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Immunomodulatory effects of Hydromethanolic Extract of Beta vulgaris Lon White Blood Cell Profile and Selected Immunoglobulins in Male Wistar Rats

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ABSTRACT
This study evaluated the immunomodulatory effects of hydromethanolic extract of Beta vulgaris L. (HMEBV) on total white blood cell (WBC) count, differential WBC count, total lymphocyte count, and selected immunoglobulin levels in male Wistar rats. Rats were divided into nine groups, receiving various treatments: HMEBV at doses of 150, 250, and 500 mg/kg body weight (BW); levamisole (40 mg/kg BW); cyclophosphamide (30 mg/kg BW); and combinations of HMEBV with cyclophosphamide.
Administration of 40 mg/kg BW levamisole significantly ($p<0.05$) increased total WBC and lymphocyte counts compared to control. Cyclophosphamide (30 mg/kg BW) led to significant reductions in total WBC count, neutrophil percentage, and lymphocyte count. HMEBV at 150 mg/kg and 500 mg/kg BW showed no significant changes in hematological parameters. However, 250 mg/kg BW HMEBV significantly ($p<0.05$) elevated total WBC count, monocyte percentage, and lymphocyte count. Combination treatments of cyclophosphamide with 150 mg/kg or 500 mg/kg BW HMEBV resulted in significant decreases in total WBC and lymphocyte counts. Notably, the combination of 250 mg/kg BW HMEBV with cyclophosphamide, while showing a decrease relative to control, significantly increased total WBC and lymphocyte counts compared to cyclophosphamide-only treatment.
Regarding immunoglobulins, levamisole significantly increased IgA and IgM levels. Cyclophosphamide reduced IgA, IgG, and IgM levels. HMEBV at 150 mg/kg and 500 mg/kg BW had no significant effect on immunoglobulin levels, whereas 250 mg/kg BW HMEBV significantly elevated IgA, IgG, and IgM levels. The combination of 250 mg/kg BW HMEBV with cyclophosphamide significantly increased IgG and IgM levels compared to both control and cyclophosphamide groups, with no effect on IgA. The 150 mg/kg combination group showed a significant increase in IgM and a decrease in IgG relative to control, with no significant effect on IgA. The 500 mg/kg combination group showed a significant decrease in IgA and IgG, but a significant increase in IgM compared to cyclophosphamide.
These findings suggest that HMEBV, particularly at 250 mg/kg BW, exhibits immunostimulatory properties by enhancing leukocyte profiles and immunoglobulin levels, and may partially mitigate cyclophosphamide-induced immunosuppression.
Keywords: Beta vulgaris L., Hydromethanolic extract, Immunomodulation, White Blood Cells, Immunoglobulins, Cyclophosphamide, Levamisole

I. BACKGROUND OF THE STUDY

The immune system is a complex network of organs, cells, and molecules that orchestrates the defense against invading pathogens while maintaining tolerance to self-antigens. A well-functioning immune system is dependent on the balance and integrity of various immune cells, notably White blood cells (WBCs) and immunoglobulins, which serve as key markers of immune competence and systemic health (Turvey & Broide, 2010). Disruptions in the normal levels or function of these components can result in increased susceptibility to infections, chronic inflammation, or autoimmune conditions.

The immune system plays a pivotal role in defending the body against pathogens, maintaining homeostasis, and preventing the onset of diseases, including cancer and autoimmune disorders (Sattler, 2017). WBCs, also known as leukocytes, are the key components of the immune system and are classified into granulocytes (neutrophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes). Their levels and differentials are vital indicators of immune status and physiological response to infections,

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inflammation, and exposure to therapeutic or toxic agents (Kumar et al., 2020).

In recent decades, there has been a resurgence of interest in plant-based therapeutics as complementary or alternative strategies for immune modulation. Among these, Beta vulgaris has emerged as a promising functional food and medicinal plant due to its rich phytochemical composition.Beta vulgaris, commonly known as beetroot, is a root vegetable belonging to the Amaranthaceae family. It has a long history of use in traditional medicine and nutrition due to its rich content of bioactive compounds such as betalains, flavonoids, polyphenols, ascorbic acid, and nitrates (Clifford et al., 2015; Kanner et al., 2001). These compounds exhibit strong antioxidant, anti-inflammatory, hepatoprotective, and chemopreventive properties (Wootton-Beard & Ryan, 2011; Georgiev et al., 2010).Beetroot is particularly abundant in betalains, including betacyanins and betaxanthins, which are responsible for its characteristic red-purple coloration and are associated with anti-inflammatory, antioxidant, and chemopreventive effects (Kujala et al., 2002). Additionally, beetroot contains essential nutrients such as folate, vitamin C, manganese, potassium, and dietary nitrates, which contribute to its broad health benefits (Clifford et al., 2015).

Among various approaches aimed at modulating the immune system, the use of natural plant-derived compounds has gained increased attention due to their safety profile, cost-effectiveness, and wide spectrum of bioactivities

Several studies have highlighted the potential of beetroot in modulating hematological parameters and enhancing immune function. Betalains, in particular, have been reported to exert immunomodulatory effects through the inhibition of inflammatory cytokines and the promotion of lymphocyte activity (Tesoriere et al., 2004). Furthermore, beetroot's polyphenolic content is known to scavenge free radicals, thereby protecting immune cells from oxidative damage (Neelwarne & Halagur, 2013). However, while the antioxidant and cardiovascular benefits of Beta vulgaris are well-documented, there remains a paucity of data on its direct influence on leukocyte dynamics and humoral immunity, especially regarding immunoglobulin production and differential WBC counts in controlled animal models.

Preliminary studies suggest that beetroot and its extracts may enhance lymphocyte proliferation, modulate cytokine production, and support the antioxidant defense systems that are critical for immune cell survival (Pavlov et al., 2002; Tesoriere et al., 2004). However, there is a need for more rigorous experimental data to confirm and extend these observations, particularly in animal models that simulate human physiological conditions.

In view of the growing interest in natural immunomodulators and the increasing burden of diseases associated with immune dysfunction, it is pertinent to explore and document the immunological effects of beetroot extracts. The hydromethanolic extraction method, which preserves both polar and non-polar phytoconstituents, offers a comprehensive profile of the plant's active compounds and is thus suitable for assessing its full biological potential (Sasidharan et al., 2011).

This study aims to investigate the immunomodulatory effect of hydromethanolic extract of Beta vulgaris on total and differential WBC counts as well as selected immunoglobulins in male Wistar rats. Through a comprehensive analysis of hematological and immunological markers, the study seeks to contribute valuable insights into the therapeutic potential of beetroot in supporting and enhancing immune function. Such findings could pave the way for the development of natural, plant-based immunotherapeutics with minimal adverse effects.

Materials and Method

Study Design

This experimental study employed a completely randomized design to investigate the effect of hydromethanolic extract of Beta vulgaris on White Blood cell profile and selected immunoglobulin levels in male Wistar rats.

Plant Material and Extract Preparation

Fresh tubers of Beta vulgaris (beetroot) were obtained from a local fruit market in Port Harcourt, Nigeria and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Nigeria, where a voucher specimen was deposited. The tubers were washed, sliced, and shade-dried at room temperature for 7–10 days. The dried samples were ground into fine powder using a mechanical blender.The hydromethanolic extract was prepared by soaking 500 grams of the powdered plant material in 2.5 liters of 70% methanol for 72 hours with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate

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was concentrated using a rotary evaporator under reduced pressure at 40°C. The semi-solid extract was stored in airtight containers at 4°C until use. The yield was calculated and expressed as a percentage of the initial dry weight.

Experimental Animals

Forty-five (45) healthy adult male Wistar rats weighing between 150–200 g were obtained from the Animal House of Department of Human Physiology, Faculty of Basic Medical Science, University of Port Harcourt, Choba, Nigeria. The rats were housed in clean, well-ventilated cages under standard laboratory conditions (12-hour light/dark cycle, temperature of 22–25°C, relative humidity 50–70%) and were acclimatized for 14 days before the experiment. They were provided with standard rat pellets and water ad libitum.

The experimental protocol was approved by the Institutional Animal Ethics Committee of University of Port Harcourt, Choba, Nigeria, and all procedures followed the guidelines for the care and use of laboratory animals as outlined by the National Institutes of Health (NIH, 2011).

Experimental Design

The animals were randomly divided into nine (9) groups (n = 5 per group) as follows:
Group 1 (Control): received 1 mL of distilled water daily.
Group 2: 40 mg/kg BW Levamisole
Group 3: 30 mg/kg BW cyclophosphamide (CPM)
Group 4 (Low dose): 150 mg/kg BW of the extract (Low dose)
Group 5: 250 mg/kg BW of the extract (Medium dose)
Group 6: 500 mg/kg BW of the extract (High dose)
Group 7: 150 mg/kg BW of the extract (Low dose) + 30 mg/kg BW cyclophosphamide (CPM)
Group 8: 250 mg/kg BW of the extract (Medium dose) + 30 mg/kg BW cyclophosphamide (CPM)
Group 9: received 500 mg/kg BW of the extract (High dose) + 30 mg/kg BW cyclophosphamide (CPM).
After administration of the extract for 28 days, 30 mg/kg BW cyclophosphamide (CPM) was administered for 3 days. All treatments were administered orally via gavage once daily.

Sample Collection

At the end of the treatment period, the animals were fasted overnight and anesthetized using intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood samples were collected via cardiac puncture into EDTA-containing tubes for hematological analysis and plain tubes for serum preparation.

Hematological Analysis

Total and differential white blood cell (WBC) counts were determined using an automated hematology analyzer (e.g., Sysmex KX-21N) according to the manufacturer's instructions. The differential count included neutrophils, lymphocytes, monocytes and eosinophils.

Immunoglobulin Assay

Serum levels of selected immunoglobulins (IgA, IgG and IgM) were quantified using enzyme-linked immunosorbent assay (ELISA) kits (e.g., from Elabscience or Thermo Fisher) following the manufacturer's protocols. Absorbance was read using a microplate reader at 450 nm.

Phytochemical Screening

Preliminary qualitative phytochemical screening of the extract was conducted to identify the presence of bioactive constituents such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics using standard procedures described by Harborne (1998).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 25.0. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences between groups. A p-value < 0.05 was considered statistically significant.

II. RESULTS

Table 1 showed the effect of the Hydromethanolic extract of Beta Vulgaris L (beetroot) on Total WBC Count, Differential WBC count and Total lymphocyte count. When compared to the control (group 1), 40 mg/kg BW Levamisole (group 2) resulted a significant ($p<0.05$) increase in the total white blood cell count and total lymphocyte count. Group 3 (30 mg/kg BW Cyclophosphamide) resulted in a significant ($p<0.05$) decrease in Total WBC Count, percent neutrophils and total lymphocyte count in comparison with the control. There was no significant difference observed in the administration of 150 mg/kg BW HMEBV (Group 4). Administration of 250 mg/kg BW of the extract (Group 5) showed a significant ($p<0.05$) increase in

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Total WBC Count, percent monocytes and Total lymphocyte count when compared with the control group. There was no significant difference observed in the administration of 500 mg/kg BW HMEBV (Group 6). A combination therapy of administration of 150 mg/kg BW HMEBV and 30 mg/kg BW Cyclophosphamide resulted in a significant ($p<0.05$) decrease in Total WBC Count and Total lymphocyte count when compared to the Control. On the other hand, the combination therapy of administration of 250 mg/kg BW HMEBV and 30 mg/kg BW Cyclophosphamide resulted in a significant ($p<0.05$) decrease in Total WBC Count and Total lymphocyte count when compared to the Control but a significant ($p<0.05$) increase in Total WBC Count and Total lymphocyte count when compared to group 3. Also, the combination therapy of administration of 500 mg/kg BW HMEBV and 30 mg/kg BW Cyclophosphamide resulted in a significant ($p<0.05$) decrease in Total WBC Count, percent neutrophils and Total lymphocyte count when compared to the Control.

Table 2 showed the effect of the Hydromethanolic extract of Beta Vulgaris L (beetroot) on selected immunoglobulins in Wistar rats. In comparison with the normal control (group 1), 40 mg/kg BW Levamisole (group 2) showed significant ($p<0.05$) increase in IgA and IgM with a slight increase, albeit not significant in IgG. The negative control (group 3) administered with 30 mg/kg BW Cyclophosphamide resulted in a significant ($p<0.05$) decrease in the levels of IgA, IgG and IgM. There was no significant ($p<0.05$) difference observed in group 4 (150 mg/kg BW of the extract) when compared to the normal control group. Group 5 (250 mg/kg BW HMEBV) showed significant ($p<0.05$) increase in the levels of IgA, IgG and IgM when compared with the normal control group. Similar to what was observed in the low dose group of the extract (150 mg/kg BW HMEBV), the high dose (group 6) administered 500 mg/kg BW of the extract had no significant ($p<0.05$) effect on IgA, IgG and IgM as compared to the normal control group. Group 7 which was a combination therapy of the immunosuppressant (30 mg/kg BW Cyclophosphamide) and low dose of the extract (150 mg/kg BW HMEBV), showed a significant ($p<0.05$) decrease in the level of IgG when compared to the normal control and a slight (not significant) increase when compared to the negative control, with a significant ($p<0.05$) increase in IgM in comparison with both the normal and negative control groups. However, no significant difference was observed in the levels of IgA. Group 8 (a combination of 250 mg/kg BW HMEBV and 30 mg/kg BW Cyclophosphamide) showed a significant ($p<0.05$) increase in IgG and IgM when compared to both normal and negative controls but no significant difference in the levels of IgA. The combination of 500 mg/kg BW HMEBV and 30 mg/kg BW Cyclophosphamide in Group 9 resulted in a significant ($p<0.05$) decrease in IgA and IgG when compared to the normal control, with a slight (not significant) increase when compared with the negative control; however, there was a significant ($p<0.05$) increase in IgM when compared to the negative control (group 3).

Table 1:Effect of the Hydromethanolic extract of Beta Vulgaris L (beetroot) on Total WBC Count, Differential WBC count and Total lymphocyte count (TCL) in Wistar rats

Groups	**Parameters**					
	WBC ($X10^9$/L)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	TLC ($X10^9$/L)
Group 1 (Negative Control)	15.30±2.95	11.67±0.88	82.67±1.45	1.33±0.33	3.00±0.58	12.67±1.99
Group 2 (40mg/kg BW Levamisole)	18.97±1.89[a]	13.00±0.58	85.67±1.20	2.00±1.00	3.67±1.20	16.25±1.50[a]
Group 3 (30mg/kg BW CPM)	5.37±0.30[a]	6.33±1.20[a]	80.67±1.76	1.33±0.33	2.67±0.33	4.33±1.12[a]
Group 4 (150 mg/kg HMEBV)	13.13±2.14	9.33±1.76	82.67±1.45	2.33±0.88	4.67±0.88	10.85±0.65

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Group 5 (250 mg/kg HMEBV)	19.33±0.84[a]	12.33±1.45	85.33±3.84	2.67±0.67	5.33±2.33[a]	16.49±1.89[a]
Group 6 (500 mg/kg HMEBV)	13.17±2.33	10.00±1.16	84.67±1.45	2.00±0.58	3.33±0.88	11.15±1.85
Group 7 (150 mg/kg HMEBV + CPM)	6.67±0.99[a]	8.67±1.20	86.33±0.88	1.33±0.33	2.67±0.33	5.75±0.33[a]
Group 8 (250 mg/kg HMEBV + CPM)	9.47±1.11[a]*	9.67±0.88	87.33±1.45	1.33±0.33	3.67±0.88	8.27±0.86[a]*
Group 9 (500 mg/kg HMEBV + CPM)	7.23±1.79[a]	7.67±1.45[a]	88.33±2.40	2.00±1.58	3.33±0.88	6.38±1.55[a]

Values expressed as mean ± standard error of the mean; [a] significant difference compared to control; *significant difference compared to group 3 ($p<0.05$)

TABLE 2: Effect of the Hydromethanolic extract of Beta Vulgaris L (beetroot) on Selected Immunoglobulins in Wistar rats

Groups	**Immunoglobulins (mg/dl)**		
	IgA	IgG	IgM
Group 1 (Negative Control)	1.25±0.14	7.33±0.88	0.63±0.14
Group 2 (40mg/kg BW Levamisole)	2.20±0.70[a]	7.43±0.75	1.23±0.10[a]
Group 3 (30mg/kg BW CPM)	0.88±0.04[a]	5.67±0.20[a]	0.44±0.02[a]
Group 4 (150 mg/kg HMEBV)	0.97±0.09	6.63±0.35	0.58±0.04
Group 5 (250 mg/kg HMEBV)	2.46±0.86[a]	14.0±4.62[a]	0.87±0.42[a]
Group 6 (500 mg/kg HMEBV)	0.99±0.05	6.53±0.20	0.51±0.02
Group 7 (150 mg/kg HMEBV + CPM)	1.61±0.41	6.10±0.38[a]	0.97±0.28[a*]
Group 8 (250 mg/kg HMEBV + CPM)	1.05±0.05	9.17±0.84[a*]	2.67±1.88[a*]
Group 9 (500 mg/kg HMEBV + CPM)	0.90±0.05[a]	5.80±0.21[a]	0.82±0.02[a*]

Values expressed as mean ± standard error of the mean; [a] significant difference compared to control; *significant difference compared to group 3 ($p<0.05$)

III. DISCUSSION

The determination of total leucocyte (WBC) and differential count are important markers of immune function. In this study, the total WBC counts of the Hydromethanolic extract of Beta vulgaris L-treated rats significantly ($p < 0.05$) increased relative to the negative control (30 mg/kg BW Cyclophosphamide). The administration of cyclophosphamide significantly reduced the Total WBC Counts, percent neutrophils and total lymphocyte count in comparison with the control, consequently validating the efficacy of cyclophosphamide has an immunosuppressive drug. The observed reduction in Total leukocyte count could be attributed to the presence of Phosphoramide, an alkylating agent produced

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through the bioactivation of cyclophosphamide (Chighizola et al., 2011). This corresponds with literature which confirms that cyclophosphamide suppresses bone marrow function, leading to a reduction in white blood cells (Ding et al., 2021; Mérida et al., 2018; Huyan et al., 2011). This is further consistent with its well-documented mechanism of action, where it induces myelosuppression and lymphocyte depletion, thereby reducing immune function (Emadi et al., 2009). The administration of levamisole on the other hand, a known immunostimulant, significantly increased total WBC and lymphocyte counts ($p<0.05$), confirming its capacity to stimulate the immune system through enhanced lymphocyte proliferation and leukocyte mobilization (Abbas et al., 2018).

The medium dose of HMEBV (250 mg/kg BW) produced a significant increase in total WBC count, percent monocytes, and lymphocyte count, suggesting a potential immunostimulatory effect at this concentration. This finding is supported by earlier reports that beetroot extracts may enhance innate and adaptive immunity through their antioxidant and anti-inflammatory properties (Wootton-Beard & Ryan, 2011). However, the lack of significant changes at both lower (150 mg/kg BW) and higher doses (500 mg/kg BW) indicates a possible biphasic dose-response curve. This phenomenon, known as hormesis, is not uncommon in natural products, where low to moderate doses exert beneficial effects while higher doses may be less effective or even suppressive (Calabrese, 2008).

The combination of 150 mg/kg HMEBV with cyclophosphamide (group 7) resulted in a significant decrease in total WBC and lymphocyte counts, similar to cyclophosphamide alone, indicating no protective or stimulatory effect at this dose. In contrast, the combination of 250 mg/kg HMEBV with cyclophosphamide (group 8) still led to a significant reduction in WBC and lymphocyte counts when compared to control, but showed a significant improvement over cyclophosphamide alone (group 3). This suggests that at this dose, HMEBV may partially attenuate the immunosuppressive effects of cyclophosphamide, potentially through its antioxidant properties, which can mitigate drug-induced oxidative damage to bone marrow progenitor cells (Kapadia et al., 2013). However, the highest dose (500 mg/kg BW) did not confer such protective effects, as the combination still significantly suppressed total WBC, neutrophils, and lymphocyte counts. This further supports the hypothesis of a non-linear dose-response and may imply that at higher concentrations, beetroot's active compounds might exert inhibitory or toxic effects on immune cell production or function (Pisoschi & Pop, 2015).

Furthermore, the determination of serum concentration of IgA, IgG and IgM showed that hydromethanolic extract of beetroot (HMEBV) exhibits a dose-dependent Immunomodulatory effect, with significant implications for both Immunostimulation and Immunoprotection. Specifically, the 250 mg/kg BW dose showed the most promising immunostimulatory activity, as evidenced by elevated levels of IgA, IgG, and IgM, suggesting an optimal dose window for the bioactive constituents. Beetroot (Beta vulgaris) is rich in a variety of phytochemicals, notably betalains (such as betanin), flavonoids, polyphenols, and nitrates. These compounds have been shown to possess strong antioxidant and anti-inflammatory activities, which are critical in modulating immune responses (Georgiev et al., 2010; Clifford et al., 2015). The elevation in IgA, IgG, and IgM in the 250 mg/kg group (Group 5) can be attributed to the enhancement of B-cell activity and plasma cell differentiation, possibly through modulation of transcription factors such as NF-κB and STAT6, which are known to regulate immunoglobulin production (Ghosh & Hayden, 2008).

The 500 mg/kg dose of HMEBV did not lead to significant increases in immunoglobulins, suggesting a non-linear dose-response relationship. This could be due to an induction of immune tolerance or downregulation of immune responses at higher concentrations. Some plant-derived compounds, especially polyphenols, have been observed to exert biphasic effects on immune cells – stimulating them at lower doses and suppressing activity at higher doses (Huang et al., 2010).

In the cyclophosphamide-induced immunosuppressed model (Group 3), a significant reduction in immunoglobulin levels was observed, consistent with the known mechanism of cyclophosphamide, which induces lymphocyte apoptosis and suppresses B-cell function through DNA crosslinking (Emadi et al., 2009). When co-administered with HMEBV, especially at 250 mg/kg (Group 8), there was a marked increase in IgG and IgM compared to both the negative and normal controls, indicating a protective or restorative effect of the extract on B-cell function. This immunorestorative effect may be due to the antioxidative properties of beetroot's betalains and

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polyphenols, which reduce oxidative stress on hematopoietic progenitor cells in the bone marrow. These antioxidants can modulate intracellular ROS levels, preventing lymphocyte apoptosis and restoring immunoglobulin synthesis pathways (Tesoriere et al., 2004; Pietrzkowski et al., 2010). The variations in individual immunoglobulin class responses suggest selective modulation of specific B-cell subsets or class-switch recombination pathways. Beetroot constituents might differentially influence cytokine environments that favor IgM (e.g., IL-6, IL-10) over IgA or IgG (Snapper et al., 1993).

IV. CONCLUSION

This study demonstrates that the Hydromethanolic extract of Beta vulgaris L. (HMEBV) possesses dose-dependent immunomodulatory properties in male Wistar rats. The 250 mg/kg BW dose notably enhanced total WBC count, lymphocyte levels, and immunoglobulin (IgA, IgG, IgM) concentrations, indicating a potent immunostimulatory and immunoprotective effect. Conversely, both lower (150 mg/kg) and higher (500 mg/kg) doses were less effective, supporting a biphasic dose-response pattern. In cyclophosphamide-induced immunosuppressed models, co-administration of 250 mg/kg HMEBV partially mitigated leukocyte and immunoglobulin suppression, likely through antioxidant-mediated protection of immune cells. These findings suggest that HMEBV, particularly at moderate doses, could serve as a natural immunomodulator with potential applications in immune support and recovery during immunosuppressive therapy.

REFERENCES

[1]. Abbas, A. K., Lichtman, A. H., & Pillai, S. (2018). Cellular and Molecular Immunology. Elsevier Health Sciences.

[2]. Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state. Oxidative Medicine and Cellular Longevity, 3(4), 228–237.

[3]. Calabrese, E. J. (2008). Hormesis: Why it is important to toxicology and toxicologists. Environmental Toxicology and Chemistry, 27(7), 1451–1474.

[4]. Chighizola, C. B., Ong, V. H., Meroni, P. L., & Hughes, G. R. V. (2011). The challenges of cyclophosphamide therapy in systemic lupus erythematosus: Current and future perspectives. Lupus, 20(5), 485–492. https://doi.org/10.1177/0961203310385240

[5]. Clifford, T., Howatson, G., West, D. J., & Stevenson, E. J. (2015). The potential benefits of red beetroot supplementation in health and disease. Nutrients, 7(4), 2801–2822.

[6]. Ding, Y., Li, L., Liu, D., Zhang, Y., Zhou, Y., Zeng, R., & Zhang, J. (2021). Protective effect of polysaccharide from Ganoderma lucidum on cyclophosphamide-induced immunosuppression in mice. International Journal of Biological Macromolecules, 182, 458–466. https://doi.org/10.1016/j.ijbiomac.2021.04.030

[7]. Emadi, A., Jones, R. J., & Brodsky, R. A. (2009). Cyclophosphamide and cancer: golden anniversary. Nature Reviews Clinical Oncology, 6(11), 638–647.

[8]. Esatbeyoglu, T., Wagner, A. E., Schini-Kerth, V. B., & Rimbach, G. (2015). Betanin—a food colorant with biological activity. Molecular Nutrition & Food Research, 59(1), 36–47.

[9]. Georgiev, V. G., Weber, J., Kneschke, E. M., Denev, P. N., Bley, T., & Pavlov, A. I. (2010). Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. Detroit Dark Red. Plant Foods for Human Nutrition, 65(2), 105–111.

[10]. Ghosh, S., & Hayden, M. S. (2008). New regulators of NF-κB in inflammation. Nature Reviews Immunology, 8(11), 837–848.

[11]. Ghosh, S., & Karin, M. (2002). Missing pieces in the NF-κB puzzle. Cell, 109(Supplement), S81–S96.

[12]. Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (3rd ed.). Springer Science & Business Media.

[13]. Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2010). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutrition and Cancer, 62(1), 1–20.

[14]. Kanner, J., Harel, S., & Granit, R. (2001). Betalains—a new class of dietary

cationized antioxidants. Journal of Agricultural and Food Chemistry, 49(11), 5178–5185.

[15]. Kapadia, G. J., Azuine, M. A., Tokuda, H., Takasaki, M., Mukainaka, T., Konoshima, T., & Nishino, H. (2013). Chemopreventive effect of red beetroot (Beta vulgaris) extract against lung and skin cancer. Phytotherapy Research, 27(2), 203–208.

[16]. Kujala, T. S., Loponen, J. M., Klika, K. D., & Pihlaja, K. (2002). Phenolics and betacyanins in red beetroot (Beta vulgaris) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. Journal of Agricultural and Food Chemistry, 50(13), 3640–3643. https://doi.org/10.1021/jf0112735

[17]. Kumar, V., Abbas, A. K., & Aster, J. C. (2020). Robbins & Cotran Pathologic Basis of Disease (10th ed.). Elsevier.

[18]. Lundberg, J. O., Weitzberg, E., & Gladwin, M. T. (2008). The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nature Reviews Drug Discovery, 7(2), 156–167.

[19]. Mérida, I., Avila-Flores, A., & Merino, E. (2018). Cyclophosphamide: From clinical use to immunological mechanisms. International Immunopharmacology, 62, 288–296. https://doi.org/10.1016/j.intimp.2018.06.009.

[20]. Neelwarne, B., & Halagur, S. B. (2013). Red beet: A potential source for phytochemicals and bioactives. In B. Neelwarne (Ed.), Red Beet Biotechnology: Food and Pharmaceutical Applications (pp. 1–26). Springer.

[21]. Pavlov, A., Berkov, S., & Stanchev, V. (2002). Effect of betanin on primary humoral immune response. Bulgarian Journal of Veterinary Medicine, 5(3), 141–146.

[22]. Pietrzkowski, Z., Nemzer, B., Spórna, A., Stalica, P., Tresher, W., Keller, R., & Studzinski, M. (2010). Influence of betalain-rich extract on reduction of discomfort associated with osteoarthritis. New Medicine, 1, 12–17.

[23]. Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry, 97, 55–74.

[24]. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines, 8(1), 1–10.

[25]. Sattler, S. (2017). The role of the immune system beyond the fight against infection. Advances in Experimental Medicine and Biology, 1003, 3–14. https://doi.org/10.1007/978-3-319-57613-8_1

[26]. Snapper, C. M., Rosas, F. R., Kiesler, P., Zelazowski, P., Nguyen, D., Tian, M., & Mond, J. J. (1993). Differential regulation of Ig isotype expression by cytokines in human B cells. International Immunology, 5(6), 637–644.

[27]. Tesoriere, L., Butera, D., Allegra, M., Fazzari, M., & Livrea, M. A. (2004). Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: potential health effects of betalains in humans. The American Journal of Clinical Nutrition, 80(4), 941–945.

[28]. Turvey, S. E., & Broide, D. H. (2010). Innate immunity. The Journal of Allergy and Clinical Immunology, 125(2 Suppl 2), S24–S32. https://doi.org/10.1016/j.jaci.2009.07.016

[29]. Wootton-Beard, P. C., & Ryan, L. (2011). A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. Journal of Functional Foods, 3(4), 329–334.