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A phytotherapic blend immunity-6™ inhibits myeloid leukemic cells 2 activation involving purinergic signaling

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ABSTRACT

Leukemia is among the most common types of hematological cancers and the use of herbal medicines to prevent and treat leukemia are under quick development. Among several molecular pathways involved in leukemia pathogenesis and exacerbations, purinergic signaling is revealed as a key component. In the present study, the effects of two doses (5 ug/mL and 10 ug/mL) of Immunity-6™, a phytocomplex composed by beta-glucan, green tea (Camelia sinensis), chamomile (Matricaria chamomilla), and ascorbic acid (vitamin C) was tested in vitro, using chronic myelogenous leukemia cell line (K-562; 5 ×104/mL/well), which were challenged with lipopolysaccharide (LPS; 1 ug/mL) for 24 h. The results demonstrated that both doses of Immunity-6™ inhibited ATP release ($p < 0.001$) and P2×7 receptor at mRNA levels expression ($p < 0.001$). Purinergic inhibition by Immunity-6™ was followed by reduced release of proinflammatory cytokines IL-1beta ($p < 0.001$) and IL-6 ($p < 0.001$), while only 5 ug/mL of Immunity-6™ reduced the release of TNF-alpha ($p < 0.001$). Beyond to inhibit the release of pro-inflammatory cytokines, both doses of Immunity-6™ induced the release of anti-inflammatory cytokine IL-10 ($p < 0.001$), while only the higher dose (10 ug/mL) of Immunity-6™ induced the release of anti-inflammatory IL-1ra ($p < 0.05$) and klotho ($p < 0.001$). Thus, Immunity-6™ may be a promising adjuvant in the treatment of leukemia and further clinical trials are guaranteed.

1. Introduction

Natural products (NPs) play an important role in the development of new treatments for infectious diseases and different types of cancer, especially by its phytochemicals effects on the modulation of the immune system and anticarcinogenic activities by interfering with cellular proliferation, apoptosis, angiogenesis, and metastasis [1–3].

Leukemia is among the most common types of cancer worldwide and the use of therapeutic herbs for treatment of leukemia is growing rapidly and presenting prominent results [4]. Chronic myeloid leukemia (CML) is a hematological malignancy and NPs and its secondary metabolites (i.e., alkaloids, flavonoids, terpenoids, saponins, peptides, etc.) offer an alternate, effective, and inexpensive option for CML therapy, revealing that many NPs inhibit CML cell proliferation and, in addition, induce cell death through apoptosis and potently could suppress tumor growth [5].

Immunity-6™ is a phytocomplex formed by different and specifics concentrations of beta-glucan, green tea (Camelia sinensis), camomile (Matricaria chamomilla), and ascorbic acid (vitamin C). Beta glucans, a yeastderived β-glucan, may be able to help treat cancer and beyond to activate immune cells triggering a defense response [6]. Green tea

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(Camellia sinensis), contains as secondary metabolites the catechins (epicatechin, epigallocatechin, etc.), which has antioxidant, anticancer, antifungal, antivirus properties [7], and act as an immunomodulator by influencing the proliferation of T lymphocyte and cytokines release [8–10]. Camomile, (Matricaria chamomilla L.) one of the most ancient medicinal herbs known to mankind, is still popular today and probably will continue to be used in the future because it contains various bioactive phytochemicals that could provide therapeutic effects as anti-inflammatory, antioxidant, anti-cancer, and anti-allergic effects [11]. Ascorbic acid (vitamin C) participates in essential functions, acting in particular, as an enzyme cofactor playing important roles in the regulation of the immune system, metabolism, and cell function [12–14].

Upon onset of leukemia, abnormal immature cells start growing very rapidly and competing with healthy cells for nutrition and space, leading to abnormal bone marrow function. Besides that, other important finds are repetitive bacterial or viral infections, which are associated with recurrent fever, runny nose, and cough, accounting to higher severity, morbidity, and mortality [15–18].

Therefore, the present study investigated whether the Immunity- 6™ could have any effect on the activation of myeloid leukemia cells induced by LPS.

2. Materials and methods

Immunity-6™ is a phytocomplex formed by different and specifics concentrations of beta-glucan (3gr), green tea (Camelia sinensis; 3gr), chamomile (Matricaria chamomilla; 2gr), turmeric (500 mg), and ascorbic acid (vitamin C; 1gr) and was kindly provided by Grow Dietary Supplements (GDS) from Brazil. Of note, these ingredients in the concentrations presented, displayed a concentration of the following molecules [gree tea (epigallocatechin gallate – EGCG – 800 mg; quercetin – 276 mg; myricetin – 137 mg); Matricaria chamomilla (apigenin – 6,5 mg)]. The chromatography of the compounds used for Immunity-6™ were previously described as follow: green tea [19], chamomile [20]. The GDS from Brazil quality control department monitored the purity and quality of the raw materials used, as well as the formulation of the Immunity-6™, which is patented under GDS rights.

2.1. Cell culture reagents

Cell culture medium Dulbecco's modified Eagle's medium (DMEM), supplemented with fetal bovine serum (FBS), penicillin-streptomycin, and phosphate-buffered saline (PBS) were obtained from Gibco BRL (Grand Island, CA, USA).

2.2. Cell line and study design

K-562 (chronic myelogenous leukemia) cell line was purchased from Cell Bank of Rio de Janeiro, Brazil, and it was cultured in Dulbecco's modified Eagles' medium (DMEM) high glucose supplemented with 10% v/v fetal bovine serum (FBS), 1% L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin, which has been maintained at 37°C in a humidified atmosphere of 5% CO2 incubator. For all the experiments, Immunity-6™ was dissolved in the culture medium in appropriate concentrations (Stock solution 10 mg/mL). Succinctly, cells (5 ×104 viable cells/well) were incubated for 24 h, and then exposed to 5 and 10 μg/mL of Immunity-6™, as described above in the presence or absence of LPS (1 μg/mL) for 60 min and incubated for another 24 h with Immunity-6™ at 37 °C.

2.3. Cell viability analysis

The cell viability of control, Lipopolysaccharides from Escherichia coli (LPS) from Sigma-Aldrich (St. Louis, Mo., USA), and Immunity-6™ at concentrations (0.1; 1; 10; 100; and 1000 μg/mL)-treated cells were measured using a standard 3-(4,5-Dimethylthiazol-2-yl)− 2,5-diphenyltetrazolium bromide (MTT) assay. Summarily, 5 × 104 viable cells were seeded into a 96-well flatbottom plates (Corning USA) and were incubated with different concentrations of the Immunity-6™ for 24 h. Following this period, 10 μL/well of MTT (5 mg/mL) were added for an additional period of 4 h. Thus, 100 μL of 10% sodium dodecyl sulfate (SDS) solution in deionized water were added to each well and left overnight. The absorbance was measured at 595 nm in a benchtop multimode reader (Spectra-Max I3, Molecular Devices, USA) [21].

2.4. Adenosine trisphosphate (ATP) measurement

K-562 cells (CML) were seeded in 24-well culture plates at a density of 5 × 104 viable cells/well and incubated for 24 h. After treatment protocol with Immunity-6™, and stimulus with LPS (1 μg/mL), the ATP concentration in the supernatants was determined by the ATPLite kit (Perkin Elmer, USA) according to the manufacturer's instructions, as previously published [22].

2.5. Cytokines measurements

Cells were seeded in 24-well culture plates at a density of 5 × 104 cells/well/mL and incubated for 24 h. The concentrations of IL-1β, IL-6, IL-10, IL1RA, TNF-α, and Klotho, in the cell culture supernatant were analyzed by using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis,

USA) following the manufacturer's instructions. Cells were pretreated with LPS (1 μg/mL) for 60 min with or without Immunity-6™ (5 μg/mL or 10 μg/mL) for 24 h. The cell culture supernatant was collected and stored at − 86ºC to determine the levels of cytokines and chemokines, according to the manufacturer's instructions, as previously published [22,23].

2.6. Statistical analysis

The obtained results were expressed as mean ± standard error of the mean (SEM) from at least three independent experiments unless stated otherwise. One-way analysis of variance (ANOVA) was used for multiple comparisons, followed by the Bonferroni test for comparison among the groups. A p value of < 0.05 was considered significant.

3. Results

3.1. Effects of immunity-6™ on cell viability and cytotoxicity

Fig. 1 displays the effect of different doses of Immunity-6™ (0.1, 1, 10, 100, 1000 μg/mL) on cell viability. Considering the minimum inhibitory concentration of 80% (IC80), the dose obtained to reduce 20% of cell viability was greater than 10 μg/mL (Fig. 1A), attesting the safety of Immunity-6™ supplementation. The effects of Immunity-6™ on LPS-induced cytotoxicity are shown in Fig. 1B, in which high cell viability can be observed after treatment with Immunity-6™ (10 μg/mL) in K-562 cells compared to the cells stimulated with LPS ($p < 0.05$).

3.2. Effects of immunity-6™ on pro-inflammatory cytokines synthesis and release

Fig. 2 shows the results of pro-inflammatory cytokines (IL-1beta, Fig. 2A; IL-6, Fig. 2B; TNF-alpha, Fig. 2C) released by K-562 cells. The results show that LPS (1 ug/mL) induced the release of IL-1beta (Fig. 2A, $p < 0.001$), IL-6 (Fig. 2B, $p < 0.001$), and TNF-alpha (Fig. 3C, $p < 0.001$) compared with control group. In addition, both doses of Immunity-6™ (5 μg/mL and 10 μg/mL) were able to reduce LPS-induced the release of IL-1beta (Fig. 2A, $p < 0.001$), and IL-6 (Fig. 2B, $p < 0.001$) by K-562 cells. However, the inhibitory effects of Immunity-6™ on LPS-induced TNF-alpha release, was observed only the dose of

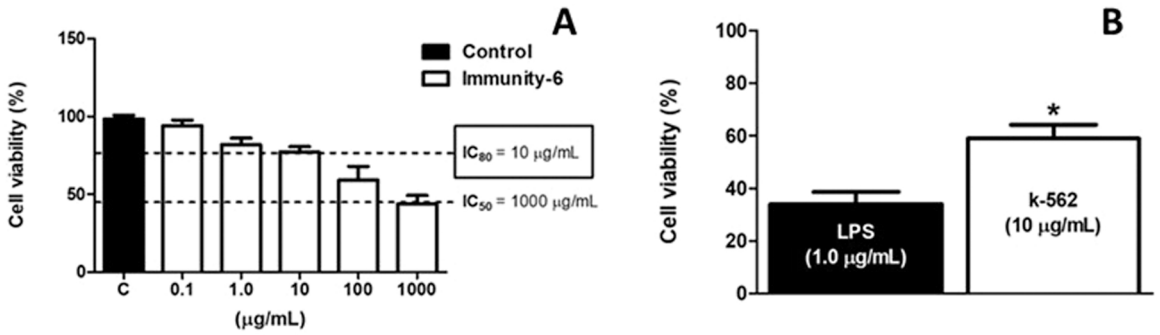

Fig. 1. Effects of Immunity-6™ on cell line viability and cell toxicity. (A) Cells were treated with different concentrations of Immunity-6™ for 24 h, and the IC80 was defined as the study test concentration (10 µg/mL) for K-562-cell line. (B) Effects of Immunity-6™ on LPS-induced cytotoxicity. *p < 0.05 indicates statistical difference (unpaired t-test).

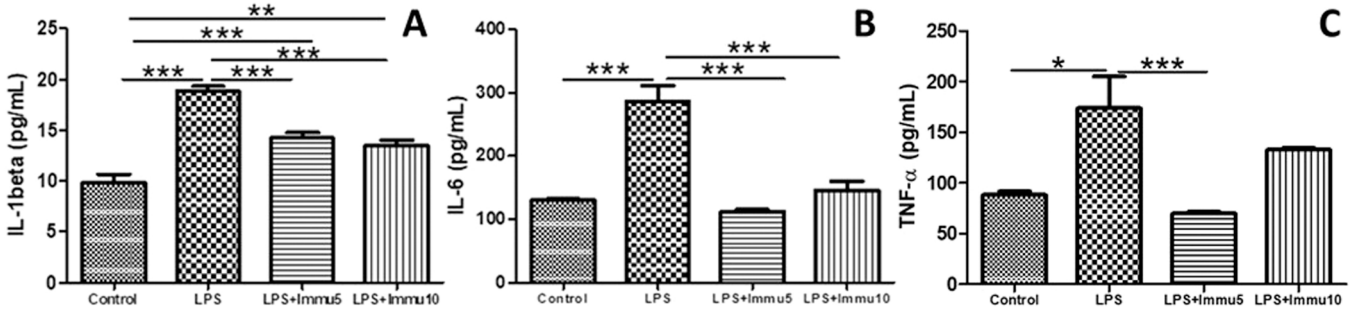

Fig. 2. Concentrations of the pro-inflammatory cytokines IL-1beta (A), IL-6 (B), and TNF-alpha (C) in cell culture supernatant. Control (medium stimulated), LPS (stimulated with 1 ug/mL of LPS), LPS+Immu5 (stimulated with 1 ug/mL of LPS and treated with 5 ug/mL of Immunity-6™), LPS+Immu10 (stimulated with 1 ug/mL of LPS and treated with 10 ug/mL of Immunity-6™). * **p < 0.001; * *p < 0.01, *p < 0.05.

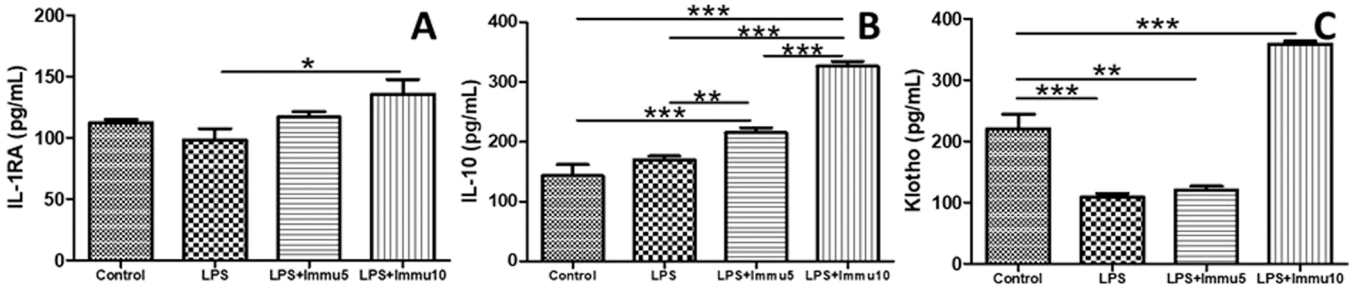

Fig. 3. Concentrations of the anti-inflammatory cytokines IL-1ra (A), IL-10 (A), and klotho protein (C) in cell culture supernatant. Control (medium stimulated), LPS (stimulated with 1 ug/mL of LPS), LPS+Immu5 (stimulated with 1 ug/mL of LPS and treated with 5 ug/mL of Immunity-6™), LPS+Immu10 (stimulated with 1 ug/mL of LPS and treated with 10 ug/mL of Immunity-6™). * **p < 0.001; * *p < 0.01, *p < 0.05.

5 µg/mL of Immunity-6™ (Fig. 2C, $p < 0.001$).

3.3. Effects of immunity-6™ on anti-inflammatory cytokines synthesis and release

Fig. 3 presents the results of the effects of Immunity-6™ (5 ug/mL or 10 µg/mL) in the concentration of anti-inflammatory mediators (IL-1ra, Fig. 3A; IL10, Fig. 3B; Klotho, Fig. 3C) released by K-562 cells. It was observed an increased release of IL-1ra in the cells treated with 10 µg/mL of Immunity-6™ compared to LPS-stimulated cells ($p < 0.05$) (Fig. 3A). Concerning IL-10 levels, both doses (5 µg/mL, $p < 0.01$ and 10 µg/mL, $p < 0.001$) of Immunity-6™ were able to increase the release of this cytokine by K-562 cells after LPS stimulation. Klotho release was increased by the dose of 10 µg/mL of Immunity-6™ (Fig. 3C, $p < 0.001$).

3.4. Effects of immunity-6™ on purinergic signaling

Fig. 4 shows the levels of ATP in cell culture supernatant and the expression of P2×7 receptor in K-562 cells. LPS induced a significant release of ATP (Fig. 4A, $p < 0.001$), which was followed by increased expression of its ligand channel, the P2×7 receptor (Fig. 4B, $p < 0.001$). On the other side, both doses of Immunity-6™ were able to reduce LPS-induce ATP release (Fig. 4A; $p < 0.001$). In addition, both doses of Immunity-6™ were also capable to inhibit LPS-induce P2×7 receptor hyperexpression (Fig. 4B; $p < 0.001$).

4. Discussion

Taken together, the results of this study showed, for the first time, that chronic myeloid leukemia cells (K-562 cells) treated with Immunity-6™ can mitigate the hyperactivation induced by LPS, involving the inhibition of purinergic signaling, as noted by decreased ATP

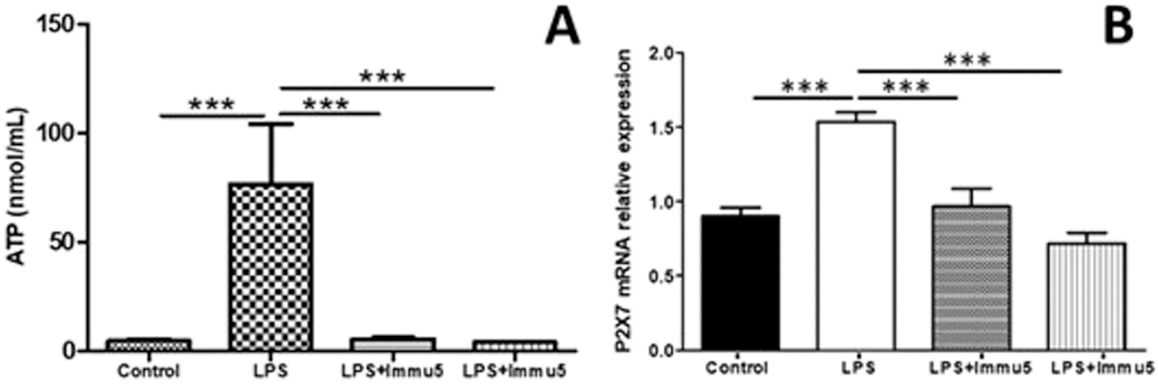

Fig. 4. Adenosine triphosphate (ATP) concentrations (A) and expression of mRNA of P2×7 receptor (B) in K-562 cells in the Control (medium stimulated), LPS (stimulated with 1 ug/mL of LPS), LPS+Immu5 (stimulated with 1 ug/mL of LPS and treated with 5 ug/mL of Immunity-6™), LPS+Immu10 (stimulated with 1 ug/mL of LPS and treated with 10 ug/mL of Immunity-6™) cells. * **$p < 0.001$.

accumulation and the lower expression of P2×7 receptor and decreased release of pro-inflammatory cytokines and increased release of anti-inflammatory mediators, thus controlling the inflammatory status.

Increases in cytokine release can stimulate an abnormal response in leukemic cells and an uncontrolled cell proliferation, which lead to an increase in the capacity of survival of these cancer cells [24]. Despite IL-1 has a ubiquitous role, being central to the regulation of normal hematopoiesis and immune responsiveness, in CML, the spontaneous production of IL-1beta can be harmful to that regulation [25]. In this respect, the signaling of pro-inflammatory cytokine IL-1beta, which has pleiotropic functions in inflammation and cancer, in the tumor tissue and in its microenvironment affect the tumor development in several aspects. The signaling of IL-1beta in the tumor tissue or in its microenvironment leads to the expression of other inflammatory mediators, such as IL-6, which leads to increased survival and proliferation of malignant cells. In addition, IL-1beta contributes to the positive regulation of gene expression associated with "stemness", through their effects on cancer stem cells, promoting proliferation and epithelial-mesenchymal transition. Another aspect that is important to point out is that IL-1beta is able to promote the production and expression of proinflammatory cytokines, chemokines, prostaglandins, growth factors, and adhesion molecules, all these contributing to a pro-survival environment and suppressing anti-tumor immune responses [26–30]. In this study, was observed increased IL-1beta release by K-562 cells after LPS stimulation, nevertheless, both doses, 5 and 10 μg/mL of Immunity-6™ significantly decrease the IL-1beta release. This result indicates the potential of Immunity-6™ to control the inflammatory response triggered in myeloid cancer cells, although these cells still show increased release compared to the control cells, the decrease in IL-1beta release may reduce the potential stimulus for the release of other inflammatory mediators, allowing the environment more appropriate for an anti-tumorigenic immune activity [26].

The IL-6 is highly up-regulated in several disorders, including cancer in which has a paramount role in favoring cell proliferation, survival, differentiation, migration, invasion, metastasis, and angiogenesis of cancer cells [31,32]. The inflammatory microenvironment induced by IL-6 contributes to tumorigenesis and metastasis via tumor-intrinsic and -extrinsic effects, which lead the IL-6 cytokine to be a target for cancer therapy [33–35]. This study showed that IL-6 release was increased in LPS cells and the Immunity-6™, both doses (5 μg/mL and 10 μg/mL), was able to inhibit the release of IL-6 by K-562 cells. This observation corroborates with the effects of Immunity-6™ on IL-1beta and reinforces the role of Immunity-6™ in the control and inhibition of the inflammatory process in chronic myeloid cancer cells [31].

Tumor necrosis factor-alpha (TNF-alpha) is a pro-inflammatory cytokine produced by macrophages and monocytes during the inflammatory process, this cytokine triggers several signaling events within cells inducing apoptosis or necrosis, in addition, is a pivotal cytokine in resistance to infections and cancer being considered a key chemical mediator in the association among cancer and inflammation [36,37]. The activation of NF-kB and AP-1 transcription factor complexes induced by TNF-alpha are the main keys that link this cytokine to cancer development and progression [37]. Despite that, TNF-alpha is used as an anti-cancer drug and present extensive cytotoxicity in many cancer cells, but on contrary, for some types of cancer, such as ovarian, and breast cancer, and also leukemia, TNF-alpha induces resistance to apoptosis, becoming a limitation for its use for certain types of cancer [37–41]. In relation to myeloid leukemia, increased plasma/serum levels of TNF-alpha are normally found [42], which stimulates the release of several cytokines, maintaining the inflammatory status [43]. In this study, the LPS stimulation increased the release of TNF-alpha, which has been abrogated by 5 μg/mL dose of Immunity-6™. This result demonstrated the potential anti-inflammatory effect of Immunity-6™ on chronic myeloid leukemia cells. Considering that part of treatment for myeloid leukemia is the downregulation of pro-inflammatory cytokines, as IL-1beta, IL-6, and TNF-alpha, our results robustly sustain the hypothesis that this phytocomplex Immunity-6™ can act as adjuvant therapy for myeloid leukemia [42].

As early described, IL-1 is a master cytokine of local and systemic inflammation, and the IL-1beta, a product of blood monocytes, tissue macrophages, and dendritic cells can be blocked by the IL-1 receptor antagonist (IL-1ra) [44]. Although IL-1beta is a pro-inflammatory cytokine, IL-1ra, a natural inhibitory molecule, has the potential to block the binding of the IL-1alpha, IL-1beta, and IL-1 receptors, blocking the pro-inflammatory effects of IL-1 proteins. In this context, IL-1ra can promote a better control of the local and systemic inflammation in several diseases, including cancer [44,45]. It was already demonstrated that the inhibition of IL-1beta activity by IL-1ra has the potential to decrease the proliferation of hematopoietic colony in chronic myeloid leukemia [46,47]. In this study, it was found that the dose of 10 μg/mL of Immunity-6™ increased release of this potent anti-inflammatory cytokine, which corroborated with decreases in the release of IL-1beta, suggesting a protective role for Immunity-6™ against the inflammatory status induced by LPS in K-562 cells [44,45].

IL-10 is an anti-inflammatory cytokine with both immuno- suppressive and antiangiogenic functions [48]. IL-10 has been pointed out as a marker of survival in chronic myeloid leukemia [49–51]. This association could be attributed to its capacity to inhibiting IL-1beta, IL-6, and TNF-alpha cytokines, all of these linked to lower survival in several diseases, and also to the capacity of IL-10 to decrease blast proliferation through inhibition of the production of granulocyte and granulocyte-macrophage colony-stimulating factors (G-CSF and GM-CSF) [52–55]. In this study, was found a significant increase in IL-10 release by K-562 cells after supplementation with both doses (5 μg/mL

and 10 μg/mL) of Immunity-6™ in relation to observed in control and in LPS-stimulated K-562 cells. So, this data reinforces the potential of Immunity-6™ to inhibit K-562 cell proliferation. It is a fact that IL-6 and TNF-a could induce IL-10 secretion, which has the potential to inhibit IL-1beta, IL-6, and TNF-alpha release. Thus, taken together, the decrease in IL-1beta, IL-6, and TNF-alpha observed herein in this study, leads to the suggestion that Immunity-6™ is driving this inflammatory control in the K-562 cells after activation by LPS [55–57].

Klotho is an anti-inflammatory and anti-apoptotic protein involved in the pathogenesis of many diseases involving oxidative stress, inflammation, and tissue injury [58], and its role in the regulation of physiological and pathophysiological processes, includes modulation of the activities of different glycoproteins on the cell surface, such as ion channels [59], insulin-like growth factor 1 (IGF-1) and the Wnt pathway [60], and also the regulation of maturation/differentiation, proliferation, phagocytosis, migration, lifespan, and survival by different cell types. Klotho is also considered an anti-cancer protein, which is decreased in different types of cancer, such as breast, gastric, and hepatocellular carcinoma [61–63]. Klotho protein has also the ability to regulate tumor cell migration in immune evasion, reducing tumor aggressiveness, beyond to have a role in the apoptosis of cancer cells [63,64]. In the light of these pieces of information, it is important to consider klotho as a key in the protection against inflammation-related cancer. In the present study, 10 μg/mL of dose of Immunity6™ significantly increases the release of klotho by K-562 cells as compared to the LPS-stimulated cells and to control cells, while the lower dose (5 μg/mL) did not affect the klotho synthesis. The increased release of klotho can lead us to suppose that the higher dose of Immunty-6™ used in this study has a potential in the control of the inflammatory microenvironment observed in myeloid leukemia, which can have a positive role in the disease prognosis [58].

It is important to highlight that diverse mechanism can be involved in the regulation of pro- and anti-inflammatory cytokine release. In this context, purinergic signaling is one of the key mediators of many cancer aspects, mainly by this capacity of up-regulate and activate P2×7 receptor, which is induced by increased ATP accumulation in the extracellular milieu. In this study, it was observed that both doses (5 ug/mL and 10 mg/mL) of Immunity-6™ mitigate the ATP release and the expression of P2×7 receptor in K-562 cells. Thus, it is clear that Immunity-6™ contributes to control the balance of pro- and anti-inflammatory cytokines, which may have been resulted from the improved purinergic signaling, as demonstrated by reduced accumulation of ATP in extracellular milieu and reduced expression of P2×7 receptor.

In conclusion, this study showed that the phytotherapic blend Immunity-6™ inhibits LPS-induced inflammation and hyperactivation of K-562 cells, which may be a promising complimentary therapy to protect patient with chronic myeloid leukemia, who normally presents an immunodeficiency.

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Credit author statement

Yanesko Fernandes Bella, Carlos Rocha Oliveira, José Roberto Mateus-Silva, Maysa Alves Rodrigues Brandao-Rangel, Anamei Silva-Reis, Juliana de Melo Batista Santos, have performed the experiments, analyzed the data and written the original version of the manuscript. Regiane Albertini, Rodrigo Alvaro Brandao Lopes-Martins, Luis Vicente Franco de Oliveira, Rodolfo P Vieira were responsible for the study design, data analysis and written the original version of the manuscript. In addition, Rodolfo P Vieira was responsible to obtain funding to perform this study.

CRediT authorship contribution statement

YFB, CRO, MARBR, ASR, JRMS have contributed to data collection and analysis and with writing the manuscript. LSZ, RABLP, RA, LVFO, JMBS, RPV have contributed to data analysis, study design and with writing the manuscript.

Conflict of interest statement

YFB and RPV received a consulting fee from the GDS company. GDS is the owner of Immunity-6™. All other authors have no conflict of interest related to this manuscript.

Data availability

Data will be made available on request.

References

[1] E. Rajesh, et al., Naturally occurring products in cancer therapy, J. Pharm. bioallied Sci. 7 (Suppl 1) (2015) S181.
[2] S. Shukla, V.K. Bajpai, M. Kim, Plants as potential sources of natural immunomodulators, Rev. Environ. Sci. Bio/Technol. 13 (1) (2014) 17–33.
[3] P. Brindha, Role of phytochemicals as immunomodulatory agents: a review, Int. J. Green. Pharm. (IJGP) 10 (1) (2016).
[4] M.M. Khalafalla, et al., Antileukemia activity from root cultures of Vernonia amygdalina, J. Med. Plants Res. 3 (8) (2009) 556–562.
[5] K.V. Khajapeer, R. Baskaran. Natural products for treatment of chronic myeloid leukemia. Anti-cancer drugsnature, synthesis and cell, 1st edn.,, Intech publications,, Croatia, 2016, pp. 1–48.
[6] A. Geller, R. Shrestha, J. Yan, Yeast-derived β-glucan in cancer: novel uses of a traditional therapeutic, Int. J. Mol. Sci. 20 (15) (2019) 3618.
[7] T. Tanaka, Y. Matsuo, I. Kouno, Biochemical and physicochemical characteristics of green tea polyphenols. Green Tea Polyphenols, CRC Press,, Boca Raton, FL, USA, 2013, pp. 19–38.
[8] D. Wu, et al., Green tea EGCG, T cells, and T cell-mediated autoimmune diseases, Mol. Asp. Med. 33 (1) (2012) 107–118.
[9] C. Di Lorenzo, et al., Correlation between catechin content and NF-κB inhibition by infusions of green and black tea, Plant Foods Hum. Nutr. 68 (2) (2013) 149–154.
[10] V. Kumar, A. Sharma, Neutrophils: Cinderella of innate immune system, Int. Immunopharmacol. 10 (11) (2010) 1325–1334.
[11] B. Al-Dabbagh, et al., Antioxidant and anticancer activities of chamomile (Matricaria recutita L.), BMC Res. Notes 12 (1) (2019) 1–8.
[12] A. Ang, et al., Vitamin C and immune cell function in inflammation and cancer, Biochem. Soc. Trans. 46 (5) (2018) 1147–1159.
[13] A.C. Carr, S. Maggini, Vitamin C and immune function, Nutrients 9 (11) (2017) 1211.
[14] S. Englard, S. Seifter, The biochemical functions of ascorbic acid, Annu. Rev. Nutr. 6 (1) (1986) 365–406.
[15] K.V. Rolston, The use of new and better antibiotics for bacterial infections in patients with leukemia, Clin. Lymphoma Myeloma 9 (2009). S357S363.
[16] S. Al Omar, et al., Bacterial infections in children with acute myeloid leukemia receiving ciprofloxacin prophylaxis, J. Pediatr. Hematol. /Oncol. 39 (3) (2017) e131–e135.
[17] S.A. Langie, et al., DNA methylation and the hygiene hypothesis: connecting respiratory allergy and childhood acute lymphoblastic leukemia, Epigenomics 11 (13) (2019) 1519–1537.
[18] A.D. Wallace, et al., Allergies and childhood acute lymphoblastic leukemia: a case–control study and meta-analysis, Cancer Epidemiol. Prev. Biomark. 27 (10) (2018) 1142–1150.
[19] H. Wang, K. Helliwell, Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography, Food Res. Int. 34 (2001) 223–227.
[20] C. Redaelli, L. Formentini, E. Santaniello, Reversed-phase high-performance liquid chromatography analysis of apigenin and its glucosides in flowers of Matricaria chamomilla and chamomile extracts, Planta Med. 42 (7) (1981) 288–292.
[21] C.R. Oliveira, H. Polonini, M.C. Marcucci, R.P. Vieira, MiodesinTM positively modulates the immune response in endometrial and vaginal cells, Molecules 27 (3) (2022 25) 782.
[22] N.C. Rigonato-Oliveira, B. Mackenzie, A.L.L. Bachi, M.C. Oliveira-Junior, A. Santos-Dias, M.A.R. Brandao-Rangel, H. Delle, T. Costa-Guimaraes, N. R. Damaceno-Rodrigues, N.R. Dulley, M.A. Benetti, C. Malfitano, C. de Angelis, R. Albertini, A.P.L. Oliveira, A. Abbasi, H. Northoff, R.P. Vieira, Aerobic exercise inhibits acute lung injury: from mouse to human evidence Exercise reduced lung injury markers in mouse and in cells, Exerc Immunol. Rev. 24 (2018) 36–44.

[23] M. Garcia, A. Santos-Dias, A.L.L. Bachi, M.C. Oliveira-Junior, A.S. Andrade-Souza, S.C. Ferreira, J.C.J. Aquino-Junior, F.M. Almeida, N.C. Rigonato-Oliveira, A.P. L. Oliveira, L.E.B. Savio, R. Coutinho-Silva, T. Müller, M. Idzko, T. Siepmann, R. P. Vieira, Creatine supplementation impairs airway inflammation in an experimental model of asthma involving P2×7 receptor, Eur. J. Immunol. 49 (6) (2019) 928–939.
[24] T. Kupsa, J.M. Horacek, L. Jebavy, The role of cytokines in acute myeloid leukemia: a systematic review, Biomed. Pap. Med Fac. Univ. Palacky. Olomouc Czech Repub. 156 (4) (2012) 291–301.
[25] A.T. Ichiki, et al., Differential regulation of interleukin-1alpha and interleukin-1beta in K-562 cells, J. Interferon Cytokine Res. 18 (12) (1998) 1045–1050.
[26] A. Malik, T.D. Kanneganti, Function and regulation of IL-1α in inflammatory diseases and cancer, Immunol. Rev. 281 (1) (2018) 124–137.
[27] Y. Li, et al., IL-1β promotes stemness and invasiveness of colon cancer cells through Zeb1 activation, Mol. Cancer 11 (2012) 87.
[28] G. Soria, et al., Inflammatory mediators in breast cancer: coordinated expression of TNFα & IL-1β with CCL2 & CCL5 and effects on epithelialto-mesenchymal transition, BMC Cancer 11 (2011) 130.
[29] E.M. Pietras, et al., Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal, Nat. Cell Biol. 18 (6) (2016) 607–618.
[30] X. Song, et al., CD11b+/Gr-1+ immature myeloid cells mediate suppression of T cells in mice bearing tumors of IL-1beta-secreting cells, J. Immunol. 175 (12) (2005) 8200–8208.
[31] K. Taniguchi, M. Karin, IL-6 and related cytokines as the critical lynchpins between inflammation and cancer, Semin Immunol. 26 (1) (2014) 54–74.
[32] C. Garbers, et al., Plasticity and cross-talk of interleukin 6-type cytokines, Cytokine Growth Factor Rev. 23 (3) (2012) 85–97.
[33] X. Yao, et al., Targeting interleukin-6 in inflammatory autoimmune diseases and cancers, Pharm. Ther. 141 (2) (2014) 125–139.
[34] S. Xu, N. Neamati, gp130: a promising drug target for cancer therapy, Expert Opin. Ther. Targets 17 (11) (2013) 1303–1328.
[35] E. Bournazou, J. Bromberg, Targeting the tumor microenvironment: JAK-STAT3 signaling, JAKSTAT 2 (2) (2013), e23828.
[36] H.T. Idriss, J.H. Naismith, TNF alpha and the TNF receptor superfamily: structure-function relationship(s), Microsc. Res. Tech. 50 (3) (2000) 184–195.
[37] F. Balkwill, TNF-alpha in promotion and progression of cancer, Cancer Metastas-.-. Rev. 25 (3) (2006) 409–416.
[38] H. Körner, et al., Tumor necrosis factor sustains the generalized lymphoproliferative disorder (gld) phenotype, J. Exp. Med. 191 (1) (2000) 89–96.
[39] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (6917) (2002) 860–867.
[40] M. Feldmann, Development of anti-TNF therapy for rheumatoid arthritis, Nat. Rev. Immunol. 2 (5) (2002) 364–371.
[41] A. Ferrajoli, et al., The clinical significance of tumor necrosis factor-alpha plasma level in patients having chronic lymphocytic leukemia, Blood 100 (4) (2002) 1215–1219.
[42] S.A. Zbaar, Serum level of TNF-alpha in patients with chronic myeloid leukemia on Imatinib therapy, Med. J. Tikrit Univ. 22 (1) (2017).
[43] F. Balkwill, Tumour necrosis factor and cancer. Nat. Rev. Cancer 9 (5) (2009) 361–371.
[44] C.A. Dinarello, J.W. van der Meer, Treating inflammation by blocking interleukin-1 in humans, Semin Immunol. 25 (6) (2013) 469–484.
[45] A.S. Yazdi, K. Ghoreschi, The interleukin-1 family, Regul. Cytokine gene Expr. Immun. Dis. (2016) 21–29.
[46] Z. Estrov, et al., Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity, Blood 78 (6) (1991) 1476–1484.
[47] W.P. Arend, et al., Biological properties of recombinant human monocytederived interleukin 1 receptor antagonist. J. Clin. Invest 85 (5) (1990), 16941697.
[48] M. Pehlivan, et al., Prognostic importance of single-nucleotide polymorphisms in IL-6, IL-10, TGF-β1, IFN-γ, and TNF-α genes in chronic phase chronic myeloid leukemia, Genet Test. Mol. Biomark. 18 (6) (2014) 403–409.
[49] L. Hempel, et al., High interleukin-10 serum levels are associated with fatal outcome in patients after bone marrow transplantation, Bone Marrow Transpl. 20 (5) (1997) 365–368.
[50] J. Martinez-Escribano, et al., Interleukin-10, interleukin-6 and interferon-γ gene polymorphisms in melanoma patients, Melanoma Res. 12 (5) (2002) 465–469.
[51] A.A. Amirzargar, et al., Cytokine gene polymorphism in Iranian patients with chronic myelogenous leukaemia, Int. J. Immunogenet. 32 (3) (2005) 167–171.
[52] Y. Asano, et al., Interleukin-10 inhibits the autocrine growth of leukemic blast cells from patients with acute myeloblastic leukemia, Int J. Hematol. 66 (4) (1997) 445–450.
[53] A.M. Tsimberidou, et al., The prognostic significance of cytokine levels in newly diagnosed acute myeloid leukemia and high-risk myelodysplastic syndromes, Cancer 113 (7) (2008) 1605–1613.
[54] R. Kurzrock, Cytokine deregulation in cancer, Biomed. Pharm. 55 (9–10) (2001) 543–547.
[55] P. Stenvinkel, et al., IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia–the good, the bad, and the ugly, Kidney Int 67 (4) (2005) 1216–1233.
[56] A. Steensberg, et al., IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans, Am. J. Physiol. Endocrinol. Metab. 285 (2) (2003) E433–E437.
[57] B. Sanchez-Correa, et al., Cytokine profiles in acute myeloid leukemia patients at diagnosis: survival is inversely correlated with IL-6 and directly correlated with IL-10 levels, Cytokine 61 (3) (2013) 885–891.
[58] N.T. Xuan, N.V. Hai, Changes in expression of klotho affect physiological processes, diseases, and cancer, Iran. J. Basic Med Sci. 21 (1) (2018) 3–8.
[59] M. Sopjani, M. Dërmaku-Sopjani, Klotho-dependent cellular transport regulation, Vitam. Horm. 101 (2016) 59–84.
[60] N.T. Xuan, et al., Regulation of dendritic cell function by insulin/IGF1/PI3K/Akt signaling through klotho expression, J. Recept Signal Transduct. Res 37 (3) (2017) 297–303.
[61] T. Rubinek, et al., Epigenetic silencing of the tumor suppressor klotho in human breast cancer, Breast Cancer Res Treat. 133 (2) (2012) 649–657.
[62] L. Wang, et al., Klotho is silenced through promoter hypermethylation in gastric cancer, Am. J. Cancer Res 1 (1) (2011) 111–119.
[63] G. Shu, et al., Restoration of klotho expression induces apoptosis and autophagy in hepatocellular carcinoma cells, Cell Oncol. (Dordr.) 36 (2) (2013) 121–129.
[64] Y. Shimoyama, et al., KLOTHO gene polymorphisms G-395A and C1818T are associated with low-density lipoprotein cholesterol and uric acid in Japanese hemodialysis patients, Am. J. Nephrol. 30 (4) (2009), 383388.