

Effect of *Cyperus rotundus* extract on the regulation of TNF- α and IFN- γ in a 4T1 triple-negative breast cancer mouse model

Wirdatun Nafisah¹, Nadia Wahyuningsih², Farida Rachmawati², Yuyun Ika Christina³, Anissa Nofita Sari⁴, Muhaimin Rifa'i³, and Muhammad Sasmito Djati^{3*}

¹Department of Biology, Universitas Negeri Surabaya, 60231 Surabaya, Indonesia

²Master Program, Department of Biology, Universitas Brawijaya, 65145 Malang, Indonesia

³Department of Biology, Universitas Brawijaya, 65145 Malang, Indonesia

⁴Research Center for Vaccine and Drug, National Research and Innovation Agency (BRIN), 16911 Bogor, Indonesia

Abstract. *Cyperus rotundus* is a traditional oriental medicine with high pharmaceutical activity. Triple-negative breast cancer (TNBC) is the most aggressive and malignant kind of breast cancer. This study aims to identify the immunomodulatory effect of *Cyperus rotundus* extract (CRE) by analyzing the production of TNF- α and IFN- γ from T cells and macrophages in 4T1-tumor-bearing mice. The extraction of CRE uses maceration methods with ethanol. This study involved 30 female BALB/c mice (*Mus musculus*), aged 6 - 7 weeks, which were acclimated for one week before being randomly assigned to six groups: (1) Normal - healthy, untreated mice; (2) Cancer - 4T1-tumor-bearing, untreated mice; (3) Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); (4) CRE1 - 4T1-tumor-bearing mice treated with *Cyperus rotundus* extract (72.5 mg/kg BW); (5) CRE2 - treated with 145 mg/kg BW; and (6) CRE3 - treated with 290 mg/kg BW. Cisplatin was administered intraperitoneally once a week for two weeks, and CRE was administered orally daily for two weeks. This study found that a dose of 145 mg/kg CRE suppressed TNF- α production in CD8 cells by 19% (from 11.47% to 9.28%, $p < 0.01$) and decreased IL-10 from Treg by 41% (from 4.65% to 2.73%, $p < 0.05$) compared to the Cancer group. Therefore, it can be concluded that CRE has immunomodulatory activity on 4T1-tumor-bearing mice as a model of TNBC in animals.

1 Introduction

Triple-negative breast cancer (TNBC) is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). TNBC is the most aggressive subtype of breast cancer, and it is difficult to cure due to the absence of receptors that apply to the target of breast cancer therapy [1]. TNBC is the only breast cancer subtype with a lack of targeted treatment and is not sensitive to endocrine

* Corresponding author: msdjati@ub.ac.id

therapy. The aggressiveness and ability to metastasis is the worst of all. TNBC is highly invasive, with distant metastasis found in approximately 46% of TNBC patients, and leads to higher mortality [2].

Inflammation is an essential condition at the initial stage of tumor development, which can recruit and activate an anti-tumor immune response. However, continuous inflammatory stimulation by immune cells causes chronic inflammation and promotes tumor growth and metastasis. Chronic inflammation contributes to immunosuppression, including by recruiting myeloid-derived suppressor cells (MDSCs) that can dampen immune cells' activities by producing cytokine suppressors such as IL-10 and TGF- β or by inducing the expression of immune checkpoint molecules such as PD-L1 and PD-L2 [1].

TNF- α and IFN- γ are cytokines with dual roles and profound pros and cons in cancer progression. These cytokines exhibit anti-cancer activities by stimulating immune cell response and tumor cell deaths. Unfortunately, this scenario is not always as expected. Some studies reported the pro-tumor activities of these cytokines, which depend on the concentration and cellular context [3]. TNF- α and IFN- γ found at very high levels in breast cancer and correlated with poor prognosis and low survival. A study reported that TNF- α stimulation increased TNBC proliferation and invasiveness [4].

Small molecule immunomodulators in cancer therapy show promising effects due to their function in modulating pro- and/or anti-tumor immune cells and their pharmacokinetic properties, such as oral availability and the ability to penetrate tumor/tissue and cell membranes [5]. Herbal medicine can be the source of small-molecule immunomodulators for cancer treatment. One of the herbs with a high immunomodulatory effect is *Cyperus rotundus*. It was known that *Cyperus rotundus* (nutgrass) became a primary staple food in predynastic populations and is currently distributed throughout tropics and subtropics countries. Besides, the ethanolic extract of this plant suppressed tumor-associated macrophages (TAMs) and the production of inflammatory cytokines [6]. Based on *in silico* study, *Cyperus rotundus* extract (CRE) bioactive compounds exhibit an inhibitory potential against PD-L1 immune checkpoint molecule [7].

In the present study, the immunomodulatory effects of CRE were evaluated by focusing on the production of TNF- α and IFN- γ from T cells and macrophages in 4T1-tumor-bearing mice. The selected doses of 72.5, 145, and 290 mg/kg body weight were adopted based on the previous study [8], which demonstrated that these dose ranges of *Cyperus rotundus* extract were effective and safe in modulating immune responses without inducing toxicity.

2 Methods

2.1 Plant extraction

The rhizome powder of *Cyperus rotundus* was sourced from UPT Laboratorium Materia Medica, Batu, East Java. The powdered sample was macerated in absolute ethanol at a ratio of 1:10 (m/v) and continuously stirred for 24 hours at room temperature. The resulting mixture was filtered through filter paper, and the filtrate was concentrated at 50°C using a rotary evaporator (IKA® RV 10, IKA Works (Asia) Sdn. Bhd., Malaysia). The resulting *C. rotundus* extract (CRE) was obtained as a thick paste and stored at 4°C until further use.

2.2 Research design

This study utilized 30 female BALB/c mice (*Mus musculus*), aged 6 - 7 weeks, which were acclimated for one week prior to experimentation. The animals were randomly divided into six groups: **Normal** (healthy, untreated mice; n=4), **Cancer** (4T1-tumor-bearing, untreated

mice; n=4), **Cisplatin** (4T1-tumor-bearing mice treated with cisplatin at 4 mg/kg BW), **CRE1** (treated with *C. rotundus* extract at 72.5 mg/kg BW), **CRE2** (treated with 145 mg/kg BW), and **CRE3** (treated with 290 mg/kg BW). Administration of *C. rotundus* extract began once tumor development was confirmed. Cisplatin was administered intraperitoneally once weekly for two weeks, whereas *C. rotundus* extract was given orally on a daily basis for two weeks.

2.3 4T1-tumor bearing BALB/c mice

The experimental protocol was approved by the Animal Care and Use Committee of Brawijaya University (Ethical Clearance No. 1152-KEP-UB) and conducted in accordance with the EU Directive 2010/63/EU on animal experimentation. The murine mammary carcinoma 4T1 cell line was obtained from the Cancer Chemoprevention Research Center (CCRC), Universitas Gadjah Mada, Indonesia. The cells were cultured in 100 mm Petri dishes containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Gibco™, Thermo Fisher Scientific, USA) in a 5% CO₂ incubator at 37°C. For tumor induction, 100 µL of the 4T1 cell suspension (7.5×10^5 cells/mL) was drawn into a 1 mL syringe and injected subcutaneously into the right flank mammary gland of each mouse once weekly for two consecutive weeks. Tumor development was monitored by palpation every three days. At the end of the second week, three mice were randomly selected and sacrificed to confirm tumor formation through histopathological examination. Tumor tissues were fixed in 4% formalin for analysis. Treatments were initiated in the remaining mice after histopathological confirmation of breast cancer was established.

2.4 Antibody staining and flow cytometry analysis

After the experimental animals were sacrificed, their spleens were collected and processed for analysis. Each spleen was mechanically dissociated and homogenized in phosphate-buffered saline (PBS; Biowest, USA). The resulting cell suspension was centrifuged (HERMLE Z 326 K) at 2500 rpm for 5 minutes at 10°C. The cell pellet was then resuspended and stained with specific monoclonal antibodies for flow cytometric analysis, including:

- FITC anti-mouse CD4 (BioLegend), PE anti-mouse CD8 (BioLegend), and PE/Cy7 anti-mouse IFN- γ (BioLegend)
- FITC anti-mouse CD4 (BioLegend), PE anti-mouse CD8 (BioLegend), and PE/Cy7 anti-mouse TNF- α (BioLegend)
- FITC anti-mouse CD4 (BioLegend), PE anti-mouse CD25 (BioLegend), and PE/Cy5 anti-mouse CD62L (BioLegend)
- FITC anti-mouse CD4 (BioLegend), PE anti-mouse CD25 (BioLegend), and PerCP/Cy5.5 anti-mouse TGF- β (BioLegend)
- FITC anti-mouse CD4 (BioLegend), PE anti-mouse CD25 (BioLegend), and PerCP/Cy5.5 anti-mouse IL-10 (BioLegend)
- FITC anti-mouse CD11b (BioLegend), PE anti-mouse TNF- α (BioLegend), PE/Cy7 anti-mouse IFN- γ (BioLegend), and PerCP/Cy5.5 anti-mouse IL-10 (BioLegend)

After antibody labeling, the cells were washed with PBS and analyzed using a BD FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA). Data acquisition and analysis were performed with FlowJo™ software.

2.5 Data analysis

Data were statistically analyzed using the One-Way ANOVA (Analysis of Variance) method, followed by multiple comparisons of the Tukey-HSD test using GraphPad Prism version 9 and SPSS version 26.0 software. Significant data were indicated by a p-value <0.05. The data was presented in mean \pm standard deviation format (SD).

3 Results

3.1 CRE suppressed TNF- α cytokine production

This study analyzed the production of TNF- α from T helper cell/CD4⁺, T killer cell/CD8⁺, and macrophages/CD11b⁺ of mice splenocytes. The production of TNF- α cytokines varied between the cell sources and groups. However, we found a significant escalation of TNF- α production level from CD4⁺, CD11b⁺, and CD8⁺ cells in the cancer group (3.91%, 3.68%, and 11.47, respectively) which was significantly different compared to control (2.67%, 0.9%, and 7.74%, respectively) (Fig.1-3). In addition, cisplatin and CRE treatment (CRE1-3 groups) can reduce CD4⁺TNF- α ⁺ levels significantly (2.27%, 2.21%, 2.42%, and 2.67%, respectively) (Fig.1). This significant decline also found in CD11b⁺TNF- α ⁺ and CD8⁺TNF- α ⁺ for CRE1 (1.81% and 9.14%) and CRE2 (1.54% and 9.28) (Fig.2-3). However, CRE3 treatment could not reduce CD11b⁺TNF- α ⁺ and CD8⁺TNF- α ⁺ (2.78% and 10.77%) in 4T1-tumor bearing mice (Fig.2-3).

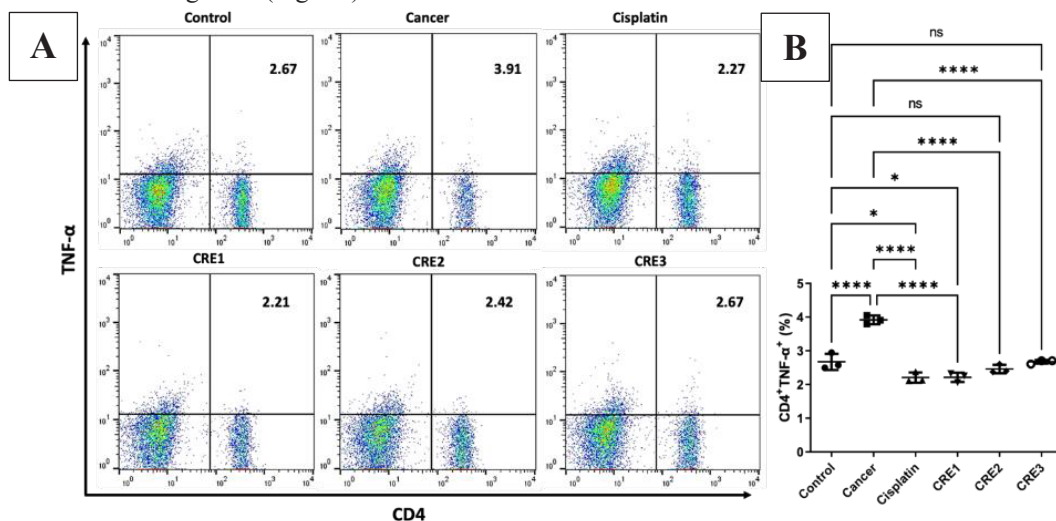


Fig. 1. TNF- α cytokine production level from CD4 T cell (CD4⁺TNF- α ⁺) of mice splenocytes. (A) FACS analysis of TNF- α relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

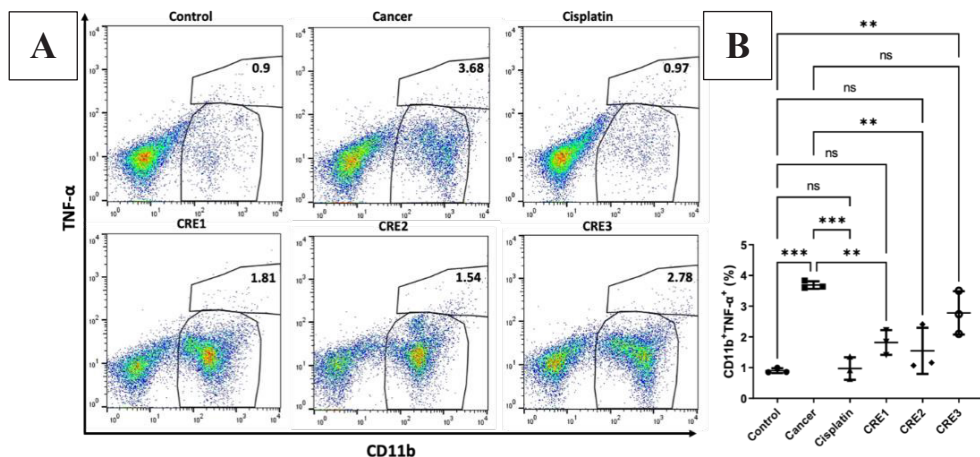


Fig. 2. TNF- α cytokine production level from macrophages (CD11b⁺TNF- α ⁺) of mice splenocytes. (A) FACS analysis of TNF- α relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

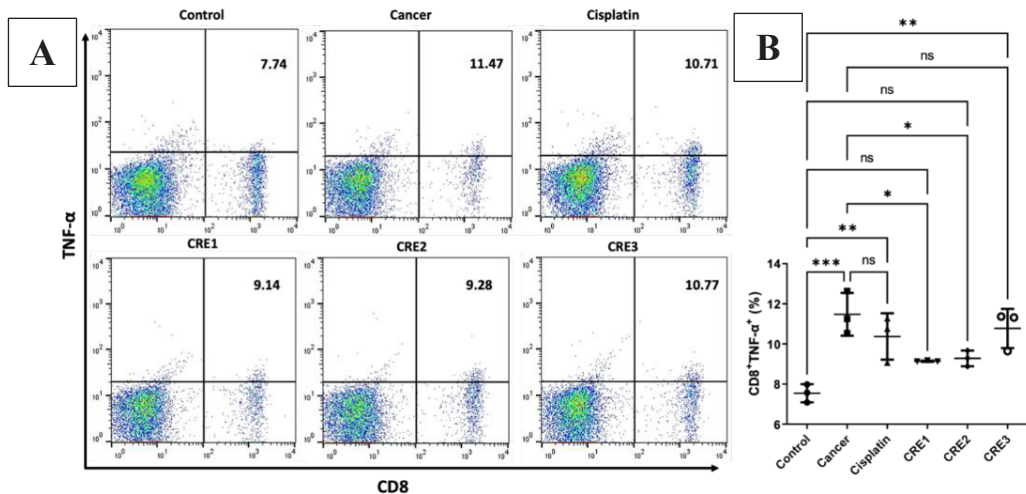


Fig. 3. TNF- α cytokine production level from CD8 T cell (CD8⁺TNF- α ⁺) of mice splenocytes. (A) FACS analysis of TNF- α relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

3.2 CRE suppressed IFN- γ cytokine production

The pro-inflammatory cytokine, IFN- γ , produced by CD4⁺, CD8⁺, and CD11b⁺ were also observed in this study. The highest production of IFN- γ cytokine from CD4⁺, CD8⁺, and CD11b⁺ was found in the cancer group, which is significantly different from the control except for CD4⁺IFN- γ ⁺ (Fig.4-6). By treating the 4T1-tumor bearing mice with cisplatin and CRE, this study found a significant reduction of CD4⁺IFN- γ ⁺, CD11b⁺IFN- γ ⁺, and CD8⁺IFN- γ ⁺ in all treatment groups (Fig.4-6). The level of CD4⁺IFN- γ ⁺ in the cancer group was 1.7% and significantly different compared to cisplatin (1.1%), CRE1 (0.56%), CRE2 (1.02%), and CRE3 (1.05%) ($p < 0.05$) (Fig. 4). A significant different also found in CD11b⁺IFN- γ ⁺ (Fig. 5) where the level of CD4⁺IFN- γ ⁺ was lower in treatment group (Cisplatin; 1.56%, CRE1; 6.39%, CRE2; 9.05%, CRE3; 9.39%) compare to cancer (16.94%) ($p < 0.05$). In addition, cisplatin and CRE treatment also caused a significant reduction of CD8⁺IFN- γ ⁺ level (Cisplatin; 3.27%, CRE1; 2.53%, CRE2; 2.91%, and CRE3; 2.61) compared to the untreated group (3.63%) ($p < 0.05$) (Fig. 6).

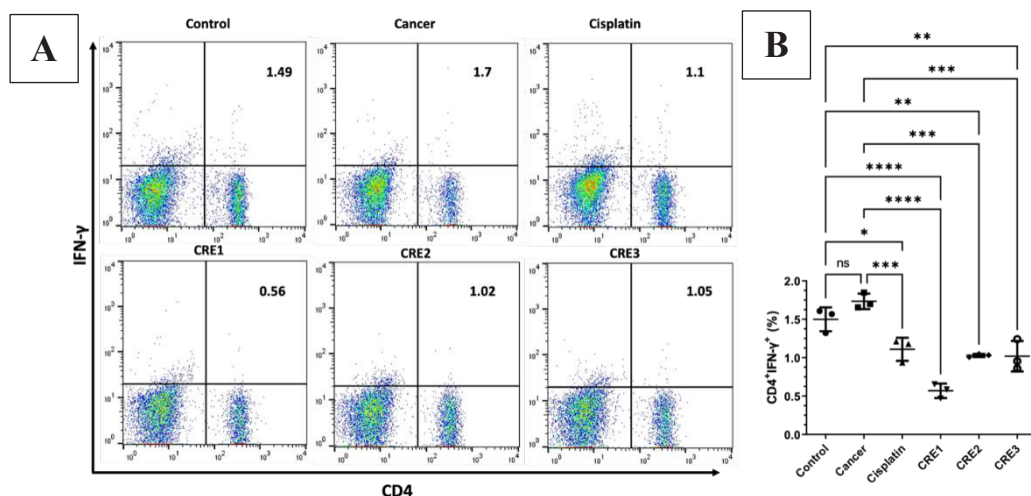


Fig. 4. IFN- γ cytokine production level from CD4 T cell (CD4⁺IFN- γ ⁺) of mice splenocytes. (A) FACS analysis of IFN- γ relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

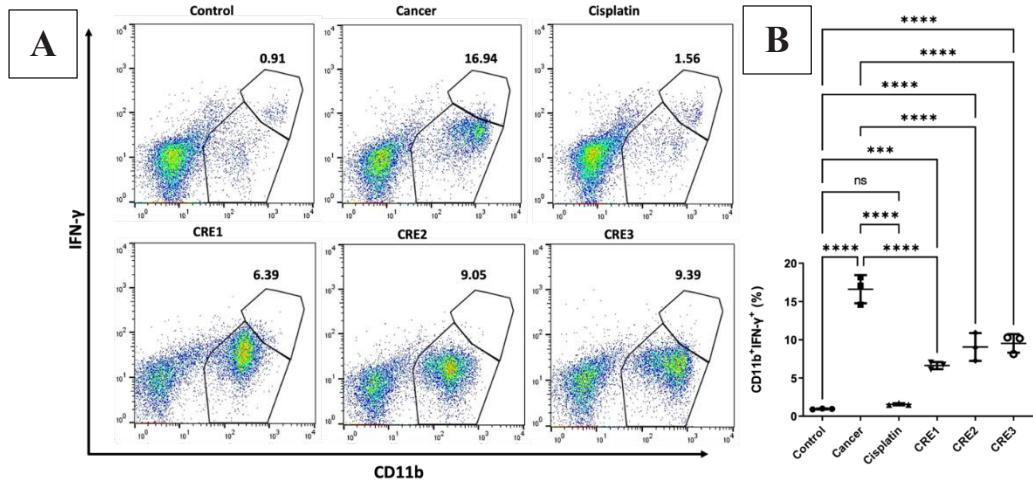


Fig. 5. IFN- γ cytokine production level from macrophages (CD11b⁺IFN- γ ⁺) of mice splenocytes. (A) FACS analysis of IFN- γ relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

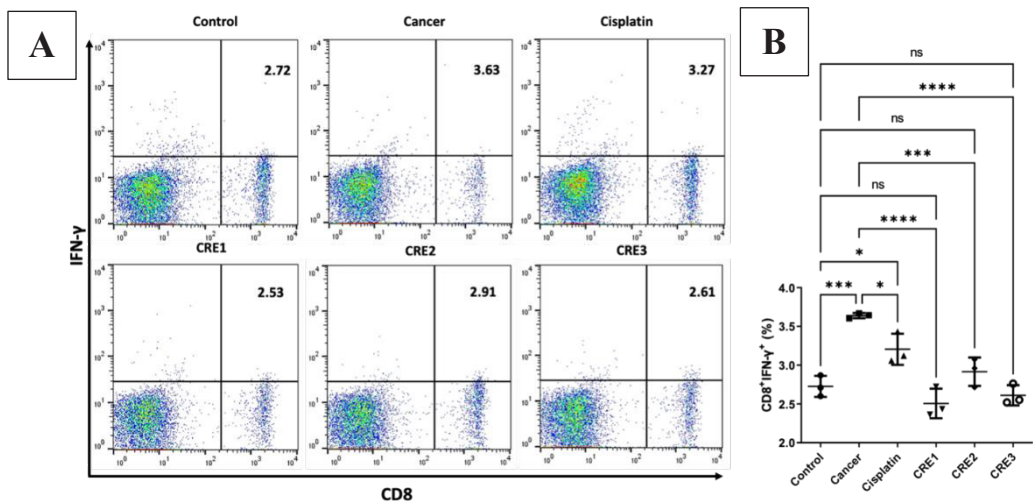


Fig. 6. IFN- γ cytokine production level from CD8 T cell (CD8⁺IFN- γ ⁺) of mice splenocytes. (A) FACS analysis of IFN- γ relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

3.3 CRE suppressed Treg activity and the production of immunosuppressive cytokine (IL-10 and TGF- β)

The activity of T regulatory cells (CD4⁺CD25⁺) and the production of IL-10 and TGF- β from Treg is analyzed in this research. The highest activity of Treg of mice splenocyte was found in the CRE1 group, which is significantly different compared to the control (Fig. 7). The production level of IL-10 and TGF- β from Treg was found lower after the treatment of CRE compared to the cancer group (Fig. 8-9). A significant difference was found in CD4⁺CD25⁺ (Fig. 7), where the level of the treatment group is lower (CRE3; 4.16%) compared to cancer (5.64%) ($p < 0.05$). In addition, CRE1 and CRE3 treatment caused a significant reduction of CD4⁺CD25⁺IL-10⁺ level (CRE1; 2.73%; CRE3; 2.5%) compared to the untreated group (4.65%) ($p < 0.05$) (Fig. 8). Other than that, a significant different found in the production level of CD4⁺CD25⁺TGF- β ⁺ in CRE1-3 (CRE1; 2.56%, CRE2; 2.5%, CRE3; 2.23%) which is lower than the control group (5.65%) ($p < 0.05$) (Fig. 9).

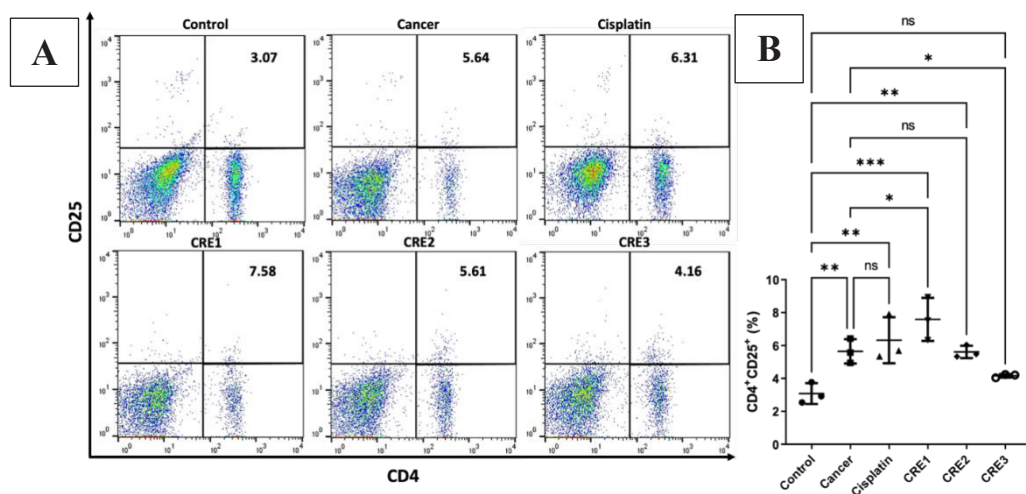


Fig. 7. The level of Treg (CD4⁺CD25⁺) of mice splenocytes. (A) FACS analysis of CD4⁺CD25⁺ relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

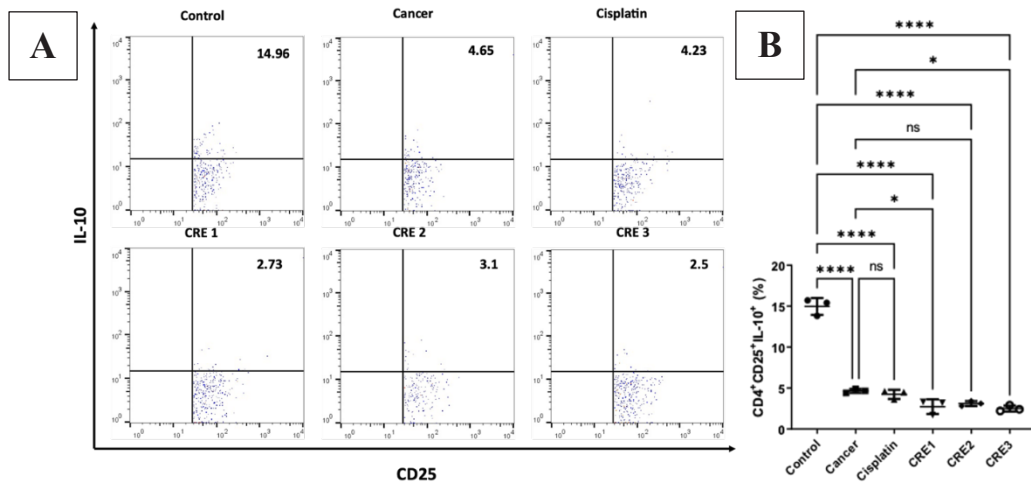


Fig. 8. The production level of IL-10 from Treg ($CD4^+CD25^+IL-10^+$) of mice splenocytes. (A) FACS analysis of $CD4^+CD25^+IL-10^+$ relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

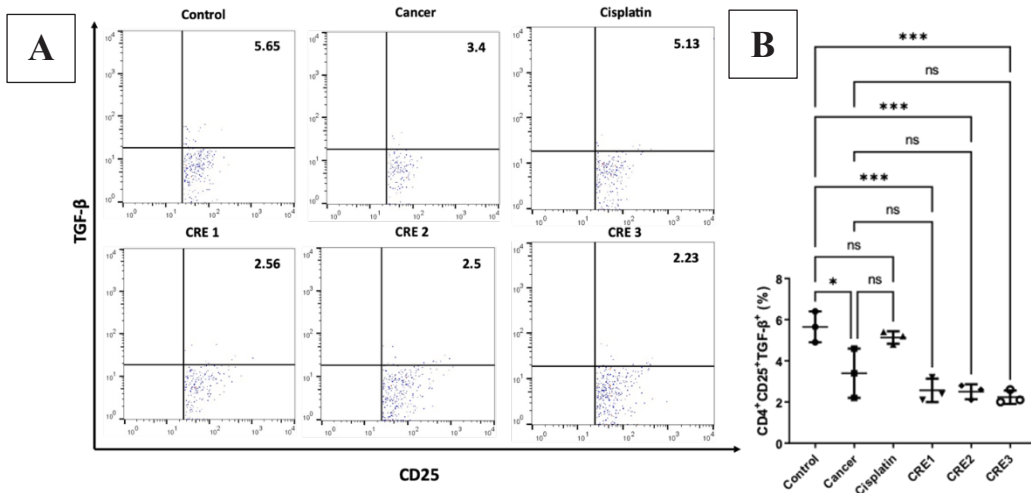


Fig. 9. The production level of TGF- β from Treg ($CD4^+CD25^+TGF-\beta^+$) of mice splenocytes. (A) FACS analysis of $CD4^+CD25^+TGF-\beta^+$ relative number showed in quadrant plots, (B) data are presented as mean \pm standard deviation (SD). Statistical significance was determined using the Tukey-HSD test, with $p < 0.05$ considered significant. **Note:** Control - healthy mice without 4T1 cell injection or treatment; Cancer - 4T1-tumor-bearing mice without treatment; Cisplatin - 4T1-tumor-bearing mice treated with cisplatin (4 mg/kg BW); CRE1, CRE2, and CRE3 - 4T1-tumor-bearing mice treated with *C. rotundus* extract at 72.5, 145, and 290 mg/kg BW, respectively.

4 Discussion

Triple-negative breast cancer (TNBC) is a type of breast cancer that has a survival mode by creating an immunosuppressive environment through Treg activation and immune checkpoint expression. P53 dysfunction is also associated with the occurrence of chronic inflammation, which promotes cancer progression. Chronic inflammation in tumors occurs due to the crosstalk between p53 and NF- κ B, followed by constitutive activation of NF- κ B while p53 activation is suppressed [9]. Thus, modulating Treg and inflammation parameters is needed in TNBC treatment.

Our previous studies have predicted the potential of CRE in blocking PD-L1 immune checkpoint protein using *in silico* research [7], and it may attributed to the enhancement of CD8⁺ T cell activation in 4T1-tumor bearing mice [8]. This study found that CRE can also inhibit pro-inflammatory cytokines such as TNF- α and IFN- γ produced by CD4⁺, CD8⁺, and CD11b⁺ in the spleen. TNF- α is a pleiotropic protein involved in the pathogenesis of multiple physiological processes such as inflammation, anti-tumor response, and immune system hemostasis. The receptors of TNF- α , TNFR1, and TNFR2 have different and opposite functions, which are associated with the paradoxical role in breast cancer, either as an anti-tumor or pro-tumor.

In TNBC, Treg cell is overproduced due to the dysfunction of p53 and becoming a survival mechanism of tumor cells. In breast cancer, Treg can create immunosuppressive conditions and inhibit T cell effector activity through several mechanisms, such as cell-to-cell contact and the production of suppressor cytokines [10]. This study found that CRE can reduce Treg cells in a especially in dose 145 and 290 mg/kg treatment groups. Treg activity was then analyzed by producing suppressor cytokines such as TGF- β and IL-10. TGF- β cytokine was commonly overproduced in the late stage of TNBC and correlated with metastasis cells through EMT induction [11]. In addition, the alteration of TGF- β can lead to drug and chemotherapy resistance [12]. The overproduction of TGF- β and TNF- α correlated with the high production of IL-10 [13]. IL-10 can induce Treg, regulator B cell, and MDSC, inhibiting anti-tumor cell T helper 1 (Th1), dendritic cell (DC), and cytotoxic T cell [14]. The inhibition of TGF- β and IL-10 production from Treg cells by CRE is a promising result of this study. The reduction of The suppression of Tregs and their cytokines is a phenomenon that can be associated with the disruption of the PD-1/PD-L1 axis. Our previous *in silico* data suggests that CRE bioactives may target PD-L1, which could be a plausible mechanism underlying our current findings [7]. However, direct experimental evidence is required to confirm this hypothesis. The PD-1/PD-L1 axis has a role in the development and function of Treg [15]. So, the blockade of PD-1/PD-L1 interaction was believed to correlate with the disruption of Treg activity.

5 Conclusion

The ethanolic extract of *Cyperus rotundus* rhizome inhibited the proinflammatory cytokines TNF- α and IFN- γ produced by T cells (CD4⁺ and CD8⁺) and macrophages (CD11b⁺) in 4T1-tumor-bearing mice. In addition to its anti-inflammatory properties, CRE also suppressed immunosuppression by modulating regulatory T cells (Tregs) and their associated suppressor cytokines, TGF- β and IL-10. Among the tested doses, **145 and 290 mg/kg BW** were identified as the optimal dose, demonstrating the most balanced immunomodulatory response. These findings suggest that CRE possesses significant immunomodulatory potential and may serve as a promising candidate for the development of adjuvant or complementary therapies in the management of triple-negative breast cancer.

The authors gratefully acknowledge the Directorate General of Higher Education, Research, and Technology (DGHERT), Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, for funding this study under contract number 015/E5/PG.02.00.PT/2022.

The authors declare that there is no conflict of interest.

References

1. T. Baram, N. Oren, N. Erlichman, T. Meshel, A. Ben-Baruch, Inflammation-driven regulation of PD-L1 and PD-L2, and their cross-interactions with protective soluble TNF α receptors in human triple-negative breast cancer. *Cancers*. **14**, 3513 (2022). <https://doi.org/10.3390/cancers14143513>
2. E. Garcia, I. Luna, K.L. Persad, K. Agopsowicz, D.A. Jay, F.G. West, M.M. Hitt, S. Persad, Inhibition of triple negative breast cancer metastasis and invasiveness by novel drugs that target epithelial to mesenchymal transition. *Sci. Rep.* **11**, 11757 (2021). <https://doi.org/10.1038/s41598-021-91344-7>
3. M.F. Mercogliano, S. Bruni, P.V. Elizalde, R. Schillaci, Tumor necrosis factor α blockade: An opportunity to tackle breast cancer. *Front. Oncol.* **10**, 584 (2020). <https://doi.org/10.3389/fonc.2020.00584>
4. H. Narasimhan, F. Ferraro, A. Bleilevens, R. Weiskirchen, E. Stickeler, J. Maurer, Tumor necrosis factor- α (TNF α) stimulates triple-negative breast cancer stem cells to promote intratumoral invasion and neovasculogenesis in the liver of a xenograft model. *Biology*. **11**, 1481 (2022). <https://doi.org/10.3390/biology11101481>
5. Y. Wu, Z. Yang, K. Cheng, H. Bi, J. Chen, Small molecule-based immunomodulators for cancer therapy. *Acta Pharm. Sin. B.* **12**, 4287 (2022). <https://doi.org/10.1016/j.apsb.2022.11.007>
6. A.H. Ramadhani, W. Nafisah, H. Isnanto, T.K. Sholeha, Y.D. Jatmiko, H. Tsuboi, M. Rifa'i, Immunomodulatory effects of *Cyperus rotundus* extract on 7,12 dimethylbenz[a]anthracene (DMBA) exposed BALB/c mice. *Pharm. Sci.* **27**, 46 (2020). <https://doi.org/10.34172/PS.2020.61>
7. W. Nafisah, F. Fatchiyah, M.H. Widyananda, Y.I. Christina, M. Rifa'i, N. Widodo, M.S. Djati, Potential of bioactive compound of *Cyperus rotundus* L. rhizome extract as inhibitor of PD-L1/PD-1 interaction: An in silico study. *Agric. Nat. Resour.* **56**, 751 (2022).
8. W. Nafisah, N. Wahyuningsih, M.F. Maulana, S.Z.K. Azmi, M. Rifa'i, N. Widodo, M.S. Djati, *Cyperus rotundus* L. rhizome extract modulates immune system and induces apoptotic in 4T1-tumor bearing mice. *J. Pharm. Pharmacogn. Res.* **11**, 674 (2023). https://doi.org/10.56499/jppres23.1604_11.4.674
9. D. Shi, P. Jiang, A different facet of p53 function: Regulation of immunity and inflammation during tumor development. *Front. Cell Dev. Biol.* **9**, 762651 (2021). <https://doi.org/10.3389/fcell.2021.762651>
10. V. Salemm, G. Centonze, F. Cavallo, P. Defilippi, L. Conti, The crosstalk between tumor cells and the immune microenvironment in breast cancer: Implications for immunotherapy. *Front. Oncol.* **11**, 610303 (2021). <https://doi.org/10.3389/fonc.2021.610303>
11. R. Vishnubalaji, N.M. Alajez, Epigenetic regulation of triple negative breast cancer (TNBC) by TGF- β signaling. *Sci. Rep.* **11**, 15410 (2021). <https://doi.org/10.1038/s41598-021-94514-9>

12. X. Xu, L. Zhang, X. He, P. Zhang, C. Sun, X. Xu, Y. Lu, F. Li, TGF- β plays a vital role in triple-negative breast cancer (TNBC) drug-resistance through regulating stemness, EMT and apoptosis. *Biochem. Biophys. Res. Commun.* **502**, 160 (2018). <https://doi.org/10.1016/j.bbrc.2018.05.139>
13. E. Sheikhpour, P. Noorbakhsh, E. Foroughi, S. Farahnak, R. Nasiri, H. Neamatzadeh, A survey on the role of interleukin-10 in breast cancer: A narrative. *Rep. Biochem. Mol. Biol.* **7**, 30 (2018).
14. B. Mirlekar, Tumor promoting roles of IL-10, TGF- β , IL-4, and IL-35: Its implications in cancer immunotherapy. *SAGE Open Med.* **10**, 1 (2022). <https://doi.org/10.1177/20503121211069012>
15. J. Cai, D. Wang, G. Zhang, X. Guo, The role Of PD-1/PD-L1 axis in Treg development and function: Implications for cancer immunotherapy. *OncoTargets Ther.* **12**, 8437 (2019). <https://doi.org/10.2147/OTT.S221340>