

IMMUNOMODULATORY EFFECTS OF ETHANOL EXTRACT OF *ARTEMISIA VULGARIS* L. IN MALE RATS

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## ABSTRACT

**Objective:** This current study was conducted to investigate the immunomodulatory effects of ethanol extract of *Artemisia vulgaris* in male rats.

**Methods:** Immunomodulatory activity was determined by measuring the carbon elimination rate, the total number of leukocytes and differential, as well as antibody titer. The treatment groups were divided into six groups, each group consisting of five male rats. The extract at doses of 50, 100, 200, and 400 mg/kg bw was administered orally for 7 days. The carbon elimination was measured using an ultraviolet-visible spectrophotometer.

**Results:** The ethanol extract of *A. vulgaris* at doses of 50, 100, 200, and 400 mg/kg body weight increased the carbon elimination and increased the number of total leukocytes and differential leukocytes, especially neutrophils and neutrophils rod segments as compared to the negative control group (CMC-Na 0.5%) ( $p < 0.05$ ).

**Conclusion:** The ethanol extract of *A. vulgaris* possesses immunomodulatory effect.

**Keywords:** Immunomodulatory, *Artemisia vulgaris*, Carbon clearance, Leukocyte.

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## INTRODUCTION

Environment around us have different kinds of pathogens such as bacteria, virus, fungus, protozoa, and parasites that can cause infections in humans. Infections that occur in normal individuals are generally short and rarely leave permanent damage. This is due to the human body has a system called the immune system to respond and protect the body against the elements pathogens that can cause disease [1].

This system serves to protect the body from foreign substances that enter the body so that the function is not impaired. The immune system is beneficial for the prevention of a disease due to infection can be obtained naturally [2]. However, the natural immune system alone is not sufficient. Thus, an artificial immune system is also required by our body. Artificial immunity may be given by means of immunization. Immune response will be modulated by administering immunomodulatory agents [3].

Disease mediated by the immune system is a significant problem, such as HIV and ebola viruses' infection that requires an aggressive and innovative approach to the development of new treatments [4], thus should be given booster or immune enhancers. Among the many herbs used, one of them is *Artemisia vulgaris* L. which is a plant of family Compositae.

Based on previous study, Binara plant (*A. vulgaris* L.) contains essential oils, saponins, flavonoids, and polyphenols. Previous studies also suggested *A. vulgaris* L. containing constituents such as tannins, polyphenols, flavonoids, saponins, minerals, and essential oil. Some of *Artemisia* spp. have been reported to have antimalarial activity, cytotoxic, antihepatotoxic, antibacterial, antifungal, and antioxidant activities [5].

Previous studies reported that *Artemisia tripartite* leaves have immunomodulatory activity [6]. *Artemisia argyi* is also widely used as antitumor and has immunomodulatory activity. Temraz and Walid suggest that *A. vulgaris* L. has antioxidant activity. James *et al.* reported that utilization of species *Artemisia* has also been widely used in the world [7]. Investigation of the effect on immune system can be performed by various methods such as hemagglutination titer

antibodies, delayed-type hypersensitivity response, and phagocytic activity test using carbon clearance method [8].

Phagocytic activity evaluation by using carbon clearance method is an established method to determine the non-specific immune system. Clearance carbon method is used to measure the activity of phagocytic cells to kill pathogens that enter the body and count the number of leukocyte cells. The hemagglutination titers of antibodies are a valuation of the specific immune system by measuring visual hemagglutination [8]. This current study was conducted to evaluate the effect of *A. vulgaris* extract on phagocytosis activity, total leukocytes and differential leukocytes, as well as antibody titer.

## MATERIALS AND METHODS

## Materials

The chemicals used in this study were ethanol (SmartLab, Indonesia) and natrium carboxymethyl cellulose (Na CMC) (Sigma, USA), imboost® (Soho, Indonesia), China ink (pelican B-17), acetic acid (SmartLab, Indonesia), and NaCl (Otsuka, Indonesia). Standard reagents for phytochemical screening were also used. A spectrophotometer (Shimadzu, Japan) was also used in this study.

## Plant materials

The plants of *A. vulgaris* were collected from Medan, Sumatera Utara, Indonesia. Then, the plant was authenticated in Research Centre for Biology, Bogor, Indonesia.

## Phytochemical screening

Phytochemical screening was performed to evaluate the presence of compounds such as alkaloids, flavonoids, glycosides, anthraquinone glycosides, saponins, tannins, and steroids/triterpenoids.

## Extraction procedure

The plant materials were allowed to dry under shade. An amount of 1 kg of dried material of plant sample was ground and macerated in ethanol. The extraction was repeated twice on the residue. The filtrates were combined and the solvent was removed under reduced pressure to obtain extract of *A. vulgaris*.

### Phagocytosis assay

The phagocytosis ability was evaluated by carbon clearance method [9]. The extract at doses of 50, 100, 200, and 400 mg/kg bw was administered orally for 7 days. On day 8, blood sample was collected as a blank (minute 0), then 0.1 ml/10 g bw carbon ink suspension was injected intravenously through a vein in the tail, then 25 µl blood samples were collected at min 5, 10, 15, and 20 after the injection of carbon. Then, 4 mL of 1% acetic acid was added to lyse the red blood cell. The measurement of absorbance was detected at a wavelength of 640.5 nm using an ultraviolet (UV)-visible spectrophotometer. After 12 h of blood collection, the rats were sacrificed; the liver and spleen of mice were taken and weighed [10].

### Measurements of total leukocytes and differential leukocyte

Figure 2 shows that *A. vulgaris* extract increased the number of total leukocytes and differential leukocytes, especially neutrophils and neutrophils rod segments as compared to the negative control group (CMC-Na 0.5%) ( $P < 0.05$ ). Examination of blood leukocyte cell count and differential leukocyte cell used blood on phagocytosis procedures. On day 8, 1 ml of blood was drawn and inserted into the tube which already contained Na-citrate to examine the number of total leukocyte and differential leukocyte cells.

### Data analysis

Data were analyzed with SPSS using one-way ANOVA to determine an average difference among treatments. If there are differences, followed by Tukey *post-hoc* test was used to determine which variables that have differences. Based on the significant, value of  $p < 0.05$  was considered statistically significant.

## RESULTS

Phytochemical screening of *A. vulgaris* is shown in Table 1.

Simplicia and extract contained chemical compounds, which include alkaloids, flavonoids, tannins, saponins, and glycosides [11]. According to Ghosh *et al.* that *A. vulgaris* L. contains flavonoids potential as an immunostimulant [12].

Constant elimination of carbon is one of the parameters used to determine the rate of phagocytosis. The greater the carbon elimination constant value, the higher the carbon clearance rate, which means faster phagocytic cells perform phagocytosis [10].

### Phagocytosis index

The extract of *A. vulgaris* increased phagocytosis activity in a dose-dependent manner. Increasing phagocytic index showed an increase in phagocytic activity of macrophages and an increase in non-specific immunity. Macrophages are responsible for conducting the process of elimination, especially in the liver and the rest are in the spleen [10].

### Stimulation index

Table 3 shows the stimulation index value of ethanol extract of *A. vulgaris* on phagocytic activity. Stimulation index is a comparison between the test group and control group. Previous research declared a substance is an immunostimulatory agent if the stimulation index  $< 1$  and is immunosuppressive if the stimulation index is smaller than 1 [13].

## DISCUSSION

Carbon elimination rate is a method used to measure the phagocytic activity in mice. Carbon will diminish in number in the blood along with the increase of time, because of the events of phagocytosis by cells of leukocytes, especially neutrophils, monocytes, macrophages, and eosinophils [13]. Carbon elimination rate was measured at minute 5, 10, 15, and 20. The elimination rate of carbon was measured by UV-visible spectrophotometer at a wavelength of 640.5 nm. The average value of the constant elimination of carbon can be seen in Table 2.

**Table 1: Results of phytochemical screening of *A. vulgaris***

No	Screening of	Dried powdered	Extracts
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannin	+	+
4	Saponin	+	+
5	Glycosides	+	+
6	Steroids/triterpenoids	-	-

+: Contains classes of compounds, -: Not containing the compound.

*A. vulgaris*: *Artemisia vulgaris*

**Table 2: Results of constant value carbon elimination ( $\pm$ SEM)**

No.	Group treatment	Carbon elimination constant (mean $\pm$ SEM)
1	CMC Na 0.5%	0.0645 $\pm$ 0.0013 (+)
2	Imboost 32.5 mg/kg	0.0041 $\pm$ 0.0002 (*, +)
3	<i>A. vulgaris</i> extract 50 mg/kg bw	0.0183 $\pm$ 0.0039 (*, +)
4	<i>A. vulgaris</i> extract 100 mg/kg bw	0.0154 $\pm$ 0.0013 (*, +)
5	<i>A. vulgaris</i> extract 200 mg/kg bw	0.0110 $\pm$ 0.0010 (*, +)
6	<i>A. vulgaris</i> extract 400 mg/kg bw	0.0085 $\pm$ 0.0004 (*, +)

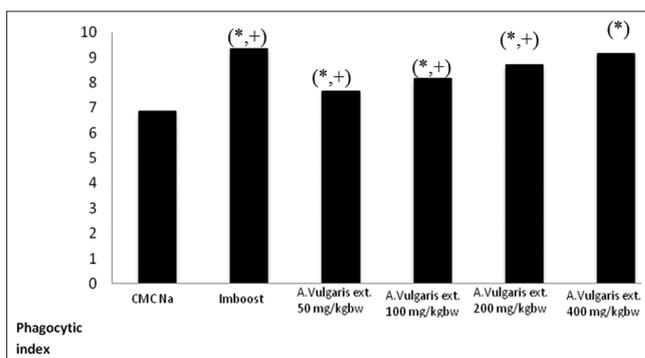
\* $p < 0.05$ , significant to the CMC Na 0.5%, + $p < 0.05$ , significantly to Imboost®.

*A. vulgaris*: *Artemisia vulgaris*, SEM: Standard error of the mean

**Table 3: Stimulation index values**

Treatment group	Stimulation index
CMC Na 0.5%	1.11
Imboost 32.5 mg/kgBB	1.36
<i>A. vulgaris</i> extract 50 mg/kg bw	1.11
<i>A. vulgaris</i> extract 100 mg/kg bw	1.19
<i>A. vulgaris</i> extract 200 mg/kg bw	1.27
<i>A. vulgaris</i> extract 400 mg/kg bw	1.34

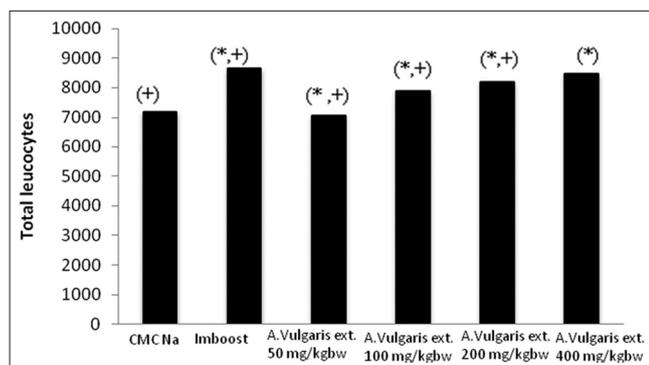
*A. vulgaris*: *Artemisia vulgaris*



**Figure 1: Phagocytic index in male rats (mean  $\pm$  standard error of the mean,  $n=5$ ) \* $p < 0.05$ , significant to the CMC Na 0.5%. + $p < 0.05$ , significant to the suspension Imboost®**

The ethanol extract of *A. vulgaris* increased the number of leukocytes, especially neutrophils. Neutrophils are the first leukocytes recruited to the site of infection. They play major role in innate immune response [15].

Fig. 1 shows that the negative control group suspensions CMC Na 0.5% to 6.8529 phagocytosis index was significantly different ( $p < 0.05$ ) with the other treatment groups. The extract at 50 mg/kg bw has 7.637 phagocytosis index which was significantly different ( $p < 0.05$ ) with the other treatment groups. The extract at 100 mg/kg bw with an index of phagocytosis 8.1513 and 200 mg/kg with 8.6964 phagocytosis index did not have a statistically significant difference ( $p > 0.05$ ) but differ significantly with 400 mg/kg with the phagocytic index of 9.1603 and Imboost® with phagocytic index of 9.3186.



**Figure 2: Result of measurement of white blood cell count of rats (mean±standard error of the mean). \*p<0.05, significant to the CMC Na 0.5%, +p<0.05, significant to the Suspensi Imboost®**

The extract at 400 mg/kg and Imboost® did not has a statistically significant difference ( $p>0.05$ ).

The extract at 50, 100, 200, and 400 mg/kg bw showed that their relationship with the dose increase stimulation index value, the greater the increase in the dose of stimulant-derived index value increases. It shows that *A. vulgaris* is a substance that is immunostimulatory agent.

Compounds that may act as an immunostimulant are flavonoids and alkaloids. This is consistent with previous study by Prastiwi *et al.*, which states that the alkaloids, saponins, and tannins have immunostimulatory activity as that alleged immunostimulatory activity of *A. vulgaris* may be caused by the flavonoids and alkaloids in the ethanol extract is known by phytochemical screening [14].

## CONCLUSION

The ethanol extract of *A. vulgaris* can increase the phagocytic activity of macrophage cells in male rats injected carbon suspension. The extract at doses of 50, 100, 200, and 400 mg/kg may affect phagocytic activity compared to the negative control 0.5% CMC-Na ( $p<0.05$ ). The extract at 400 mg/kg gives phagocytic effect similar to the positive control Imboost®. Extract ethanol of herb Binara (*A. vulgaris* L.) increases the total number of cells of leukocytes and neutrophils part differential leukocyte cells and stem segments in male rats.

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