UMYU Scientifica, Vol. 1 NO. 1, September 2022, Pp 30 –35
https://doi.org/10.56919/usci.1122.005
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Immunotoxicity and proliferation of human peripheral blood mononuclear cells of HIV-positive patient after treatment with aqueous extract of polyherbal formulation

Usman Abubakar Babangida [1], Gana Samuel Eniola [1], Hamid Kabir Magaji [1], Abubakar Nura Kaoje [1,2], Abdullahi Hamisu [1], Isah Sulaiman Yahaya [3], Aliyu Mansur [4,*]

[1] Department of Immunology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria
[2] Department of Health Services, Federal University Birnin Kebbi, P.M.B. 1157, Kebbi, Nigeria
[3] Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria.
[4] Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria.

ABSTRACT

Medicinal plants have been widely used for medicinal purposes in the treatment of various ailments including human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). This study evaluated the effect of aqueous extracts of polyherbal formulation (PHF) on peripheral blood mononuclear cells (PBMCs) of an HIV$^+$ patient. In this study, 4 ml of blood was collected from HIV$^+$ patient who has not commenced antiretroviral treatment. PBMCs were isolated using the Ficoll-Paque method and counted using the trypan blue assay. The isolated PBMCs were treated with different concentrations of the PHF (25 50, 100, and 200 µg/ml). Cytotoxicity and proliferation were determined using the WST-8 assay. The percentage viability in this study was 96.74%, whereas the non-viable cell percentage was 3.26%. The lowest percentage of cytotoxicity for the PHF was 0.05% at 25 µg/ml of the extract, while the highest was 5.35% at 200 µg/ml. The mean absorbance scores of the formulation's various concentrations and the control group did not significantly differ from one another ($F=0.622$, $df = 5$, $p=0.69$). There was significant difference ($t= -4.887$, $df =6$, 95% CI= -0.1566 to -0.0521, $p = 0.003$) in mean absorbance scores between control and treatment. The polyherbal formulation exerts the lowest cytotoxicity of 0.05% and significant proliferation on the PBMCs at 25 µg/ml. The PHF has potential immunoproliferative activity with a good safety margin. An in-depth study of the bioactivity of the PHF on individual immune cells is recommended.

ARTICLE HISTORY

Received August 5, 2022
Accepted August 20, 2022
Published on xxx

KEYWORDS

Polyherbal formulation, immunotoxicity, proliferation, peripheral blood mononuclear cells, human immunodeficiency virus

INTRODUCTION

The knowledge of herbal products (leaves, seeds, bark, roots, fruits, secretions) usage for medicinal purposes stems from several years of struggle to find remedies to illnesses, which include infectious and non-infectious ailments (Petrovska, 2012; Anywar *et al.*, 2020). The majority of therapeutic herbs are obtained locally from the wild, where they naturally thrive (Kankara *et al.*, 2015). About 60% of the world's population and 80% of the population of developing countries rely on traditional medicine (Mussarat *et al.*, 2014) and it was estimated that 25% of routinely prescribed medications contain plant-derived components (Mukhtar *et al.*, 2008). Several chemicals extracted from plant species have subsequently been employed as medications in chemically altered forms, while others have served as templates for synthetic counterparts. Purified chemicals from higher plant species have been utilized directly as pharmaceuticals (Lutoti, 2020). In developing countries, the majority of people, especially those living in rural areas, are still obliged to use traditional medicines to treat their disease conditions due to poverty, ignorance, and a lack of access to modern health facilities

Correspondence: Aliyu, M.; Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria. ✉ maliyu.med@buk.edu.ng ; +234 803 846 8616
How to cite: Usman, A. B., Gana, S. E., Hamid, K. M., Abubakar, N. K., Abdullahi. H., Isah, S. Y., Aliyu, M. (2022). Immunotoxicity and proliferation of human peripheral blood mononuclear cells of HIV-positive patient after treatment with aqueous extract of polyherbal formulation. UMYU Scientifica, 1(1), 30 – 35. https://doi.org/10.47430/usci.1122.005

(Muthu *et al.*, 2006). Several viral diseases have been treated with a range of medicinal herbs, some of which have broad-spectrum antiviral action (Mukhtar *et al.*, 2008). In Nigeria, many patients use herbal remedies for the management of HIV/AIDS, without sufficient scientific proof that those herbs possess anti-HIV activity (Ajaiyeoba and Ogbole, 2006). The traditional healthcare system is not only easily available but also provides an affordable, customized, and socially acceptable alternative to the expensive allopathic medical system (Isola, 2013). According to Norisugi *et al.* (2014), herbal medications are being on practice globally. Thus, numerous medicinal plants have been found to have biological effects, including immunomodulation of mammalian cells (Nhu *et al.*, 2019). Many people who use plant products for medicinal purposes lack the necessary information or training for using herbal products safely (Ala *et al.*, 2018).

The blood cells known as peripheral blood mononuclear cells (PBMCs) comprise groups of lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic cells (Pourahmad and Salimi, 2015). In humans, PBMCs subpopulation frequencies vary from person to person, but typically, lymphocyte frequencies range between 70 to 90 %, monocyte frequencies between 10 to 20 %, and dendritic cell frequencies from 1 to 2 %. The PBMCs being the major cells that constitute the body's immune system, respond selectively to non-self (Pourahmad and Salimi, 2015).

A polyherbal formulation (PHF) is a global practice that entails the use of more than one herbal product to achieve a synergistic effect with targeted efficacy and lower toxicity (Karole *et al.*, 2019). The PHF used for this study was previously reported by Isah *et al.* (2018). The PHF was locally used under the name "*Garjin*" where the herbalists claim it has medicinal properties, it consisted of baobab (*Adansonia digitata* (L.) Gaertn) leaves, orchid (*Bauhinia rufescens* Lam: *Piliostigma rufescens* (Lam) bark, gum Arabic *Acacia senegal L. Willd.* (*Senegalia senegal* (L) Britton) bark, black cutch (*Acacia polyacantha* willd: *Senegalia polyacantha* (Willd) Seigler & Ebinger)bark, and garlic (*Allium sativum* (L): *Foeniculum vulgare* Kazakova) yellow bulb. Previous studies on the PHF have shown improvements in neutrophil adhesion and T-dependent antibody response (Isah *et al.*, 2018), and exerted immunostimulatory activity on the production of Th1/Th2 cytokines and the rat cell-mediated immune response (Hamid *et al.*, 2021a). The PHF also confers immunomodulatory activity on mice's macrophage function and humoral immunity to sheep red blood cells (Hamid *et al.*, 2021b). It exhibits protective activity in cyclophosphamide-induced immunosuppression (Abubakar *et al.*, 2021). However, the effect of the PHF on the PBMCs, which form a significant part of the immune system is unknown. Leukopenia and more significantly lymphopenia due to a massive reduction of $CD4^+$T cells characterized advanced stages of HIV/AIDs (Bhardwaj et al., 2020). Hence, drugs with the potential to induce lymphocyte proliferation might stand a chance to improve HIV/AIDs cytopenia. Consequently, the purpose of this study is to evaluate the impact of the PHF on the PBMCs of HIV^+ patient by evaluating the PHF's cytotoxic and proliferative activity.

MATERIALS AND METHODS

Polyherbal Formulation

The PHF aqueous extract described by Isah *et al.* (2018) was first kept and preserved at 5°C in airtight bottles and marked as PHF aqueous extracts by the Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto. The PHF is made up of five plant extracts: baobab (*Adansonia digitata* (L.) Gaertn) leaves, orchid bush (*Bauhinia rufescens* Lam: *Piliostigma rufescens* (Lam) bark, gum Arabic *Acacia senegal L. Willd.* (*Senegalia senegal* (L) Britton) bark, black cutch (*Acacia polyacantha* willd: *Senegalia polyacantha* (Willd) Seigler & Ebinger)bark, and garlic (*Allium sativum* (L): *Foeniculum vulgare* Kazakova) yellow bulb.

Source of the PBMCs

The Monovette vacutainer system containing lithium heparin as an anticoagulant was used to collect approximately four millilitres (4 ml) of whole blood from a voluntary donor who was recently diagnosed HIV-positive, had not commenced antiretroviral treatment, and had consented to participate in the study. The blood sample collected was immediately processed. Ethical clearance for this study protocol was issued by the Hospital Ethics and Research Committee of Specialist Hospital Sokoto with reference number SHS/SUB/133/vol.1. The research was carried out in line with the Helsinki declaration (Rickham, 1964).

Isolation of PBMCs

The procedure for the isolation of PBMCs was carried out according to the manufacturer's instructions and described in Hamid *et al.* (2021c). Briefly, a 15 ml falcon tube was loaded with 3 ml of Histopaque-1077 (Sigma-Aldrich® Co. United Kingdom) at room temperature. Then, 3 ml of whole blood was carefully transferred onto the Histopaque-1077. It was centrifuged at 400 × g for 30 min at room temperature, the PBMCs-containing middle layer was then carefully aspirated with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells and was transferred into a clean conical centrifuge tube. The cells were mixed by gently pulling in and out of a Pasteur pipette after being gently washed by adding 10 ml of isotonic phosphate-buffered saline solution. We washed the cells by centrifugation at 250 g for 10 min, and the supernatant was discarded. The cells pellets were suspended in 5 ml of isotonic phosphate-buffered saline solution and gently mixed by drawing in and out of the pipette. Following that, the cells were washed as described above and the step was repeated two more times. The cells pellets were re-suspended in 2 ml of RPMI-1640 (Beijing Solar bio Science and Technology Co. Ltd, China) and treated with 10% heat-inactivated foetal bovine serum (FBS).

Trypan blue assay

The protocol was carried out as directed by the manufacturer and described in Hamid *et al.* (2021c). Ten microliters (10 µl) of 0.4% trypan blue (Sigma-Aldrich® Co. United Kingdom) solution (w/v) and 10 µl of the PBMCs suspension were transferred into a cryovials tube (dilution factor = 2) and mixed, it was then allowed to stand for a maximum of 5 minutes. 10 µl of the trypan blue-PBMCs suspension mixture was added to both chambers of the haemocytometer while the cover-slip was in place. With the use of a light microscope, the number of both live and dead cells was counted. Viable cells are colourless, whereas non-viable ones were stained blue. The percentage of viable cells was calculated.

Determination of percentage cytotoxicity

Cytotoxicity of the PHF extracts on PBMCs was determined using WST-8 cell proliferation assay kit (Beijing Solar bio Science and Technology Co. Ltd, China) and the procedure was carried out according to manufacturers' instructions. The cells (100 µl/well) were seeded in a 48-well microplate with RPMI-1640 medium at a concentration of 1 x 10^5 cells/well and then kept for 24 hours in a humid incubator at 37°C with 5% CO_2. The 48-well microplate's cells were then exposed to various amounts of treatment (10 µl/well) of the PHF extract (25, 50, 100, and 200 µg/ml). The treated cells were then placed in a humidified incubator and incubated for 4 hours at 37°C and 5% CO_2. After the incubation period, 10 µl of WST-8 reagent was added to the treated cells also the control and was incubated for 1 hour at 37°C and 5% CO_2 in a humidified incubator. The absorbance was read using an ELISA microplate reader at 450 nm and cytotoxicity was calculated. All steps for the cytotoxicity test were done in duplicate, as described in the manufacturer's protocol. The PBMCs suspended in RPMI-1640 were used as the negative control, RPMI-1640 and FBS were used as a media background control, PBS only was used as control, and an empty well as blank. The percentage cytotoxicity of the different concentrations of the extract was determined by calculation:

$$\% \text{ cytotoxicity} = \frac{(100 \times (\text{Control} - \text{Sample}))}{\text{Control}}$$

Determination of PBMCs proliferation

For the proliferation of PBMCs, a WST-8 cell proliferation assay kit (Beijing Solar bio Science and Technology Co. Ltd, China) was used. The experiment was carried out as directed by the manufacturer. The PBMCs suspension (100 µl) was dispensed in 48-well microplates at a density of 1 x 10^5 cells/well in RPMI-1640 and kept at 37°C in a 5% CO_2 humidified incubator for 24 hours. Phytohaemagglutinin (Sigma-Aldrich® Co., United Kingdom) (PHA: 10 µg/ml) was added to each of the PBMCs cultures with 10 µl of the PHF extracts at IC_{50} concentration (25 µg/ml) incubated at 37°C and 5% CO_2 in a humidified incubator for 4 hours. Then 10 µl /well of WST-8 cell proliferation assay reagent was added and then incubated for 1 hour at 37°C and 5% CO_2 in a humidified incubator. The PBMCs not treated with the extract, RPMI-1640, FBS, and PBS were considered controls. All treatments were performed in duplicate, as described in the manufacturer's protocol. The absorbance was read using an ELISA microplate reader at 450 nm. The amount of absorbance is proportional to cell number (proliferation).

Statistical analysis

The acquired results were entered into IBM's SPSS version 21 (US) for analysis. While categorical data were expressed in percentages, continuous variables were expressed as mean and standard deviation (SD). To compare across groups, a one-way analysis of variance (ANOVA) with a post-hoc test (Bonferroni) was used. To compare the mean absorbance of the control and treatment groups on PBMC proliferation, an independent sample t-test was used. Statistical significance was defined as a *p*-value of less than 0.05.

RESULTS

PBMCs count and percentage viability

The total number of viable PBMCs counted before treatment with the PHF was 1664 x 10^4 cells/ml (96.74%), whereas non-viable cells were 56 x 10^4 cells/ml (3.26%).

Immunotoxicity of PBMCs

The lowest percentage of cytotoxicity for the PHF was 0.05% at 25 µg/ml of the extract concentration, while the highest was 5.35% at 200 µg/ml. The percentage of cytotoxicity tends to increase with an increase in extract concentration. The minimum concentration with the lowest cytotoxicity was 25 µg/ml of the extract and this is equivalent to inhibitory concentration (IC_{50}) (Figure 1).

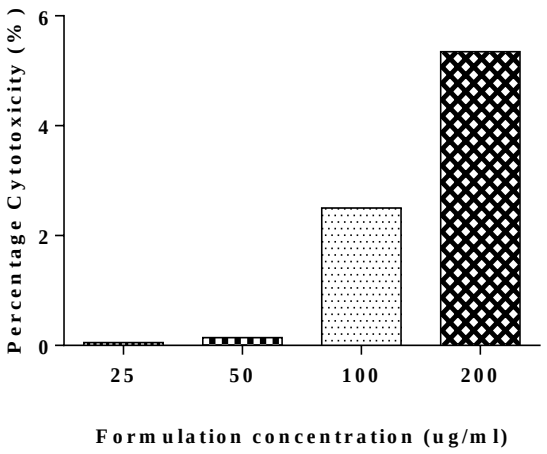

Figure 1: Percentage cytotoxicity of different concentrations of the PHF on PBMCs.

Comparison of different concentrations of the PHF on the PBMCs

As shown in Figure 2, the mean absorbance scores and SD of control were M=1.842, and SD=0.107. While the mean absorbance of the different concentration of the PHF were 25 µg/ml (M=1.841, SD= 0.104), 50 µg/ml (M=1.839, SD=0.102), 100 µg/ml (M=1.796, SD= 0.103), and 200 µg/ml (M=1.743, SD=0.106). A one-way between-groups analysis of variance was conducted to compare the effect of different concentrations of the PHF on PBMCs. There was no significant difference in the mean absorbance scores of control and that of different concentration of the (i.e. 25, 50, 100 and 200 µg/ml) ($F = 0.622$, $p = 0.69$).

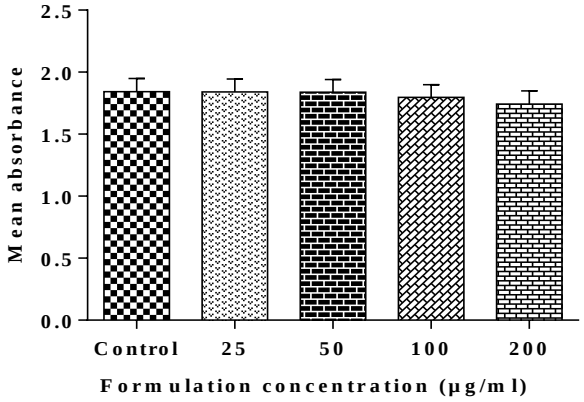

Figure 2: Effects of different concentrations of the PHF on PBMCs

Proliferation of PBMCs

As depicted in Figure 3, the mean absorbance score for control was M=1.776, SD=0.141, while that of treatment was M=1.880, SD= 0.122. The higher the mean absorbance the higher the proliferation. An independent sample t-test was conducted to compare the effect of the PHF extracts on PBMCs proliferation between control and treatment after treatment with a 25 µg/ml (IC50) concentration of the extract. When the mean absorbance scores between control and treatment were compared, there was significant difference (t=-4.887, 95% CI= -0.1566 to -0.0521, $p = 0.003$).

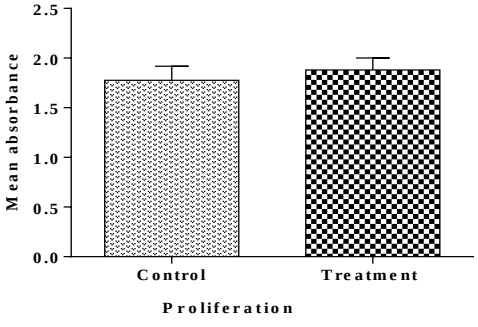

Figure 3: Effect of PHF on human PBMCs proliferation.

DISCUSSION

In the current study, we investigated the *in vitro* activity of aqueous extract of the PHF on human PBMCs of a patient with confirmed HIV infection. The PBMCs viability reported in this study was within the expected normal range. Our viability score is in agreement with previous studies (Hamid *et al.*, 2021c; Kalgo *et al.*, 2020; Nguyen and Le, 2021). This also agrees with the viability test done by Kim *et al.* (2011) using the trypan blue dye exclusion method which says that the manual method of viability testing gives accurate results as with other techniques such as microscopic cell counter with microchip and flow cytometry for assessing cell viability.

This study demonstrated that there was no cytotoxic difference between control PBMCs and those PBMCs treated with different concentrations of the PHF. The indifference, as well as the toxicity at the lowest PHF concentration, indicated that the PHF possesses potentially safe constituents that can be used safely in future drug formulation. The PHF has minimal toxicity on the PBMCs even at the highest concentration. This contradicts the studies done by Sudeep *et al.* (2017) and Kalgo *et al.* (2019) where different plant extracts of similar concentrations caused a decrease in the viability of the normal neutrophils and lymphocytes showing that the extracts were toxic to the cells.

When the PBMCs were exposed to the lowest PHF concentration, we recorded significantly higher cells number in comparison to the control. Besides, a research has shown that a lower concentration of a plant extract can cause an increase in cell viability while a higher concentration can lead to the cytotoxicity of cells (Nemudzivhadi and Masoko, 2014). This might be a result of low dose-concentration of PHF on the cells which did not exert cytotoxicity as reported by Aldbass *et al.* (2021) who observed that the dose concentration of plant extracts is a function of cytotoxicity. A test substance's potential toxicity, including that of plant extracts or biologically active compounds isolated from plants, could be determined through cytotoxicity studies. The successful development of a pharmaceutical or cosmetic preparation depends on the test substance having little to no toxicity. In this context, studies on cellular toxicity are essential for determining a safe dose that is not harmful to cells (McGaw *et al.*, 2014).

Accordingly, the concentration of the studied PHF induces proliferation in the cells, hence increasing their viability. Our finding is in line with the findings of Swee *et al.* (2011) who reported that *Rhaphidophora korthalsii* methanol extract induced proliferation and activation of PBMCs, which in turn induce apoptosis of effector cancer cell (K562). Another study has shown that *Echinacea purpurea* extract (Cichoric acid) increased the proliferation and cell cycle activities of PBMCs (Wang *et al.*, 2020). Makgatho *et al.* (2015) reported that leaf extract of *Albizia gummifera* had growth-promoting activities on PBMCs. Furthermore,

scientists reported varying effects of different plants extracts on PBMCs, some promote proliferation (*Glycyrrhiza glabra* L and *Astragalus membranaceus*), and some inhibit proliferation (*Cassia alata* L. *Wedelia chinensis*, *Lonicera japonica*, *Caesalpinia sappan* L, *Schefflera heptaphylla*, and *Piper betle*) (Nguyen and Le, 2021).

Furthermore, different plants extract have different effect(s) on the proliferation and cytotoxic effects on PBMCs at possibly different concentration. This phenomenon could be based on the phytochemical content and combination in the studied plant products. As such, these individual constituents need to be studied in more detail, in order to have a specific therapeutic agent with desired activity against the desired target condition.

CONCLUSION

The polyherbal formulation exerts the lowest cytotoxicity of 0.05% and significant proliferation on the PBMCs of the patient living with HIV at 25 µg/ml concentration. The PHF has potential immunoproliferative activity on the PBMCs with a good safety margin. These qualities indicated that the PHF has potential for therapeutic formulation. An in-depth study of the bioactivity of the individual immune cells will invariably provide an avenue for drug development.

CONFLICT OF INTEREST

Authors declare no competing interest

ACKNOWLEDGEMENT

The Garjin® PHF was initially donated by Mr. Sani Muhammad Umar (Nagge) of Al-Mustakshif Medical Health Centre, Kano, Nigeria (RC: 1393615), and the authors appreciate his cooperation.

REFERENCES

Abubakar, N. K., Hamid, K. M., Haruna, M., Abdullahi, M. I., Usman, A. B., Isah, S. Y., and Aliyu, M. (2021). Protective activity of a polyherbal formulation in cyclophosphamide-induced immunosuppression. *Ethiopian Pharmaceutical Journal,* 37, 33-44. https://doi.org/10.4314/epj.v37i1.3

Ajaiyeoba, E., and Ogbole, O. (2006). A phytotherapeutic approach to Nigerian anti-HIV and immunomodulatory drug discovery. *African Journal of Medicine and Medical Sciences,* 35, 71-76.

Ala, A., Olotu, B., and Ohia, C. (2018). Assessment of cytotoxicity of leaf extracts of *Andrographis paniculata* and *Aspilia africana* on murine cells in vitro. *Archives of Basic Applied Medicine,* 6(1), 61.

Aldbass, A., Amina, M., Al Musayeib, N. M., Bhat, R. S., Al-Rashed, S., Marraiki, N., Fahmy, R., and El-Ansary, A. (2021). Cytotoxic and anti-excitotoxic effects of selected plant and algal extracts using COMET and cell viability assays. *Scientific Reports,* 11(1), 1-11. https://doi.org/10.1038/s41598-021-88089-8

Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., and Oryem-Origa, H. (2020). Indigenous traditional knowledge of medicinal plants used by herbalists in treating opportunistic infections among people living with HIV/AIDS in Uganda. *Journal of Ethnopharmacology,* 246, 112205. https://doi.org/10.1016/j.jep.2019.112205

Bhardwaj, S., Almaeen, A., Ahmed Wani, F., and Thirunavukkarasu, A. (2020). Hematologic derangements in HIV/AIDS patients and their relationship with the CD4 counts: a cross-sectional study. *International Journal of Clinical and Experimental Pathology,* 13(4), 756-763.

Hamid, K. M., Isah, S. Y., Kalgo, M. U., Isiyaku, A., Usman, A. B., Yeldu, M. H., Yusuf, A., Abubakar, N. K., Garba, Y. B., and Muhammad, H. Y. (2021a). Imunostimulatory activity of aqueous extract of polyherbal formulation on Th1/Th2 cytokines secretion and cell mediated immune response in rats. *Saudi Journal of Medical and Pharmaceutical Sciences* 10, 11. https://doi.org/10.36348/sjmps.2021.v07i01.013

Hamid, K. M., Shehu, A. A., Kalgo, M. U., Isiyaku, A., Alkali, S., Muhammad, T., Usman, A. B., Isah, S. Y., and Abubakar, N. K. (2021b). Immunomodulatory activity of aqueous extract of polyherbal formulation on macrophage function and humoral immune response against SRBCs in mice. *Nigerian Journal of Pure and Applied Science,* 34(2), 3989-3997. https://doi.org/0.48198/njpas/21.a01

Hamid, K. M., Yakubu, A., Sani, S. A., Kalgo, M. U., Isiyaku, A., Umahi, N. P. F., Garba, Y. B., Aliyu, M., Isah, S. Y., and Abubakar, U. (2021c). Immunocytotoxic effect of aqueous leaf extract of *Cassia occidentalis* on human peripheral blood mononuclear cells and neutrophils. *UMYU Journal of Microbiology Research,* 6(2), 30-36. https://doi.org/10.47430/ujmr.2162.005

Isah, Y. S., Yeldu, M. H., Hamid, K. M., Musa, U., and Kalgo, M. U. (2018). Effect of polyherbal formulation on neutrophil function and TDAR in Wistar albino rat. *European Journal of Pharmaceutical and Medical Research,* 5(12), 563-568.

Isola, O. I. (2013). The" relevance" of the african traditional medicine (alternative medicine) to health care delivery system in Nigeria. *The Journal of Developing Areas* 319-338. https://doi.org/10.1353/jda.2013.0004

Kalgo, M. U., Hamid, K. M., Muhammad, U. A., Balarabe, A., Yeldu, M. H., Yahaya, I. S., Kalgo, Z. M., Aliyu, B., and Bala, Y. G. (2019). Effects of aqueous stem bark extract of *Vitellaria paradoxa* on human neutrophil function and viability. *Internaional Journal of Biological and Medical Research,* 10(3), 6782-6787.

Kalgo, M. U., Hamid, K. M., Balarabe, A., Muhammad, U. A., Bala, Y. G., Raji, H. Y., Yusuf, A., Isiyaku, A.,

Bagudo, I. A., and Usman, A. (2020). Cytotoxic effect of aqueous stem bark extract of *Vitellaria paradoxa* on human peripheral blood mononuclear cells. *IOSR Journal of Dental and Medical Sciences,* 12(2), 48-52.

Kankara, S. S., Ibrahim, M. H., Mustafa, M., and Go, R. (2015). Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. *South African Journal of Botany,* 97, 165-175. https://doi.org/10.1016/j.sajb.2015.01.007

Karole, S., Shrivastava, S., Thomas, S., Soni, B., Khan, S., Dubey, J., Dubey, S. P., Khan, N., and Jain, D. K. (2019). Polyherbal formulation concept for synergic action: a review. *Journal of Drug Delivery and Therapeutics,* 9(1-s), 453-466. https://doi.org/10.22270/jddt.v9i1-s.2339

Kim, J. S., Nam, M. H., An, S. S. A., Lim, C. S., Hur, D. S., Chung, C., and Chang, J. K. (2011). Comparison of the automated fluorescence microscopic viability test with the conventional and flow cytometry methods. *Journal of Clinical Laboratory Analysis,* 25(2), 90-94. https://doi.org/10.1002/jcla.20438

Lutoti, S. (2020). An appraisal of plants as a natural source of conventional drugs. *Journal of Pharmacology and Drug Research,* 3(3), 366-372.

Makgatho, M. E., Mashele, S., Makhubele, T., Sedibane, M., Bagla, V., Mashinya, F., Mokgotho, M., Masoko, P., Makola, R., and Matsebatlela, T. (2015). In vitro immune-modulatory potential of crude extract of leaf of *Albizia gummifera* against stimulated peripheral blood mononuclear and raw cells. *African Journal of Traditional, Complementary and Alternative Medicines,* 12(3), 85-90. https://doi.org/10.4314/ajtcam.v12i3.10

McGaw, L. J., Elgorashi, E. E., and Eloff, J. N. (2014). Cytotoxicity of African medicinal plants against normal animal and human cells. In *Toxicological Survey of African Medicinal Plants* (pp. 181-233): Elsevier. https://doi.org/10.1016/B978-0-12-800018-2.00008-X

Mukhtar, M., Arshad, M., Ahmad, M., Pomerantz, R. J., Wigdahl, B., and Parveen, Z. (2008). Antiviral potentials of medicinal plants. *Virus Research,* 131(2), 111-120. https://doi.org/10.1016/j.virusres.2007.09.008

Mussarat, S., Abdel-Salam, N. M., Tariq, A., Wazir, S. M., Ullah, R., and Adnan, M. (2014). Use of ethnomedicinal plants by the people living around Indus River. *Evidence-Based Complementary and Alternative Medicine,* 2014. https://doi.org/10.1155/2014/212634

Muthu, C., Ayyanar, M., Raja, N., and Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *Journal of Ethnobiology Ethnomedicine,* 2(1), 1-10. https://doi.org/10.1186/1746-4269-2-43

Nemudzivhadi, V., and Masoko, P. (2014). In vitro assessment of cytotoxicity, antioxidant, and anti-inflammatory activities of *Ricinus communis* (Euphorbiaceae) leaf extracts. *Evidence-Based Complementary and Alternat Medicine,* 2014, 625961. https://doi.org/10.1155/2014/625961

Nguyen, T. T. M., and Le, N. T. T. (2021). Inhibitory effect of medicinal plants on in vitro human peripheral blood mononuclear cell proliferation, interleukin-2 secretion and antioxidant activity. *MedPharmRes,* 5(1), 22-29. https://doi.org/10.32895/UMP.MPR.5.1.5

Nhu, T. Q., Hang, B. T. B., Vinikas, A., Hue, B. T. B., Quetin-Leclercq, J., Scippo, M.-L., Phuong, N. T., and Kestemont, P. (2019). Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based in vitro tests. *Fish & Shellfish Immunology,* 93, 296-307. https://doi.org/10.1016/j.fsi.2019.07.064

Norisugi, O., Yoshihisa, Y., Shimizu, K., and Shimizu, T. (2014). In vitro cytokine expression by peripheral mononuclear cells in herbal drug-induced skin eruption. *Acta Dermato-Venereologica,* 94(1). https://doi.org/10.2340/00015555-1631

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews,* 6(11), 1. https://doi.org/10.4103/0973-7847.95849

Pourahmad, J., and Salimi, A. (2015). Isolated human peripheral blood mononuclear cell (PBMC), a cost effective tool for predicting immunosuppressive effects of drugs and xenobiotics. *Iranian Journal of Pharmaceutical Research,* 14(4), 979.

Rickham, P. (1964). Human experimentation. Code of ethics of the world medical association. Declaration of Helsinki. *British Medical Journal,* 2(5402), 177-177. https://doi.org/10.1136/bmj.2.5402.177

Sudeep, N., Nithya, M., and Kiranmayee, P. (2017). Evaluation of in vitro cytotoxic effects of three medicinal plants on peripheral blood mononuclear cells (PBMC). *Journal of Chemical and Pharmaceutical Research* 9, 18-26.

Swee, K. Y., Noorjahan, B. M. A., Wan, Y. H., Abdul, R. O., Abdul, M. A., Boon, K. B., and Abdul, H. N. Y. (2011). Immunomodulatory role of *Rhaphidophora korthalsii* methanol extract on human peripheral blood mononuclear cell proliferation, cytokine secretion and cytolytic activity. *Journal of Medicinal Plants Research,* 5(6), 958-965.

Wang, M., Raza, S. H. A., Wu, Q., Xue, C., Liu, J., Zhang, L., Zhang, W., Wang, A., and Wu, H. (2020). Cichoric acid from extracted *Echinacea purpurea* induces the proliferation and apoptosis of peripheral blood mononuclear cells from yaks. *Electronic Journal of Biotechnology,* 47, 17-28. https://doi.org/10.1016/j.ejbt.2020.06.003