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Research Article

IMMUNOMODULATORY EFFECTS OF ETHANOL EXTRACT OF *CURCUMA MANGGA* RHIZOMES IN MICE

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ABSTRACT

Objective: This study was conducted to evaluate the immunomodulatory effects of ethanol extract of *Curcuma mangga* by *in vivo* study.

Methods: The ethanol extract of *C. mangga* was comprised to carbon clearance method for its immunomodulatory potential. The extract was administered orally at doses of 100, 200, and 400 mg/kg BW to mice for 7 days. On day 8, carbon ink was injected, and the blood was collected for measurement of elimination of carbon. Total leukocyte count was also determined.

Results: The evaluation of immunomodulatory potential of ethanol extract of *C. mangga* revealed a dose-dependent increase in phagocytosis ability. The phagocytic index of ethanol extract of *C. mangga* was more than those of negative control, indicating the immunostimulatory activity of *C. mangga.* It showed low stimulation on total leukocyte count.

Conclusion: The results indicate that ethanol extract of *C. mangga* rhizomes possesses immunomodulatory activity and has therapeutic potential for the treatment of infectious diseases.

Keywords: *Curcuma mangga*, Phagocytosis, Total leukocyte count, Immunomodulatory.

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INTRODUCTION

Immune system is a complex system, but its components are interrelated act in a highly coordinated and specific manner. Thus, most infections caused by pathogens in most individuals are short-lived with minor permanent damage. Innate immunity is the most universal and the most rapidly acting, which is largely mediated by professional immunocytes (neutrophils, monocytes, and macrophage cells) [1]. Phagocytosis is an effective innate internal defense and in activating the adaptive immune response. At the site of infection, phagocytes engulf and destroy the foreign substances by microbicidal agents [2]. There are various toxic molecules for microorganisms generate from superoxide anion (O_2^-) such as hypochlorous acid ($HOCl^-$), hydroxyl radicals (OH^-), and singlet oxygen. In addition, nitric oxide (NO^-) which is produced by macrophages during the respiratory burst reacts with O_2^- to form peroxynitrite, a strong antimicrobial [3,4].

Several plant extracts and their isolates have been reported for their immunomodulatory activity [5-7]. The extracts of many herbs such as *Panax ginseng, Tinospora cordifolia*, *Phyllanthus amarus*, *Centella asiatica, Trigonella foenum graecum*, *Pouteria cambodiana*, *Picrorhiza scrophulariiflora*, *Garcinia mangostana, Thymus guyonii, Salvia verbenaca, Capparis spinosa,* and *Stachys circinata* were able to upregulate or downregulate both innate and adaptive arms of the immune response [8-11].

The plants belonging to the genus Curcuma (family: *Zingiberaceae*) are widely distributed in most tropical countries [12]. Among them, *Curcuma mangga* is widely used in traditional medicine to treat various diseases such as stomach disorders, fever, and cancer-related diseases [13]. The variety of its organic compound of medicinal importance such as β-sitosterol, curcumin, demethoxycurcumin, and bisdemethoxycurcumin has emphasized to evaluate its immunomodulatory activity [13,14]. The extracts and compounds isolated from *C. mangga* have revealed a wide spectrum of pharmacological activities, including analgesic, anti-inflammatory, antioxidant, anticancer, antifungal, and nitric oxide inhibitory activities [15-18]. In addition, the ethanol extract of *C. mangga* did not induce significant short-term toxicity as reported in our previous study [19]. However, there are little studies to validate the traditional use of *C. mangga* leaves to treat diseases related to the immune system.

Previous *in vitro* study has indicated that the methanol extracts of *C. mangga* displayed strong immunomodulatory effects on polymorphonuclear neutrophils and macrophage cells. However, *in vivo* study is necessary to elaborate its immunomodulatory activity. This study was conducted to investigate the effects of ethanol extract of *C. mangga* on phagocytosis ability of mice leukocytes as well as total leukocyte count.

METHODS

Chemicals and reagents

The chemicals used in this study were ethanol (Smart Lab, Indonesia) and natrium carboxymethylcellulose (Na-carboxymethylcellulose [CMC]) (Sigma, USA), imboost® (Soho, Indonesia), China ink (pelican B-17), acetic acid (Smart Lab, Indonesia), and NaCl (Otsuka, Indonesia). A spectrophotometer (Shimadzu, Japan) was also used in this study.

Plant materials

The rhizomes of *C. mangga* were collected from Medan, Sumatera Utara, Indonesia. Then, the plant was authenticated in Herbarium Medanense, Universitas Sumatera Utara, Indonesia.

Extraction procedure

The plant materials were allowed to dry under shade. 350 g of dried material of plant sample was ground and macerated in ethanol at the ratio of 1:10 (w/v). The extraction was repeated twice on the residue. The filtrates were combined, and the solvent was removed

under reduced pressure to obtain an extract of *C. mangga* (38.4 g, 10.95% w/w).

Phagocytosis response

The phagocytosis ability was evaluated by carbon clearance method as described previously with slightly modification [20]. The animals were treated with ethanol extract of *C. mangga* at doses of 100, 200, and 400 mg/kg BW for 7 days. Meanwhile, the negative control group received Na CMC 0.5% as vehicle. Imboost® was used as positive control at a dose of 32.5 mg/kg BW. On day 8, all the animals received the treatment of an intravenous injection of (0.1 ml per 10 g) China ink dispersion via tail vein. Thereafter, 25 µL of blood samples were collected from each animal at an interval of 5, 10, 15, and 20 minutes after the injection of ink dispersion. Blood samples were added to 4 ml of 1% acetic acid to lyse the erythrocytes. Absorbance of the samples was measured at 640.5 nm using spectrophotometer. After 12 hrs of blood, collection animals were sacrificed and the livers and spleens were collected and weighed.

Rate of carbon clearance (K), and phagocytic index (α) were calculated using following formula:

$$\text{Rate of carbon clearance (K)} = \frac{\text{Log OD5 - log OD20}}{\text{t2 - t1}}$$

$$\text{Phagocytic index } (\alpha) = \frac{K^{1/3} \times \text{body wt of animal}}{\text{Liver wt} + \text{spleen wt}}$$

Where, OD5 is the log absorbance of blood at 5 minutes; OD20 is log absorbance of blood at 20 minutes; t_2 is the last time point of blood collection; t_1 is the first time point of blood collection. The use of mice was approved by the Animal Research Ethics Committees of Universitas Sumatera Utara (approval number 599/KEPH-FMIPA/2016).

Total leukocyte count

After the treatment with extracts for 7 days and injection with carbon ink dispersion, the blood sample of all animals was also collected for determination of total leukocyte count.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 15.0. Each sample was measured in triplicate and the data presented as mean±standard error of the mean. Data were analyzed using a one-way analysis of variance for multiple comparisons and followed by Tukey *post hoc* test. p<0.05 was considered to be different significantly.

RESULTS AND DISCUSSION

Phagocytosis response

Phagocytosis is performed using pseudopodia which are extended to surround an organism or particle and followed by intracellular destruction [21]. The effect of ethanol extract on phagocytosis ability of mice leukocytes was determined by the removal of carbon from bloodstream. The enhanced clearance rate of carbon particle from blood flow indicates the increment of phagocytosis activity of leukocytes. The rate of carbon clearance of the *C. mangga* extract at different doses (100, 200, and 400 mg/kg) was higher than the negative control (p<0.05), signifying that they were increasing the percentage of carbon ingestion and thus stimulating the phagocytic cells (Table 1). The stimulation on carbon engulfment of *C. mangga* was in a dose-dependent manner. The ethanol extract of *C. mangga* at a dose of 400 mg/kg BW demonstrated the strongest stimulant with phagocytic index of 6.71 which was comparable with those of positive control, Imboost® with phagocytic index of 6.82 (Fig. 1). Imboost® is a marketed drug to enhance an immune system which contained *Echinacea purpurea* 250 mg, black elderberry 400 mg, and zinc picolinate 10 mg. The previous study reported the ability of *E. purpurea* to stimulate cytokine release from macrophages [22].

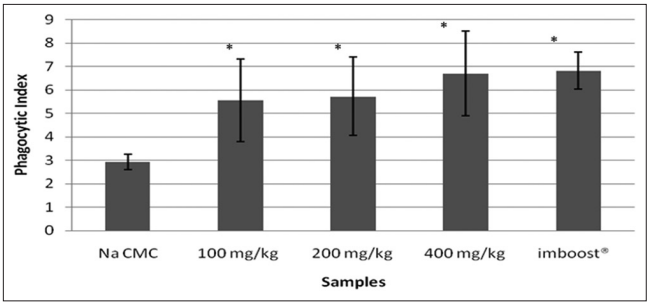

Fig. 1: Effect of administration of ethanol extract of *Curcuma mangga* rhizomes on phagocytic index. Data are mean±standard error of the mean (*n*=5); *p<0.05 compared to the respective control

Table 1: Effect of ethanol extract of *C. mangga* rhizomes on rate of carbon clearance

S.No.	Samples	Rate of carbon clearance
1	Na CMC	0.0059±0.0027
2	100 mg/kg BW	0.0283±0.0065*
3	200 mg/kg BW	0.0301±0.0051*
4	400 mg/kg BW	0.0411±0.0084*
5	Imboost®	0.0494±0.0167*

Data were analyzed by one-way ANOVA, and followed by Tukey *post hoc* test. *p<0.05 compared to the respective control, CMC: Carboxymethylcellulose, *C. mangga: Curcuma mangga*

Table 2: Effect of *C. mangga* extract on total leukocyte count (mean±SEM, *n*=5)

S.No.	Samples	Total leukocyte count (10^9/L)
1	Na CMC	6.24±0.73
2	*C. mangga* extract 100 mg/kg	8.26±0.66
3	*C. mangga* extract 200 mg/kg	8.03±0.87
4	*C. mangga* extract 400 mg/kg	7.85±0.71
5	Imboost®	7.70±0.59

Data were analyzed by one-way ANOVA, and followed by Tukey *post hoc* test. None of the sample showed p<0.05 . *C. mangga: Curcuma mangga*, CMC: Carboxymethylcellulose

The enhancement of phagocytosis activity to eradicate pathogens is markedly increase the immune system to protect the body from bacterial infection [23]. Fig. 1 shows that the phagocytic index of ethanol extract of *C. mangga* was more than those of negative control, indicating the immunostimulatory effect of *C. mangga* by enhancing phagocytosis ability. The result was in agreement with the previous study which reported the ability of methanol extract of *C. mangga* to enhance the phagocytosis response of human neutrophils by *in vitro* study [24].

Total leukocyte count

Cells of the immune system are generated from pluripotent hematopoietic stem cells in bone marrow. Thereafter, various immune cells are circulating in the blood stream, lymph, gastrointestinal system, and respiratory tract. The presence of pathogen-derived chemotactic factors attracts leukocytes to the site of infection [25]. Ethanol extract of *C. mangga* demonstrated low stimulation on the total leukocyte count as compared to negative control (p>0.05) (Table 2). The previous study was also reported the inhibition effect of *C. mangga* on the migration of neutrophils to the site of infection [26].

CONCLUSION

The ethanol extract of *C. mangga* was able to modulate the innate immune response especially phagocytosis ability of mice phagocytes. In addition, the plant extracts revealed low stimulation on the total leukocyte count. The highest phagocytic index was observed when ethanol extract was administered at a dose of 400 mg/kg. Hence, from the results obtained, it can be concluded that *C. mangga* has therapeutic potential and could be served as an effective immunomodulatory candidate. However, further studies are required to elucidate their activities on other mechanisms of immunomodulatory responses.

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