ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2022; 7(2): 5 - 13

SJMLS-7(2)-001

Immunomodulation of IFN-γ, IL-4, and IL-10 Cytokines by Polyherbal Formulation in Cyclophosphamide-Induced Immunosuppression among Wistar Rats

Hamid, K.M. [1], Abubakar, N.K. [1,4], Yeldu, M.H. [2], Abdullahi, M.I. [3], Usman A.B. [1], Isah, S.Y. [5], Aliyu, M. [6]*

Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto [1], Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto [2], Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto [3], Department of Health Services, Federal University Birnin Kebbi, P.M.B. 1157, Kebbi [4], Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University, P.M.B. 3011, Kano [5], Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Bayero University, P.M.B. 3011, Kano [6].

Author for correspondence*: maliyu.med@buk.edu.ng/ +234- 803- 846- 8616/ ORCID Number: 0000-0002-4223-2788.

https://dx.doi.org/10.4314/sokjmls.v7i2.1

Abstract

This study evaluated the immunomodulatory potential of aqueous extract of polyherbal formulation (PHF) in cyclophosphamide (CP)-induced immunosuppression in Wistar albino rats. The rats were randomly divided into six (6) groups of four (4) rats each. The normal control group received 10 ml/kg b.w of normal saline for 28 days orally. The Positive control group received 50mg/kg b.w of Levamisole Hydrochloride for 28 days orally. Cyclophosphamide (CP) control group had 10 mg/ kg b.w of CP for three days subcutaneously. While the Treatment control group received 1500 mg/ kg b.w of PHF orally for 28 days. Treatment 1 group received 10 mg/ kg b.w of CP for three days subcutaneously, followed by oral administration of 1500 mg/ kg b.w of PHF for 28 days. Lastly, Treatment 2 group received 1500 mg/ kg b.w of PHF for 28 days orally, followed by subcutaneous administration of 10 mg/ kg b.w of CP for three days. On the first day prior to any intervention, 1 ml of the blood sample was collected from each rat which serve as a pre-treatment sample. At the end of the experiment (after 28 days), a blood sample was collected from each rat which serve as a post-treatment sample. The serum concentration of IFN-γ, IL-4, and IL-10 were determined using the ELISA technique. For pre-treatment samples, there was no statistically significant difference in the mean serum concentration of the cytokines across the groups ($p > 0.05$). For post-treatment samples, there was a statistically significant difference in the mean serum concentration of IFN-γ ($p < 0.0001$) and IL-4 ($p<0.001$) across the groups. There was a significant difference when normal controls were compared with other groups in IFN-γ and IL-4 ($p <0.05$). No significant difference was observed in IL-10. The formulation seems to neutralise the immunosuppressive condition and induce immunostimulatory activity on the immune cells responsible for the secretion of IFN-γ and IL-4.

Keywords: Cyclophosphamide, Cytokines, Immunomodulation, Immunosuppression, Murine, Polyherbal formulation

Introduction

An immunomodulator is a substance that can enhance, dampens, or regulate the immune system (Das *et al.,* 2014). The immunomodulators can stimulate cytokines (Alamgir and Uddin, 2010; Catanzaro *et al.,* 2018). Across the world, immunomodulation is viewed as the primary target for the treatment and prevention of various diseases, including common cold, various infections, and cancer (Ye *et al.,* 2015). Researchers are developing an interest in the experimental study of the effect of herbs on the immune system. Several sources including mushrooms are being screened for immunomodulatory compounds that can be used to enhance cancer chemotherapy (Kyakulaga *et al.,* 2013).

The serum concentration of various cytokines may give information on the presence, or predictive value of inflammatory processes in various disease conditions , as well as immunomodulatory effects of foods or drugs . Cytokines are now seen to be crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis, and developmental as well as repair processes (Spelman *et al.,* 2006). The Cyclophosphamide (CP) belongs to nitrogen mustard a subclass of alkylating agents which induces bone marrow suppression.

Bone marrow suppression is caused by the alkylation of DNA and therefore interferes with DNA synthesis and functions (Bhatt *et al.,* 2017).

Herbal medicine, sometimes called traditional or natural medicine, entails the use of plant products for medicinal purposes, a practice that dated back millennia (Kumar *et al.,* 2012; Shaista and Ahmad, 2011). Which can be more effective, less toxic, and safer than conventional medicines and could be administered for a long period (Vinothapooshan and Sundar, 2011). Herbal medicines with immunomodulatory activity alter the immune function through the dynamic regulation of molecules such as cytokines and chemokines (Arreola *et al.,* 2015). Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific as well as non-specific immunity (Hajra *et al.,* 2012).

Polyherbal formulations (PHF), comprises various herbal products with assumed additive effects such herbal constituents with diverse pharmacological activities principally work together in a dynamic way to produce maximum therapeutic benefits with minimum side effects (Barik *et al.,* 2015). The PHF has been used for the treatment of different conditions (Petchi *et al.,* 2015). It is believed that the mixture of different herbal species in these PHFs shows a better therapeutic effect than either of the species on its own (Subhasree *et al.,* 2015). The immune system protects the human host from foreign invasion. It is involved in the recovery from diseases determined by the host's immune status such as cancer, obesity, diabetes, tuberculosis, etc. Thus, the body's immune function is vitally important for the prevention and recovery from these immune-mediated diseases (Kumar *et al.,* 2014). The study of medicinal plants and the knowledge of their traditional use can provide useful information for the discovery of new drugs that could be less expensive than most commercially available synthetic drugs. Moreover, natural products, either pure compounds or standardized plant extracts, provide unlimited opportunities for the development of new leads in drug discovery (Cecilio *et al.,* 2012).

A large proportion of African inhabitants live in rural communities and strongly depend on local plant products for their livelihood. However, literature on traditional plant use is still scarce as the comprehensive and scientific basis for most medicinal herbs is still missing (Zizka *et al.,* 2015). Also, the formulations and sales are poorly regulated (Oshikoya *et al.,* 2013). Immunomodulative efficacy and safety data for some of the imported herbal medicines are available in the literature to guide their use (Choi *et al.,* 2013) , however, such important information is not available in our locally produced herbal medicines in Nigeria. Previous studies (sah *et al.,* 2018; Hamid *et al.*, 2021) on the PHF showed that it has immunostimulatory activity. However, its activity in immunosuppressed conditions was not assessed. Therefore this study was aimed at determining the therapeutic and protective potential of the PHF in the cyclophosphamide-induced immunosuppression model. It will particularly provide an insight into the effect of a high dose of the PHF in neutralizing the immunosuppressed condition using cytokines parameter.

Materials and Methods

Plant Materials

Al-Mustakshif Medical Health Center, Kano (RC: 1393615) provided the herbal materials as previously reported (Isah *et al.,* 2018). The PHF comprises five plant materials namely: *Adansonia digitata* (Baobao-leaves: UDUH/ANS/0194), *Acacia senegal* (Gum Arabic tree-Bark: UDUH/ANS/0195), *Allium sativum* (Garlic-Yellow-bulb: UDUH/ANS/0196), *Bauhinia rufescens lam* (Orchid Hedge-Bark: UDUH/ANS/0197) and *Acacia polyacentha* wild (Black Cutch-Bark: UDUH/ANS/0198). The plant taxonomic identification was carried out at the Herbarium section of the Botany Unit, Department of Biological Sciences, Faculty of Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The individual plant pharmacological potential was reported in our previous study (Isah *et al.,* 2020).

Polyherbal extract

Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto supplied the aqueous extract of the PHF. The extract was initially labelled as PHF aqueous extracts then stored and preserved at 5°C in airtight bottles. About 0.1 g of the dried extract was dissolved in 1 ml of distilled water and this served as stock. The stock was prepared in batches (Hamid *et al.,* 2021).

Experimental Animals

Twenty-four (24) Albino Wistar rats of 10-12 weeks old male and female that weighted averagely 145g to 155g were purchased from Ahmadu Bello University, Zaria's Faculty of Veterinary Medicine. Animals were housed at Usmanu Danfodiyo University, Sokoto's Faculty of Pharmaceutical Sciences with alternating 12 hours of light and dark cycles at ambient temperature. Food and water were available to the rats *ad libitum*, animals were allowed two weeks to acclimatize. Animals were handled according to the guidelines for handling laboratory animals and ethical guidelines of the Animal Ethics Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, who approved the study with reference number: PTAC/PF/AE/ OT/11-19.

Grouping of Animals and Treatment

We randomly divided the rats into six (6) groups of four (4) rats each, Normal control (NC) group received 10 ml/kg b.w of normal saline for 28 days orally. The positive control (PC) group received 50mg/kg b.w of Levamisole Hydrochloride (LEV) (Beijing Solarbio Science and Technology Co., Ltd., China) for 28 days orally. Cyclophosphamide (CP) control (CC) group had 10 mg/ kg b.w of CP (Beijing Solar Bio Science and Technology Co., Ltd, China) for three days subcutaneously. The Treatment control (TC) group received 1500 mg/ kg b.w of PHF orally for 28 days. Treatment 1 (T1) group (CP+PHF) received 10 mg/ kg b.w of CP for three days subcutaneously, followed by oral administration of 1500 mg/ kg b.w of PHF for 28 days. Lastly, the Treatment 2 (T2) group (PHF+CP) received 1500 mg/ kg b.w of PHF for 28 days orally, followed by subcutaneous administration of 10 mg/ kg b.w of CP for three days.

Immunosuppression Induction

Immunosuppression was induced among group CC, T1, and T2 through administration of CP 10 mg/kg body weight subcutaneously for three consecutive days. After the last dose, complete clinical signs of immunosuppression such as weakness, weight loss, spontaneous loss of hair, and diarrhoea were observed.

Sample Collection and Processing

On the first day prior to any intervention, one milliliter (1 ml) of the blood sample was collected from each rat in the groups through the tail vein. The blood sample collected was dispensed into a plain tube and allowed to clot within 30 minutes of the collection. It was centrifuged at 4000 rpm for 20 minutes to obtain a neat serum. The supernatant was transferred into specimen bottles and labelled as a pre-treatment sample for cytokines analysis. At the end of the experiment (after 28 days), the blood sample was collected from each rat via cardiac puncture under 50 mg/kg ketamine and 10 mg/kg xylazine administered intramuscularly. The samples were processed as stated above and labelled as post-treatment samples for cytokines analysis.

Measurement of Serum Cytokines Concentration

Blood samples for serum cytokine analysis were collected at two different intervals (i.e., Pre-treatment and post-treatment) across the groups. The serum concentrations of cytokines (IFN-γ, IL-4, and IL-10) were determined using a sandwitch enzyme-linked immunosorbent assay (ELISA) purchased from Beijing Solarbio Science and Technology Co., Ltd (China). The procedure was performed according to the manufacturer's instructions. The assay sensitivity was 19 pg/ml, 7 pg/ml, and 15 pg/ml for IFN-γ, IL-4, and IL-10 respectively.

Statistical Analysis

Data obtained were analyzed using SPSS version 25. Continuous variables were reported as mean and standard deviation (SD). One way between-groups of analysis of variance (ANOVA) was used to explore differences in mean serum concentration of the cytokines across the groups. A post hoc test, Bonferroni was used to compare between the groups. A p-value $\leq$ 0.05 was considered statistically significant.

Results

The effect of PHF on IFN-γ across the groups was explored using ANOVA. As depicted in Table 1, for pre-treatment sample there was no statistically significant difference in mean serum concentration of IFN-γ across the groups ($F = 1.14$, $p = 0.391$). However, in post-treatment sample there was a statistically significant

difference in mean serum concentration of IFN-γ across the groups (F = 15.30, p < 0.0001). Post-hoc comparisons using the Bonferroni tests indicated that the mean serum concentration of IFN-γ for Normal control (NC) (M = 22.55 pg/ml, SD = 1.65) was significantly different from that of Postive control (PC) (M = 38.89 pg/ml, SD = 1.32), 95% CI: -31.24 to -1.42 (p =0.02); Cyclophosphamide control (CC) (M = 12.80 pg/ml, SD = 1.14), 95% CI: -5.15 to -24.66 (p =0.04) ; Treatment control (TC) (M = 38.33 pg/ml, SD = 1.32), 95% CI: -30.68 to -0.86 (p =0.03) ; Treatment (T1) (M = 39.88 pg/ml, SD = 1.38), 95% CI: -32.23 to -2.41 (p=0.01) and Treatment (T2) (M=32.50pg/ml, SD= 1.48), 95%CI: -30.01 to -0.19 (p=0.04). However, there was no statistically significant difference when other groups were compared (p > 0.05).

Table 1: Serum Concentration of IFN -γ at Pre- and Post- Treatment with aqueous extract of PHF

Grouping & Dose (kg b. w) (N = 24)	IFN-γ (pg/ml)	
	Pre-treatment Mean (SD)	Post-treatment (Mean SD)
NC (Normal saline 10 ml)	23.98 (1.85)	22.55 (1.65)
PC (Levamisole Hydrochloride 50 mg)	20.82 (1.86)	38.89 (1.32) [a*]
CC (Cyclophosphamide 10 mg)	18.21 (1.83)	12.80 (1.14) [b*]
TC (PHF 1500 mg)	21.96 (1.45)	38.33 (1.32) [c*]
T1 (CP 10 mg + PHF 1500 mg)	19.99 (1.58)	39.88 (1.38) [d*]
T2 (PHF 1500 mg + CP 10 mg)	18.19 (1.74)	32.50 (1.48) [e*]
	p=0.391	p<0.0001

a= I vs II, b= I vs III, c=I vs IV, d=I vs V, e= I vs VI *p ≤0.04. NC - Normal control, PC- Positive control, CC-Cyclophosphamide control, TC -Treatment control, T - Treatment

As depicted in Table 2, for the pre-treatment sample there was no statistically significant difference in the mean serum concentration of IL-4 across the groups (F = 1.07, p = 0.423). However, in the post-treatment sample, there was a statistically significant difference in the mean serum concentration of IL-4 across the groups (F = 532.43, p < 0.001). Post-hoc comparisons using the Bonferroni tests indicated that the mean serum concentration of IL-4 for Normal control (NC) (M = 134.53 pg/ml, SD = 1.66) was significantly different from that of positive control (PC) (M = 270.10 pg/ml, SD = 1.49), 95% CI: -152.05 to -119.08 (p<0.001); Cyclophosphamide control (CC) (M = 115.40 pg/ml, SD = 1.86), 95% CI: 2.64 to 35.62 (p=0.02) ; Treatment control (TC) (M = 266.70 pg/ml, SD = 1.19), 95% CI: -148.65 to -115.68 (p<0.001) ; Treatment (T1) (M = 269.63 pg/ml, SD = 1.56), 95% CI: -151.58 to -118.61 (p<0.001) and Treatment (T2) (M = 262.13 pg/ml, SD = 1.84), 95% CI: -144.08 to -111.11 (p<0.001). However, there was no statistically significant difference when other groups were compared (p > 0.05). Table 3 show that in both pre- and post-treatment samples there was no statistically significant difference in mean serum concentration of IL-10 across the groups (p > 0.05).

Table 2: Serum Concentration of IL-4 at Pre- and Post- Treatment with aqueous extract of PHF

Grouping and Dose (kg b. w) (N = 24)	IL-4 (pg/ml)	
	Pre-treatment Mean (SD)	Post-treatment (Mean SD)
NC (Normal saline 10 ml)	130.33 (1.78)	134.53 (1.66)
PC (Levamisole Hydrochloride 50 mg)	134.40 (1.95)	270.10 (1.49)[a***]
CC (Cyclophosphamide 10 mg)	133.73 (1.55)	115.40 (1.86)[b*]
TC (PHF 1500 mg)	127.90 (1.29)	266.70 (1.19)[c***]
T1 (CP 10 mg + PHF 1500 mg)	129.06 (1.98)	269.63 (1.56)[d***]
T2 (PHF 1500 mg + CP 10 mg)	128.96 (1.49)	262.13 (1.84)[e***]
	p=0.423	p<0.001

a=I vs II, b= I vs III, c= I vs IV, d= I vs V, e= I vs VI *p= 0.02, ***p<0.001 . NC- Normal control, PC- Positive control, CC-Cyclophosphamide control, TC-Treatment control, T- Treatment

Table 3: Serum Concentration of IL-10 at Pre- and Post- Treatment with aqueous extract of PHF

Grouping & Dose (kg b. w) (N = 24)	IL-10 (pg/ml)	
	Pre-treatment Mean (SD)	Post-treatment (Mean SD)
NC (Normal saline 10 ml)	1015.6 (1.51)	1028.3 (1.64)
PC (Levamisole Hydrochloride 50 mg)	1028.7 (1.90)	1034.0 (1.28)
CC (Cyclophosphamide 10 mg)	1065.0 (1.47)	1028.0 (1.51)
TC (PHF 1500 mg)	1013.1 (1.44)	1041.3 (1.83)
T1 (CP 10 mg + PHF 1500 mg)	1009.0 (1.12)	1086.6 (1.60)
T2 (PHF 1500 mg + CP 10 mg)	1019.5 (1.12)	1005.4 (1.02)
	p=0.203	p=0.87

NC- Normal control, PC - Positive control, CC -Cyclophosphamide control, TC -Treatment control, T - Treatment

Discussion

A substance that can stimulate or suppress the immunne system is referred to as an immunomodulator. The use of these immunomodulators to control disease conditions is widely accepted globally (Nfambi *et al.,* 2015). Therefore, regulation of the normal function of the immune system depends on the balance between stimulation and suppression of immune cells (Bagwan *et al.,* 2017). Checking this balance regularly is desirable depending on the interest. However, the immune system can be modulated to improve disease conditions which generate research interest (Tripathi, 2012).

This study demonstrated a significant increase in serum levels of IFN-γ and IL-4 in the samples collected after treatment with PHF (i.e., post-treatment samples) across the groups. However, no significant difference was observed in IL-10 concentration in the post-treatment sample. The observed changes may be attributed to the effect of the PHF. This suggests that CP's immunosuppressive effect on IFN-γ and IL-4 level was neutralised by the PHF. This is an indication that the PHF has a protective and/or therapeutic tendency against the CP effect on the immune cells responsible for the secretion of these cytokines. Some formulations have been reported to stimulate the desired immunological response (Sulaiman *et al,* 2010).

In comparison between Treatment groups (i.e., T1 and T2) and positive control and/or treatment control groups, no significant difference was observed. The indifference is due to the potential role of the PHF in neutralizing the effect of CP, thus rendering the activity of the drug ineffective. This emphasizes the immunostimulatory activity of the formulation on immune cells responsible for the secretion of IFN-γ and IL-4 cytokines. Further study will be required to provide information on the specific cells responsible for the cytokine secretion. It also, implies that the formulation possesses phytoconstituents can stimulate immune cells.

Several plant constituents can induce the secretion of cytokines. For instance, saponins stimulate lymphocytes and IFN-γ (Cho *et al.,* 2002). Polysaccharides in herbs have been proven to possess a potent immunostimulating effect by significantly increasing IFN-γ production in mice (Li *et al.,* 2003). Flavonoid, phenol, and glycoside improve immunity and augments both cell-mediated and humoral immune responses (Chiang *et al.,* 2003). Likewise, secondary metabolites like flavonoid, phenol, and glycoside enhance IFN-γ production, inhibits apoptosis, enhance NK cell activity and promote antibody-dependent cell-mediated cytotoxicity (Ganju *et al.,*2003). Phytophenols enhance the IFN-γ levels (Murali and Kuttan, 2016). Quercetin a flavonoid has been reported to reduce IL-4 and increased IFN-γ concentration in bronco-alveolar lavage fluid (BALF) of mice (Park *et al.,* 2009).

We reported the presence of secondary metabolites including alkaloids, terpenoids,

steroids, cardiac glycosides, flavonoids, saponins, tannins, polyphenols, carbohydrates, phytosterols, proteins, and vitamins in the aqueous extract of this polyherbal formulation previously (Isah *et al.,* 2020). Therefore, these observed biological activities may be attributed to some of the above metabolites.

Our findings corroborate that of Jin *et al.* (1994), which reported that aqueous extract of hi-ka-ron a PHF induce an increase in IFN-γ production, and that of Shin *et al.* (2003) who reported that a PHF called PG 201 increase IL-4 production. Contrastingly Wang *et al.* (2005), reported that aqueous extract of Shen-fu-tang a PHF decreases IFN-γ production. Also, Kurokawa *et al.* (2002), also reported contrary findings.

The IFN-γ is regarded as a proinflammatory cytokine, associated with Th1 cells stimulating macrophage function and cytotoxic T-cell function (Saroj *et al.,* 2012). IFN-γ is one of the most vital cytokines that induced immune shift toward Th1 (Zhu and Paul, 2010) and plays a crucial role in clearing pathogens and preventing allergic inflammation (Schroder *et al.,* 2004). IL-4 is the signature cytokine associated with Th2 helper T cell subtypes that are linked to antibody responses. It has been suggested that specialized T cells that express NK cell markers and secrete large amounts of IL-4 immediately after T cell receptor stimulation produce the IL-4 that promotes Th2 cell differentiation (Mathers and Cuff, 2004).

The indifference in serum concentration of IL-10 as revealed by this study implies that the PHF has no effect on the cells responsible for the secretion of the cytokine. This study findings are in line with that of Jeong *et al.* (2003), which reported that an aqueous extract of PHF called Jeo-Dang-Tang decrease or stabilized IL-10 production. However a study by Luo *et al.* (2004), disagreed with our findings, it reported that aqueous extract of PHF (Qing-huo-bai-du-yin) induces an increase in IL-10 production. Administration of CP can damage the Th1/Th2 balance, induce a reduction in the absolute counts of T cells and B cells (Yu *et al.,* 2015), and causes a decrease in the lineage of blood cells and their functional products including cytokines (Yang *et al.,* 2018). Interleukin-10 is an important regulatory cytokine that is produced by almost every immune cell, limiting excessive immune responses in both cell-mediated and humoral immune responses, and it inhibits the effects of pro-inflammatory cytokines such as IL-2, IL-4, and IFN-γ (Ng *et al.,* 2013). It displays both immunosuppressive and immunostimulating activities (Mannino *et al.,* 2015).

Conclusion

There is a significant increase in serum concentration of IFN-γ and IL-4 among the immunosuppressed Wistar albino rats treated with PHF. This indicates that the PHF has neutralise the immunosuppressive condition and induced immunostimulatory activity on the immune cells responsible for the secretion of the cytokines. There is no significant increase in serum concentration of IL-10 among the immunosuppressed Wistar albino rats treated with PHF. Therefore, no significant immunomodulatory activity on the cytokine.

Acknowledgments

We sincerely appreciate the contribution of Mr. Sani Muhammad Umar (Nagge) of Al Mustakshif Medical Health Centre, Kano, Nigeria (RC: 1393615) for the Garjin® PHF. We likewise appreciate the exertion of Mr. Aliyu Abubakar Garba, Mr. Abdullahi Suleiman, Mr. Abdulrasheed Ahmad, and Mr. Salmanu Makama from the Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto, Nigeria, for the technical help.

Conflict of Interest

The authors declare no conflict of interest

Funding

This project has no funding

References

Alamgir, M. and Uddin, S.J. (2010). Recent advances on the ethnomedicinal plants as immunomodulatory agents. Ethnomedicine: a source of Complementary Therapeutics; 37: 2.

Arreola, R., Quintero-Fabián, S., López-Roa, R.I., *et al.* (2015). Immunomodulation and anti-inflammatory effects of garlic compounds. Journal of Immunology

Research; 2015: . 401630

Bagwan, S., Naikwade, N. and Manure, J. (2017). Comparative Study of Immunomodulatory Activity of Marketed Ayurvedic Formulation. Indo American Journal of Pharmaceutical Sciences; 4: 4776.

Barik, C., Kanungo, S., Tripathy, N., *et al.* (2015). A review on therapeutic potential of polyherbal formulations. International Journal of Pharmaceutical Sciences and Drug Research; 7: 211-228.

Bekisz, J., Sato, Y., Johnson, C., *et al.* (2013). Immunomodulatory effects of interferons in malignancies. Journal of Interferon and Cytokine Research; 33: 154-161.

Bhatt, L., Sebastian, B. and Joshi, V. (2017). Mangiferin protects rat myocardial tissue against cyclophosphamide induced cardiotoxicity. Journal of Ayurveda and Integrative Medicine; 8: 62-67.

Catanzaro, M., Corsini, E., Rosini, M., *et al.* (2018). Immunomodulators inspired by nature: a review on curcumin and echinacea. Molecules (Basel, Switzerland); 23: 2778.

Cecílio, A.B., De Faria, D.B., De Carvalho Oliveira, P., *et al.* (2012). Screening of Brazilian medicinal plants for antiviral activity against rotavirus. Journal of Ethnopharmacology; 141: 975-981.

Chiang, L.-C., Ng, L.T., Chiang, W., et al. (2003). Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. Planta Medica; 69: 600-604.

Cho, J.Y., Kim, A.R., Yoo, E.S., *et al.* (2002). Ginsenosides from Panax ginseng differentially regulate lymphocyte proliferation. Planta Medica; 68: 497-500.

Choi, J., Kim, T.-H., Choi, T.-Y., *et al.* (2013). Ginseng for health care: a systematic review of randomized controlled trials in Korean literature. PloS One; 8: e59978.

Das, S., Bordoloi, R. and Newar, N. (2014). A review on immune modulatory effect of some traditional medicinal herbs. Journal of Pharmaceutical, Chemical and Biological Sciences; 2: 33-42.

Ganju, L., Karan, D., Chanda, S., *et al.* (2003). Immunomodulatory effects of agents of plant origin. Biomedicine and Pharmacotherapy; 57: 296-300.

Gberikon, G., Adeoti, I. and Aondoackaa, A. (2015). Effect of ethanol and aqueous solutions as extraction solvents on phytochemical screening and antibacterial activity of fruit and stem bark extracts of Tetrapleura tetraptera on Streptococcus salivarus and Streptococcus mutans. International Journal of Current Microbiology and Applied Sciences; 4: 404-410.

Hamid, K.M., Isah, S.Y., Kalgo, M.U., *et al.* (2021). Immunostimulatory 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; 7: 46-70

Hajra, S., Mehta, A. and Pandey, P. (2012). Immunostimulating activity of methanolic extract of Swietenia mahagoni seeds. International Journal of Pharmacy and Pharmaceutical Sciences; 4: 442-445.

Isah, S.Y., Mohammed, H., Hamid, K.M., *et al.* (2018). Effect of Polyherbal Formulation on Neutrophil function and TDAR in Wistar albino rat. European Journal of Pharmaceutical and Medical Research; 5: 563-568.

Isah, S.Y., Hamid, K.M., Muhammad, H.Y., *et al.* (2020). Phytochemical Screening, Acute Toxicity Study and Immunomodulatory Activity of Aqueous Extract of Polyherbal Formulation in Albino Wistar Rats. World Journal of Pharmaceutical Research; 9: 165-177.

Jeong, H.-J., Chung, H.-S., An, H.-J., *et al.* (2003). Immune-enhancement effect of the herbal combination Allergina. Clinica Chimica Acta; 337: 77-84.

Jin, R., Wan, L.L., Mitsuishi, T., *et al.* (1994). Effect of shi-ka-ron and Chinese herbs on cytokine production of macrophage in immunocompromised mice. The American Journal of Chinese Medicine; 22: 255-266.

Kokkonen, H., Söderström, I., Rocklöv, J., et al. (2010). Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis and Rheumatism; 62: 383-391.

Kumar, D., Arya, V., Kaur, R., *et al.* (2012). A

review of immunomodulators in the Indian traditional health care system. Journal of Microbiology, Immunology and Infection; 45: 165-184.

Kumar, S., Sharma, G., Sidiq, T., *et al.* (2014). Immunomodulatory potential of a bioactive fraction from the leaves of Phyllostachys bambusoides (Bamboo) in BALB/C mice. EXCLI Journal; 13: 137.

Kurokawa, M., Tsurita, M., Brown, J., et al. (2002). Effect of interleukin-12 level augmented by Kakkon-to, a herbal medicine, on the early stage of influenza infection in mice. Antiviral Research; 56: 183-188.

Kyakulaga, A., Ogwang, P., Obua, C., et al. (2013). Immunomodulatory effects of aqueous extracts of auricularia sp and pleurotus sp mushrooms in cyclophosphamide-immunosuppressed wistar rats. Journal of Pharmaceutical Research International: 3: 662-670.

Li, R.W., Myers, S.P., Leach, D.N., et al. (2003). A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. Journal of Ethnopharmacology; 85: 25-32.

Luo, C.-Q., Zhou, J.-D. and Chen, D.-J. (2004). Effect of Chinese traditional medicine mixture on inflammatory response in rats with severe burn. Zhong nan da xue xue bao. Yi xue ban= Journal of Central South University. Medical Sciences; 29: 32-34.

Mannino, M.H., Zhu, Z., Xiao, H., et al. (2015). The paradoxical role of IL-10 in immunity and cancer. Cancer Letters; 367: 103-107.

Mathers, A.R. and Cuff, C.F. (2004). Role of interleukin-4 (IL-4) and IL-10 in serum immunoglobulin G antibody responses following mucosal or systemic reovirus infection. Journal of Virology; 78: 3352-3360.

Murali, V.P. and Kuttan, G. (2016). Curculigoside augments cell-mediated immune responses in metastatic tumor-bearing animals. Immunopharmacology and Immunotoxicology; 38: 264-269.

Nfambi, J., Bbosa, G.S., Sembajwe, L.F., *et al.* (2015). Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats. Journal of Basic and Clinical Physiology and Pharmacology; 26: 603-611.

Ng, T.H., Britton, G.J., Hill, E.V., *et al.* (2013). Regulation of adaptive immunity; the role of interleukin-10. Frontiers in Immunology; 4: 129.

Oshikoya, K.A., Oreagba, I.A., Ogunleye, O.O., *et al.* (2013). Herbal medicines supplied by community pharmacies in Lagos, Nigeria: pharmacists' knowledge. Pharmacy Practice; 11: 219.

Park, H.-J., Lee, C.-M., Jung, I.D., *et al.* (2009). Quercetin regulates Th1/Th2 balance in a murine model of asthma. International Immunopharmacology; 9: 261-267.

Petchi, R.R., Parasuraman, S., Vijaya, C., *et al.* (2015). Antiarthritic activity of a polyherbal formulation against Freund's complete adjuvant induced arthritis in Female Wistar rats. Journal of Basic and Clinical Pharmacy; 6: 77.

Saroj, P., Verma, M., Jha, K., *et al.* (2012). An overview on immunomodulation. Journal of Advanced Scientific Research; 3: 7-12.

Schroder, K., Hertzog, P.J., Ravasi, T., *et al.* (2004). Interferon-γ: an overview of signals, mechanisms and functions. Journal of Leukocyte Biology; 75: 163-189.

Shaista, K. and Ahmad, D. (2011). Opportunities and potentials for herbal pharmaceutical products development in Pakistan. Managing Natural Resources for Sustaining Future Agriculture; 2: 1-14.

Shin, S., Jin, M., Jung, H., *et al.* (2003). Suppressive effects of PG201, an ethanol extract from herbs, on collagen-induced arthritis in mice. Rheumatology; 42: 665-672.

Spelman, K., Burns, J., Nichols, D., et al. (2006). Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. Alternative Medicine Review; 11: 128.

Subhasree, N., Kamella, A., Kaliappan, I., et al. (2015). Antidiabetic and antihyperlipidemic activities of a novel polyherbal formulation in high fat diet/streptozotocin induced diabetic rat model. Indian Journal of Pharmacology; 47: 509.

Sulaiman, S., Rajashekhar, G., Prakash, P., et al. (2010). Immunoprophylactic activity of immunol, a polyherbal formulation against dexamethasone induced

immunosuppression in rats. Journal of Pharmacology and Toxicology; 5: 275-287.

Tripathi, S. (2012). Immunomodulatory property of ethanolic extract of Trigonella foenum-graeceum leaves on mice. Scholar Research library; 4: 708-713.

Vinothapooshan, G. and Sundar, K. (2011). Immunomodulatory activity of various extracts of Adhatoda vasica Linn. in experimental rats. African Journal of Pharmacy and Pharmacology; 5: 306-310.

Wang, S.-Y., Chen, P.-F. and Chang, S.-T. (2005). Antifungal activities of essential oils and their constituents from indigenous cinnamon (Cinnamomum osmophloeum) leaves against wood decay fungi. Bioresource Technology; 96: 813-818.

Yang, H., Kim, M., Kang, H., *et al.* (2018). Immunomodulating properties of Polygonum multiflorum extracts on cyclophosphamide-induced immunosuppression model. Indian Journal of Pharmaceutical Sciences; 80: 749-755.

Ye, L., Jia, Y., Ji, K., *et al.* (2015). Traditional Chinese medicine in the prevention and treatment of cancer and cancer metastasis. Oncology Letters; 10: 1240-1250.

Yu, Q., Nie, S.-P., Wang, J.-Q., *et al.* (2015). Molecular mechanism underlying chemoprotective effects of Ganoderma atrum polysaccharide in cyclophosphamide-induced immunosuppressed mice. Journal of Functional Foods; 15: 52-60.

Zhu, J. and Paul, W.E. (2010). Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. Immunological Reviews; 238: 247-262.

Zizka, A., Thiombiano, A., Dressler, S., *et al.* (2015). Traditional plant use in Burkina Faso (West Africa): a national-scale analysis with focus on traditional medicine. Journal of Ethnobiology and Ethnomedicine; 11: 1-10.

Citation: Hamid, K.M., Abubakar, N.K., Yeldu M.H., Abdullahi, M.I., Usman A.B., Isah, S.Y., Aliyu, M. Immunomodulation of IFN-γ, IL-4 and IL-10 Cytokines by Polyherbal Formulation in Cyclophosphamide-Induced Immunosuppression among Wistar Rats. ***Sokoto Journal of Medical Laboratory Science; 7(2):5 - 13.*** https://dx.doi.org/10.4314/sokjmls.v7i2.1