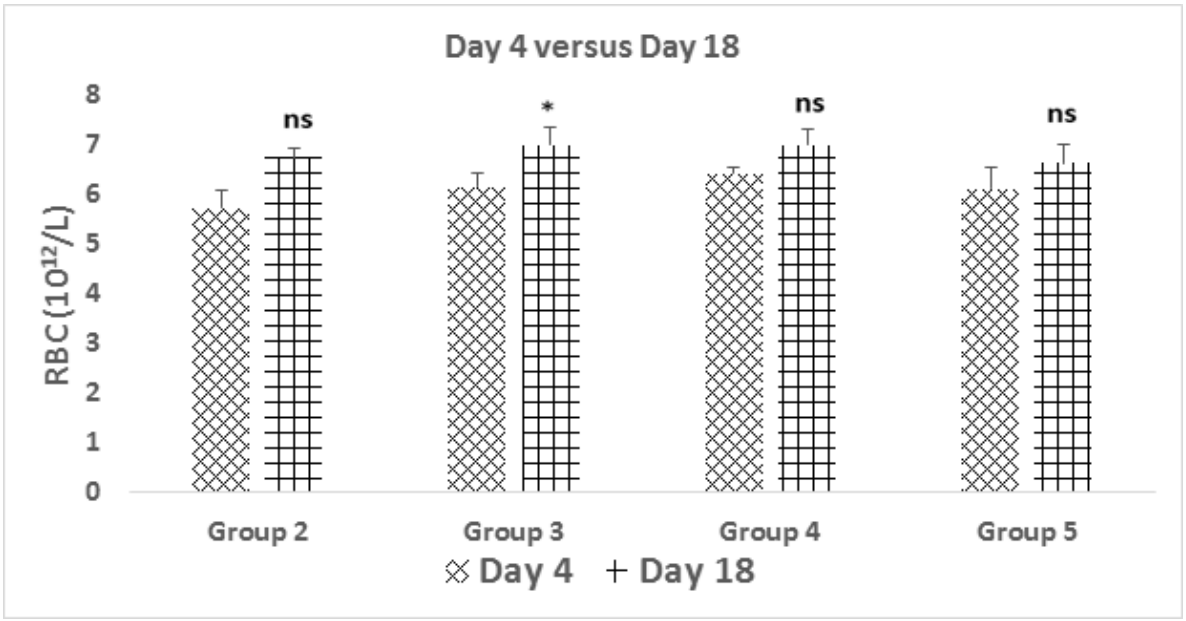

Figure 2e: After CYP treatment and 2 weeks administration (Day 4 and Day 18) Paired t-test comparison of red blood cell (RBC) counts showing RBC stimulation. * $p < 0.05$; ^{ns} $p > 0.05$

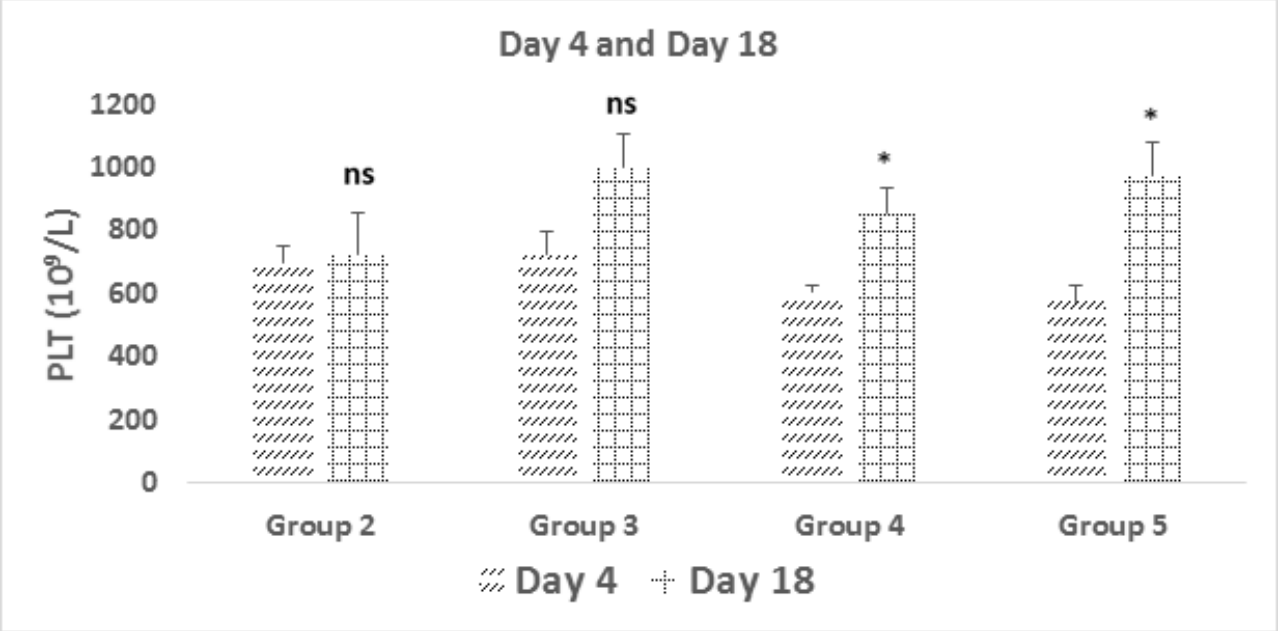

Figure 2f: After CYP treatment and 2 weeks administration (Day 4 and Day 18) Paired t-test comparison of platelet (PLT) counts showing PLT stimulation. * $p < 0.05$; ^{ns} $p > 0.05$.

DISCUSSION

This study evaluated the haematopoiesis-stimulating activity of the methanol leaf extract of *Tapinanthus globiferus* at 50 mg/Kg, 100 mg/Kg, and 150 mg/Kg on cyclophosphamide-induced immunosuppressed rats with the ultimate aim of assessing its suitability as a possible source of immunnostimulatory/immunomodulatory agents. Tween 80 (i.e., extract vehicle) and 5 mg/Kg levamisole were carried along as negative and positive controls respectively. Preliminary phytochemical screening of the extract revealed reducing sugars, cardiac glycosides, phenolic compounds, tannins, flavonoids, steroids, anthraquinones, and a trace of saponins. Kabiru *et al*.[24] confirmed the absence of anthraquinone and the heavy presence of alkaloids using Wagner's test, but their findings also indicated its absence by Meyer's test. On the other hand, Tabe *et al*.[14] confirmed the presence of alkaloids and the absence of terpenoids. This variation in secondary metabolites of the extract is supported by the fact that environmental factors and habitat affect the secondary metabolites of a plant sampled from a different location. It can also be affected by the solvent extraction method employed.[25] In addition, it has been proven that the biological activities of mistletoe extracts are host tree and seasonal dependent.[26,27] Also, climate, parts, and age of plant used have significantly influenced the plant's protein, polypeptide, and carbohydrate contents.[27]

The first toxicological investigation step is carrying out an acute toxicity study. It should be noted that LD50 must not be regarded as a biological constant as varying results are obtained on repetition or when the studies are carried out in different laboratories.[20] An acute toxicity test was carried out according to Lorke's method,[23] and no mortality was recorded upon administration of 10, 100, and 1000 mg/Kg of methanol leaves extract of T. globiferus. However, death was recorded about 2 hours after administration of 1600 mg/Kg, immediately after administration of 2900 mg/Kg, and during the administration of 5000 mg/Kg of the extract. With the highest dose that gave no mortality as 1000 mg/Kg and the lowest that produced mortality as 1600 mg/Kg, the lethal dose was calculated using equation 1 to be approximately 1265 mg/Kg. According to Loomis and Hayes,[28] substances with lethal doses between 0.5 and 5 g/Kg are slightly toxic. Hence, there should be great caution in consuming this plant extract.

Haematological parameters are functional indices explaining blood-related functions of a chemical compound or plant extract. As such, complete blood counts (CBC) help assess the status of the innate and

adaptive immune cells because all the immune system cells originate from the bone marrow. The CBC quantifies the number of white blood cells, red blood cells, platelets, and the concentration of haematocrit and haemoglobin with differential.[29] Of all the cells quantified, the key player in the immune system is the white blood cells, which can travel throughout the body via blood vessels.[30] The three-part haematology automated analyser used in this study differentiates the WBC (white blood cells) into LYMPH (lymphocytes), MID (middle-sized cells consisting of the monocytes), and GRAN (granulocytes which majorly consist of neutrophils but also basophils, and eosinophils)[31] and it sums this differential to give the total WBC.

To investigate the effect of the plant extract on the bone marrow, cyclophosphamide (CYP), an anticancer drug, was used to induce myelosuppression. Blood samples were collected from each rat at three different time intervals and subjected to paired t-test analysis. This analysis eliminates variation between the samples that could be caused by anything other than what is being tested by subjecting data gotten from the same animals at different time intervals to the analysis. Immunosuppression was confirmed in all groups prior to the two weeks of treatment by subjecting the baseline (day 0) and pre-treatment values (day 4) of each immune-related haematological parameter to paired t-test (Figures 1a-1d). In addition, anaemia was confirmed by subjecting each group's baseline and pre-treatment RBC values to paired t-test analysis (Figure 1e). Although leukopenia, anaemia, loss of appetite, and thrombocytopenia are reported side effects associated with CYP,[32,33] thrombocytopenia was not induced in any of the groups at the dose used (Figure 1f). The effect of the plant extract was then measured by subjecting day 4 and day 18 values of each haematopoietic parameter in each group to paired t-test analysis (Figures 2a-2f).

In group 1, where immunosuppression was induced and not treated, there was insufficient day 18 data because 80% mortality was recorded during the 14 days of vehicle administration. This mortality is due to the severe suppressive activity of CYP on the animals, which could not be reversed by the vehicle. The extract countered this mortality caused by the severe myelosuppression of CYP. A lower mortality rate was observed in the groups treated with the extract because only 20% of death was recorded in groups 3 and 5. Ukpo *et al*.[32] reported 100% mortality on the administration of 100mg/kg and 200mg/kg Averon®, an herbal immune booster, for 17 days to rats induced with 30 mg/kg of CYP. This mortality explains the impact of the toxicity of CYP. The inability of Averon® to reverse the severe myelosuppression which led to death indicates that methanol leaves extract of *Tapinanthus globiferus* exerts better immunostimulatory activity on cyclophosphamide-induced myelosuppression.

On the other hand, levamisole, an anthelmintic and the reference immunostimulant used could only significantly increase the lymphocytes and the total white blood cells. It also prevented mortality in group 2. This effect shows that levamisole stimulates the white blood cells by stimulating the lymphocytes, which agrees with Olusi *et al*,[34] where levamisole was observed to influence cell-mediated immunity by stimulating the thymus gland production of the thymic hormones where T lymphocytes are developed. The lymphocytes are immune cells fundamental to cellular and humoral immunity, and representing 20 to 45% of WBC in the blood, belonging to the B or T systems.

The statistically significant increase observed in the lymphocyte count after treatment in group 3 ($p < 0.01$), group 4 ($p < 0.001$), and group 5 ($p < 0.05$) (Figure 2b) indicates the extract's ability to recruit immune cells against viruses. The middle-sized cells, consisting of the monocytes and known to be activated against bacterial infections and chronic inflammatory diseases,[31] were significantly increased ($p < 0.05$) upon administration of 100 mg/Kg of the extract (Figure 2c). A significant increase ($p < 0.05$) in the granulocytes of rats administered 100mg/Kg and 150mg/Kg of the extract is an indication of the ability of the extract to stimulate the neutrophils which fight against fungi and bacterial infections, the basophils which are activated in response to inflammatory reactions, especially those causing allergic symptoms, and the eosinophils which respond to parasite infections or allergic reactions (Figure 2d).[29] Furthermore, there was a significant increase ($p < 0.05$) in the overall white blood cells of all groups treated with the extract (Figure 2a). These findings agree with previous studies that have reported mistletoes to contain agents that stimulate the production of white blood cells.[35,36]

Although CYP did not induce thrombocytopenia ($p > 0.05$) in any of the groups, a significant increase in platelet count ($p < 0.05$) was observed in groups 4 and 5 after treatment (Figure 2f). In contrast, Olusola *et al*.[37] who experimented with high doses of Viscum album, reported thrombocytopenia and anaemia as possible side effects of mistletoes from cocoa, kola, and coffee. However,

lower doses used in our study increased RBC levels, as only 50 mg/kg of the extract showed a significant difference (Figure 2e). These varying observations indicate that mistletoes exert dose-dependent effects on platelets and red blood cells. Haemoglobin and heme, internal components of the red blood cells play significant roles in innate immunity by generating antimicrobial reactive oxygen species against invading microbes and promoting pathologic inflammatory and auto-immune responses.[10] The platelets are also known to impact the immune system by supporting the induction of progenitor cells and white blood cells to the vascular injury or pathogen permeation site and endothelial cells. They interact with immune cells such as neutrophils, monocytes, and lymphocytes and form platelet-leukocyte aggregates, paralysing pathogens and preventing their spread.[11] The synergy between the platelets, red blood cells and white blood cells and the observed increase in these cells after treatment with the extract could be responsible for the plant's ability to reverse the myelosuppression and curtaining the associated mortality.

Interestingly, only 100 mg/Kg of the extract significantly increased all the differentials of WBC and effectively countered the induced immunosuppression and anaemia such that no mortality was recorded in group 4. Since all doses of the extract significantly increased the lymphocytes, this indicates the plant's action in immunomodulating the lymphoid cells. Hence, the stimulatory activity of this plant may be on the effectors cells of the immune system.[37]

CONCLUSION

The ability of plant extracts to restore health or fasten convalescence could be linked to their actions on the immune system. Our results showed that the administration of 50 mg/kg, 100 mg/kg, and 150 mg/kg of methanol leaves extract of *T. globiferus* caused a significant increase in the mobilisation of leukocytes by stimulating the lymphocytes, indicating that it may provide adaptive immunity to its host.

Therefore, our study showed that the methanol leaves extract of *Tapinanthus globiferus* possess immunostimulatory activity. In addition, it showed that the plant extract could stimulate the production of red blood cells and platelets, an indication of an increase in the function of the bone marrow. This extract may be used as a cancer-supportive therapy to improve the quality of life of cancer patients. However, only a few compounds, such as 2-(3'4'-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromane-4-one (quercetin), and 2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-3-(3-methylhexyl)-4H-chromen-4-one which are flavonoids,[38] and 3-hydroxylup-20(29)-ene (lupeol), a triterpenoid[39] have been isolated from the plant.

Thus, further research is needed to isolate and characterise the bioactive compounds present in the plant extract. Furthermore, its immunomodulation mechanism of action and effect on biochemical and histological parameters should be evaluated.

ACKNOWLEDGEMENT

The authors wish to acknowledge the effort of Dr Ehianeta Teddy of Academia Sinica for providing guidance based on his similar research. The tutorials provided by Dr Akinsola Oluwatosin of the department of Community Health and Primary Care, UNILAG, and Mr Mikail Abideen of Page Capital Limited, Lagos as regards the right statistical tool to be used is highly appreciated. We also acknowledge the kind help extended to the success of this research by Abdullahi Musa of the Central Research Laboratory, College of Medicine, UNILAG.

REFERENCES

1. Hirakawa H, Kurushima J, Hashimoto Y, Tomita H (2020). Progress overview of bacterial two-component regulatory systems as potential targets for antimicrobial chemotherapy. *Antibiotics*, 9(10): 635.
2. Horn D, Duraisingh MT (2014). Antiparasitic chemotherapy: from genomes to mechanisms. *Annual Review of Pharmacology and Toxicology* 54:71-94.
3. Dickens E, Ahmed S (2018). Principles of cancer treatment by chemotherapy. Surgery (Oxford) 36(3):134-8.
4. Gonzalez LC, Ghadaouia S, Martinez A, Rodier F (2016). Premature aging/senescence in cancer cells facing therapy: good or bad?. *Biogerontology* 17(1):71-87.
5. Emmenegger U, Man S, Shaked Y, Francia G, Wong JW, Hicklin DJ, Kerbel RS (2004). A comparative analysis of low-dose metronomic cyclophosphamide reveals absent or low-grade toxicity on tissues highly sensitive to the toxic effects of maximum tolerated dose regimens. *Cancer Research* 64(11):3994-4000.
6. Jantan I, Ahmad W, Bukhari SN (2015). Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. *Frontiers in Plant Science* 6:655.

7. Williams BG, Dye C (2003). Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. *Science* 301(5639):1535-7.
8. Ukpo G, Ogbonnia S, Ehianeta T, Bashir S (2013). Immunostimulatory and biochemical effects of ethanolic extract of Mangifera indica stem bark on dexamethasone-induced immunosuppressed male rats. *International Journal of Pharmacy and Pharmaceutical Sciences* 5(2):569-72.
9. Manzia TM, Angelico R, Gazia C, Lenci I, Milana M, Ademoyero OT, Pedini D, Toti L, Spada M, Tisone G, Baiocchi L (2019). De novo malignancies after liver transplantation: The effect of immunosuppression-personal data and review of literature. *World Journal of Gastroenterology* 25(35):5356.
10. Anderson HL, Brodsky IE, Mangalmurti NS (2018). The evolving erythrocyte: red blood cells as modulators of innate immunity. *The Journal of Immunology*, 201(5):1343-1351.
11. Trzeciak-Ryczek A, Tokarz-Deptula B, Deptula W (2013). Platelets-an important element of the immune system. *Polish Journal of Veterinary Sciences*, 16(2):407-413.
12. Jayathirtha MG, Mishra SH (2004). Preliminary immunomodulatory activities of methanol extracts of Eclipta alba and Centella asiatica. *Phytomedicine* 11(4):361-5.
13. Kumolosasi E, Ibrahim SN, Shukri SM, Ahmad W (2018). Immunostimulant activity of standardised extracts of Mangifera indica leaf and Curcuma domestica rhizome in mice. *Tropical Journal of Pharmaceutical Research* 17(1):77-84.
14. Tabe NN, Ushie OA, Jones BB, Kendenson AC, Muktar M, Ojeka CU (2019). Phytochemical Analysis of Methanolic Extract of Mistletoe Leaf. *International Journal of Advanced Research in Chemical Science* 5(7):7-11.
15. Mann A, Ifarajimi OR, Adewoye AT, Ukam C, Udeme EE, Okorie II, Sakpe MS, Ibrahim DR, Yahaya YA, Kabir AY, Ogbadoyi EO (2011). In vivo antitrypanosomal effects of some ethnomedicinal plants from Nupeland of north central Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines* 8(1): 15-21
16. Adekunle AS, Oyewo BE, Afolabi OK (2012). Therapeutic efficacy of Tapinanthus globiferus on acetaminophen induced nephrotoxicity, inflammatory reactions and oxidative stress in albino rats. *International Research Journal of Biochemistry and Bioinformatics* 2(2):41-5.
17. Adekunle AS, Oluba A, Babatola LJ, Kamdem JP, Adesokan A (2014). Antiatherogenic, hypolipidemic and anti-inflammatory benefits of black tea and Zanthoxylum zanthoxyloid. *British Journal of Medicine and Medical Research* 4(9):1923-37.
18. Misra LN, Wouatsa NV, Kumar S, Kumar RV, Tchoumbougnang F (2013). Antibacterial, cytotoxic activities and chemical composition of fruits of two Cameroonian Zanthoxylum species. *Journal of Ethnopharmacology* 148(1):74-80.
19. Kelter G, Schierholz JM, Fischer IU, Fiebig HH (2007). Cytotoxic activity and absence of tumor growth stimulation of standardized mistletoe extracts in human tumor models in vitro. *Anticancer Research* 27(1A):223-33.
20. Wahab OM, Ayodele AE, Moody JO (2010). TLC phytochemical screening in some Nigerian Loranthaceae. *Journal of Pharmacognosy and Phytotherapy* 2(5):64-70.
21. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). Veterinary research forum: *an International Quarterly Journal* 5(2):95-100.
22. National Research Council. (2010). Guide for the care and use of laboratory animals. Washington, DC: National Academy Press.
23. Lorke D (1983). A new approach to practical acute toxicity testing. Archives of toxicology 54(4):275-87.
24. Kabiru M, Usman M, Manga SB, Opaluwa SA, Nura A, Spencer TH (2017). Phytochemical screening and antibacterial activity of the crude extract and fractions of Tapinanthus globiferus leaves on the bacterial isolates of wound. *World Journal of Pharmaceutical Research* 6(7):209-38.
25. Borokini TI, Ayodele AE (2012). Phytochemical screening of Tacca leontopetaloides (L.) Kuntze collected from four geographical locations in Nigeria. *International Journal of Modern Botany* 2(4):97-102.
26. Osadebe PO, Okide GB, Akabogu IC (2004). Study on anti-diabetic activities of crude methanolic extracts of Loranthus micranthus (Linn.) sourced from five different host trees. *Journal of Ethnopharmacology* 95(2-3):133-8.
27. Omeje E, Osadebe PO, Okoyea FB, Agwu AK, Esimone CO (2008). Immunomodulation and Nigerian mistletoe immunomodulatory activities of n-hexane and methanol extracts of Loranthus micranthus Linn. parasitic on Parkia biglobosa. *Asian Pacific Journal of Tropical Medicine* 1(3):48-54.

28. Loomis TA, Hayes AW (1996). Loomis's essentials of toxicology In: Numbers in toxicology, 4th edn. Elsevier, pp 25.
29. Kuethe JW, Mintz-Cole R, Johnson III BL, Midura EF, Caldwell CC, Schneider BS (2014). Assessing the immune status of critically ill trauma patients by flow cytometry. *Nursing research* 63(6):426.
30. Chowdhury MA, Hossain N, Kashem MA, Shahid MA, Alam A (2020). Immune response in COVID-19: A review. *Journal of Infection and Public Health* 13(11):1619-29.
31. Boule. Hematology analyzers: 3-part or 5-part, that is the question (2019). Available at: https://boule.com/knowledge-center/literature/whitepaper-3p-or-5p-hematology-analyzers/ Accessed Jan 2, 2021.
32. Ukpo GE, Ehianeta TS, Adegoke AY, Salako OA (2013). Evaluation of the haematological and biochemical effects of Averon®, A herbal formulation, against cyclophosphamide-induced immunomodulated male rats. *International Journal of Pharmaceutical Sciences and Research* 4(9):3556-62.
33. Diwanay S, Chitre D, Patwardhan B (2004). Immunoprotection by botanical drugs in cancer chemotherapy. *Journal of Ethnopharmacology* 90(1):49-55.
34. Olusi SO, Jessop WJ, Shoroye A (1979). Effects of levamisole on the immune responses of experimentally malnourished rats. *Pediatric Research* 13(11):1237-9.
35. Al-Mamary MA (2002). Antioxidant activity of commonly consumed vegetables in Yemen. *Malaysian Journal of Nutrition* 8:179-189.
36. Imoru JO, Eno AE, Unoh FB, ENkanu E, Ofem OE, Ibu JO (2005). Haematopoietic agents in the crude extractsfrom the leaves of Viscum album (mistletoe) *Nigerian Journal of Health and Biomedical Sciences* 4(2):139-145.
37. Olusola L, Matthew O, Oluwatosin A (2015). Comparative study on the effects of aqueous extracts of viscum album (mistletoe) from three host plants on hematological parameters in albino rats. *African Health Sciences* 15(2):606-12.
38. Abubakar H, Musa AM, Abdullahi MI, Mzozoyana V, Yusuf AJ (2020). Isolation and characterization of some flavonoids from the leaf of Tapinanthus globiferus growing on Vitex doniana. *Brazilian Journal of Biological Sciences*, 7(17): 239-245.
39. Emaikwu V, Ndukwe IG, Mohammed R, Iyun ORA, Anyam JV (2020). Isolation and Characterization of Lupeol from the Stem of Tapinanthus globiferus (A Rich.) and its Antimicrobial Assay. *Journal of Applied Sciences and Environmental Managemen*t, 24(6), 1015-1020.