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Original Research Article

Immunomodulatory effect of *Artemisia vulgaris* L. ethanol extract and its marker compound on antibody and cytokine release in non-immunosuppressed and immunosuppressed rats

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ABSTRACT

Artemisia vulgaris L., a medicinal herb from Tanah Karo, North Sumatra, has been traditionally utilized for treating diseases. The purpose of this study was to assess the immunomodulatory effects of *Artemisia vulgaris* L. extract on rats. The effects were assessed by measuring the total and differential leukocyte count, as well as Interleukin 2 (IL-2) and Immunoglobulins G (IgG) production. The rats were divided into two groups: non-immunosuppressed and immunosuppressed rats. Both groups received the extract daily from day 1 to day 14. On day 4, all rats were inoculated with *Staphylococcus aureus* bacteria, while the immunosuppressed group also received cyclophosphamide (70 mg/kg body weight/BW) on days 8 and 13. The extract given at doses of 50, 100, 200, and 400 mg/kg as well as quercetin as a marker compound, increased the total and differential leukocyte count in both non-immunosuppressed and immunosuppressed rats. The highest dose of 400 mg/kg BW showed the greatest effects, with increased activity in lymphocytes (25.66±0.15), neutrophils (49.76±0.03), basophils (2.32±0.07), eosinophils (1.35±0.10), and monocytes (2.69±0.15) in non-immunosuppressed rats. In the immunosuppressed rats, the same dose led to increases in lymphocytes (24.18±0.05), neutrophils (43.14±0.02), basophils (2.32±0.07), eosinophils (2.14±0.04), and monocytes (2.11±0.06). Additionally, the levels of IL-2 and IgG were higher in both groups treated with the extract and quercetin as compared to those of the negative control. The extract at the dose of 400 mg/kgBW and quercetin demonstrated stronger immunostimulatory effects than levamisole. The study concluded that *Artemisia vulgaris* L. exhibits immunomodulatory activity, acting as an immunostimulatory agent. with potential for development as a therapeutic treatment.

Keywords: *Artemisia vulgaris* L., *Leukocyte, IL-2, IgG, Imunostimulatory activity.*

Introduction

Pathogenic microbes, widely present in the environment, can cause infections in humans. The immune system's physiological regulation may weaken under certain conditions, necessitating innovative approaches for developing new drug candidates. These include immunomodulators that can modulate the immune system.[1,2]

Macrophages play a crucial role in phagocytosis and as antigen-presenting cells (APCs), making them vital to the immune system. They engulf foreign material and present these antigens to T cells and B cells.[3] Following vascular changes, neutrophils adhere to endothelial cells and migrate out of the vessels into tissues.[4] Activated macrophages release various mediators involved in inflammatory reactions, while neutrophils ingest antigens and release cytokines and other mediators crucial for the inflammatory response. Activated tissue macrophages also release cytokines.[5,6]

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The assessment of immune system activity involves its specific effects on secondary metabolites and immune functions. Studies on natural immunomodulators have been traditionally used to treat various immune-mediated diseases.[7] *Artemisia vulgaris* L., used empirically to treat skin infections and hypertension, contains saponins, flavonoids, and polyphenols. Its reported benefits include antimalarial, antihelmintic, antibacterial, antioxidant, and antidiabetic properties.[8] Traditionally used in China as both medicine and food, *Artemisia vulgaris* L. has anti-inflammatory, antibacterial, antitumor, antiviral, antioxidant properties, and is used in treating herpes simplex.[9] However, its immunomodulatory effect on non-immunosuppressed and immunosuppressed state is very rare reported.[10] IL-2, identified as a crucial cytokine, controls immune responses and maintains tolerance. Its absence or blocked signal transmission can cause autoimmune diseases due to a deficiency in T-suppressors. IL-2 is crucial for the strength and longevity of both primary and memory immune responses. It plays a key role in suppressing immune reactions and is necessary for the growth of activated T cells through its binding to a high-affinity membrane receptor. IL-2 also affects various cellular functions, such as natural killer cell activity and antibody production, acting as both a growth factor and a signal for differentiation. In most humoral antibody responses, whether targeting IgG mediated effector functions play a role in responding to viral or cellular pathogens. Immunoglobulins D (IgD) mainly found in the tonsils, has an enigmatic function.[12] This research was carried out to examine the immunomodulatory effects of *Artemisia*

vulgaris L. and its marker compound on IgG and IL-2 release in non-immunosuppressed and immunosuppressed rats.

Materials and Methods

Chemicals and Reagents

The chemicals employed consisted of cyclophosphamide (Dankos), Quercetin (Sigma Chemical), levamisole® (Konimex Pharmaceutical Laboratories, Solo-Indonesia), Phosphate Buffer Saline (Irvine Scientific), wash buffer, assay diluent, standard protein Ig-G, standard protein IL-2, streptavidin-HRP conjugate, tetramethylbenzidine (TMB), and stop solution (all from Komabiotech).

Plant Materials

The plants of *Artemisia vulgaris* L. (2.5 kg) were collected in Kabanjahe, Karo Region, (2º50'N, 97º55'E), Sumatera Utara, Indonesia, and the sample was identified in Indonesian Institute of Sciences, with voucher specimen number of 1889/IPH.1/DI.05.07/If.07/VIII/2017 in 4 August 2017 . The plant material was air-dried for 20 days at 25°C, and powdered with a mechanical grinder

Extraction procedure

Five hundred grams of the dried powder underwent was macerated in 5 L ethanol for 24 hours, with initial stirring for the first 6 hours, followed by resting for 18 hours. The filtrate was then collected and evaporated.[13]

Animals

Male Wistar rats with a weight of 200-300 g were used and the rats were acclimatized in the experimental room for 7–14 days at room temperature with the conditions of 12 h of light and 12 h of darkness. All procedures were evaluated by the Animal Research Ethics Committee (AREC) of the University of Sumatera Utara (0135/KEPH-FMIPA/2021). The rats Seven formulas were prepared and divided into two treatment groups: non-immunosuppressed and immunosuppressed rats. The rats received the extract from the first to the 14th day. All groups were sensitized to *S. aureus* (1 x 10^8 cells/mL) by intraperitoneal injections on day 4. The immunosuppressed group received cyclophosphamide (70 mg/kgBW) on days 8 and 13. Treatments included a negative control, *Artemisia vulgaris* L. extract (50, 100, 200 mg/kgBW), levamisole, and Quercetin. On the 15th day, the rats were dissected, and from the heart for serum and plasma separation. Blood samples were collected. in Green Vac-Tubes and centrifuged (Centrifugase Gemmy, PLC 05, Taiwan) at 3000-4000 rpm for 15 minutes to separate the supernatant and precipitate.[14]

Measurement of Total Leukocyte Count and Leukocyte Differential

On the 15th day, 1 mL of blood was collected in an anticoagulant tube for total leukocyte count and differential analysis. [25]

Measurement of IgG and IL-2 Levels

The supernatant was transferred into a microtube after centrifugation of the blood samples at 3000-4000 rpm for 15 minutes. For the assay, the plate was washed three times with 300 µL wash buffer in each well. Following the final wash, the plate was flipped upside down to drain any remaining solution. Then, 100 µL of standards, *Artemisia vulgaris* L. extract (50, 100, 200 mg/kg body weight), and negative control were added to each well, and the plate was incubated for 2 hours at 37°C in the dark. After washing the plate four times with wash buffer, 100 µL of detection antibodies were added to each well and incubated for an in the dark room. The color change was observed upon adding 100 µL SDS to each well. Absorbance was measured using a microplate reader (Biorad, Model 680, USA) at 450 nm, and levels of IgG and IL-2 were calculated accordingly.[14]

Statistical Analysis

All results were presented as mean ± standard error of the mean (SEM), and statistical comparisons were conducted using GraphPad Prism 5 software (San Diego, CA, USA). Statistical significance between groups was assessed using a one-way ANOVA followed by Tukey's post-hoc analysis.

Results and Discussion

Simplicia and extract from *Artemisia vulgaris* L. extract contained chemical compounds, which include alkaloids, flavonoids, tannins, saponins, and glycosides. According to Marbun et al. that *Artemisia vulgaris* L. contains flavonoids potential as an immunostimulant.[26] Leukocytes are white blood cells produced by the hemopoietic tissue for granulated cells (polymorphonuclear) and the lymphatic tissue for non-granulated (mononuclear) cells, which contribute to the body's defense system against microbial invasion.[16] leukocyte count varies in response to the number of invading microbes, adjusting to levels that the body can tolerate without functional impairment.

The results of the leukocyte count are presented in Table 1. It showed *Artemisia vulgaris* L. extract differs significantly from negative control group in non-immunosuppressant and suppressant rats ($p < 0.05$). It can increase leukocyte production. This research demonstrated that cyclophosphamide significantly reduced the total leukocyte count. This decrease is a well-known acute effect of cyclophosphamide administration, mainly due to its suppressive impact on the bone marrow, which interferes with the production of blood cells, including leukocytes.[17] However, the study found that the extract, rich in flavonoids, was effective in increasing the total leukocyte count by activating the lymphatic system, thereby boosting white blood cell production. Moreover, the extract led to an increase in various leukocyte types, such as lymphocytes, neutrophils, eosinophils, basophils, and monocytes, in both immunosuppressed and non-immunosuppressed rats. This effect is attributed to the flavonoid compounds in the extract, particularly quercetin, this can affect the action of lymphokines produced by T cells, thereby stimulating phagocytic cells to boost their phagocytic response. The stem cells then differentiate into two main pathways: myeloid and lymphoid. The myeloid pathway gives rise to polymorphonuclear granulocytes, including basophils, neutrophils, eosinophils, macrophages, and platelets.[19,20] Influence of extract on these counts in non-immunosuppressed and immunosuppressed rats are depicted in Figures 1 and 2. Figures 1 and 2 showed extract differs significantly from negative control in non-immunosuppressant and suppressant rats. *Artemisia vulgaris* L. extract can increase basophils, neutrophils, eosinophils, and monocytes/macrophages production. The effect of *Artemisia vulgaris* L. on IgG levels were examined using the ELISA method, which involved reading absorbance with a microplate reader at a wavelength of 450 nm. IgG levels against *Artemisia vulgaris* L. treatment (50; 100; and 200 mg/kgBW), results were obtained by measuring absorbance with the addition of a standard solution of 7.8125; 15.625; 31.25; 62.5; 125; 250; and 500 ng/mL. The effect of the extract on IgG levels in non-immunosuppressed and immunosuppressed rats is shown in Figure 3. Figure 3 showed effect of *Artemisia vulgaris* L. increasing Ig-G antibody levels in immunosuppressed rat infected with *S, aureus* differed significantly with negative controls ($p < 0.05$).

The impact of treatment on IgG levels in immunosuppressed and non-immunosuppressed animals revealed a significant effect on IgG level changes. Specifically, the extract led to notable alterations in IgG levels in both groups of rats. The rise in immunoglobulin G levels may be due to the flavonoid and saponin content in the extract.[20] Flavonoids, possessing hydroxyl groups, can donate electrons and act as antioxidants.[21] Previous research has highlighted the role of flavonoids and saponins as immunostimulators, as demonstrated in studies using ceplukan leaf infusion, which is known to increase B lymphocyte proliferation and differentiation, thereby boosting immunoglobulin production. Adaptive immunity can be acquired either through the transfer of preformed antibodies from another host (passive immunity) or through contact with environmental or microbial antigens (active immunity). Antibodies, also known as Ig specifically bind to a single antigen through the interaction between the Fab region of the antibody molecule binds to the antigen, while other regions of the antibody interact with various components of the immune system, such as phagocytes.[22] Examination of the effect of the extract on IL-2 was read with a microplate reader at 450 nm. IL-2 levels following treatment with extract (50; 100; 200; and 400 mg/kgBW) were determined by

Table 1: Results of Leukocyte Count in Non-Immunosuppressed and Immunosuppressed Rats (n = 4; Data Presented as Mean ± SEM).

Group of treatment	Leukocyte 10^9/L (Mean±SEM)	
	Non-immunosuppressant	Immunosuppressant rat
Negative Control	10.11 ± 0.04	9.82± 0.02
Levamisole®	24.29 ± 0.06*	22.32± 0.02*
Quercetin	25.57±0.15*	23.93± 0.07*
Artemisia vulgaris L. 50 mg/kg BW	10.28±0.14*	8.41± 0.01*
Artemisia vulgaris L. 100 mg/kg BW	11.35± 0.09*	9.93± 0.07*
Artemisia vulgaris L. 200 mg/kg BW	18.65±0.08*	15.05± 0.02*
Artemisia vulgaris L. 400 mg/kg BW	24.30±0.03*	23.93± 0.008*

Information:
*Sig (P) < 0.05, there are significant differences with the negative control group.

Table 2: Effect of *Artemisia vulgaris* L. Extract on IL-2 Levels in Non-Immunosuppressed and Immunosuppressed Rats (n=4; Data Presented as Mean ± SEM)

Group of treatment	IL-2 levels 10^9/L (Mean±SEM)	
	Non-immunosuppressant	Immunosuppressant rat
Negative Control	244.72±9.02	234.62±3.02
Levamisole®	846.02±5.23*	816.02±4.1*
Quercetin	871.86±5.44*	791.86±4.2*
Artemisia vulgaris L. 50 mg/kg BW	435.51±3.72*	398.22±4.6*
Artemisia vulgaris L. 100 mg/kg BW	580.72±4.05*	520.31±4.02*
Artemisia vulgaris L. 200 mg/kg BW	653.13±4.02*	623.22±5.01*
Artemisia vulgaris L. 400 mg/kg BW	796.01±4.44*	746.14±5.2*

Information:
*Sig (P)<0.05, there are significant differences with the negative control group.

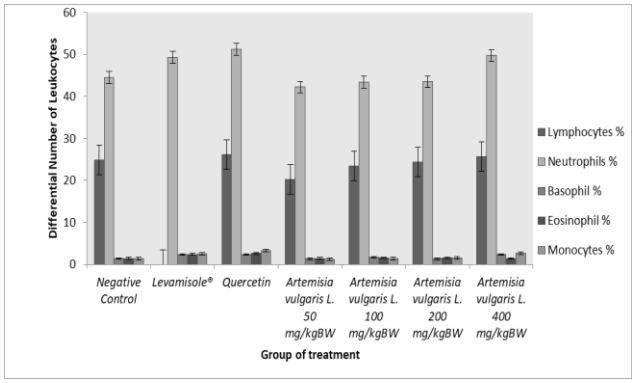

Figure 1: Influence of *Artemisia vulgaris* L. Extract on Differential Leukocyte Count in Non-Immunosuppressed Rats.

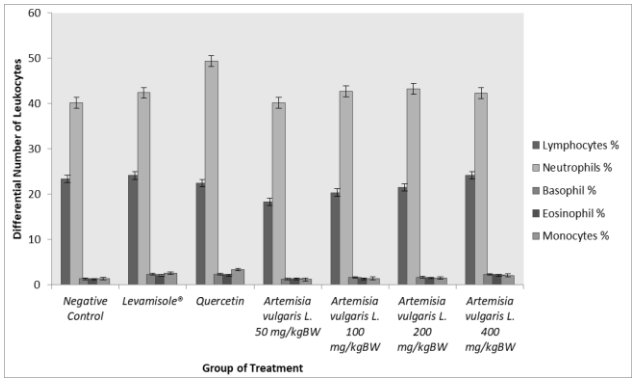

Figure 2: Influence of *Artemisia vulgaris* L. Extract on Differential Leukocyte Count in Immunosuppressed Rats

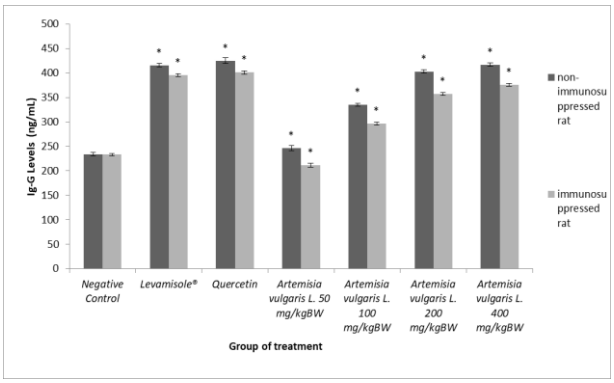

Figure 3: Comparison of IgG Levels in Non-Immunosuppressed and Immunosuppressed Rats (Data: Mean ± SD, n=4; *P*<0.05 Significant Compared to Negative Control).

measuring absorbance after adding standard solutions of 62;125; 250; 500; 1000; 2000; and 4000 pg/mL. The effect of extract on IL-2 levels in non-immunosuppressed and immunosuppressed rats can be seen in Table 2. It showed effect of *Artemisia vulgaris* L. increasing IL-2 antibody levels in immunosuppressed rat infected with *Staphylococcus aureus* differed significantly with negative controls ($p < 0.05$). Regarding the impact on IL-2 levels, the treatment showed a significant effect on IL-2 level changes in both immunosuppressed and non-immunosuppressed animals. This study found that cyclophosphamide markedly reduced IL-2 levels, which aligns with the acute responses to its administration. The notable factors contributing to cyclophosphamide's immunosuppressive effects include reduced T cell proliferation and diminished Th1 cell cytokine secretion.[23] The boost in IL-2 levels could be attributed to the Quercetin content. As a flavonol compound derived from flavonoids, Quercetin is vital in boosting the immune system, particularly by enhancing IL-2 activity and promoting lymphocyte proliferation. IL-2 is a crucial cytokine for regulating the immune response and acts as a mitogen for T cells.[24] Different

secondary metabolites found in *Artemisia vulgaris* L. could potentially be accountable for its stimulatory activity, such as flavonoids, saponins, and terpenoids. A previous study indicated that flavonoid boost the immune response. The mechanism by which flavonoids and saponins contribute to an increase in IgG involves augmenting IL-2 activity and promoting lymphocyte proliferation.[25] The stimulation was noted in both the healthy rats and the cyclophosphamide-induced rats, suggesting that *Artemisia vulgaris* L. has the potential to increase protection against harmful microorganisms in both healthy and compromised immune responses. IL-2 is essential for promoting the differentiation and proliferation of B-lymphocytes. This process of B-lymphocyte differentiation is crucial during the developmental stage, as it regulates the production of immunoglobulin (Ig). It has the potential to be developed into an effective agent for modulating the immune system.[26]

Conclusion

The findings of this research suggest that *Artemisia vulgaris* L. is capable of increasing the total and differentials leukocyte count, IgG, and IL-2 levels in both immunosuppressed and non-immunosuppressed rats. The extract of *Artemisia vulgaris* L. shows promise as a candidate for development into a commercially viable immunomodulating product. However, to validate its effectiveness in humans, further research, specifically clinical trials, is necessary.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Conflict of Interest

The authors declare no conflict of interest.

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