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Macrophage Activity and Capacity Following Oral Administration of Cocoa Extract to Mice

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Abstract

The activity of an ethanolic extract from cocoa bean (*Theobroma cacao* L.,) towards the non-specific immune response in mice being challenged with *Staphylococcus epidermidis* was studied. Mice (Swiss-Webster, 12 weeks old, 35 ± 1.9 g) received oral administration of cocoa extract (CE), positive control or negative control, every day for seven consecutive days. Cocoa extract (CE) was in three different doses, i.e. CE1 7.14 mg/30 g body weight (BW), CE2 14.28 mg/30 g BW, and CE3 28.57 mg/30 g BW. The positive control was *Phyllanthus niruri* Linn. (PN) extract (Stimuno®) 17.55 mg/30 g BW, while the negative control was sterile water (SW). On day 8, mice were given intraperitoneal injections of *S. epidermidis* suspension (0.5 ml, 105 CFU). After being settled for one hour, mice were sacrificed and peritoneal fluid was withdrawn for staining and microscopy observation. The number of macrophages performing phagocytosis and number of bacterial cells being recruited were counted. CE increased the number of active macrophages as well as enhanced macrophage phagocytic capacity against *S. epidermidis* cells. Various doses of CE increased the number of active macrophages from 46 ± 5% (SW) to 73 ± 3% (CE1), 76 ± 3% (CE2), and 85 ± 12% (CE3). Phagocytic capacity was elevated more than 2-fold after consumption of CE, from 215 ± 25 cells (SW) to 437 ± 9 cells (CE1), 452 ± 4 cells (CE2), and 511 ± 6 cells (CE3). CE3 with the highest dose had activity equal to that of PN (p = 0.68; α = 0.05). This research suggests a potential use of CE as an immunostimulant. This study indicates macrophage activity and capacity in mice were enhanced by oral consumption of cocoa extract.

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Keywords: cocoa; macrophage; immunomodulator; S. epidermidis; *Phyllanthus niruri*

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1. Introduction

Cocoa bean as the raw material of chocolate is a very important ingredient for food and beverage preparation. The indispensable chocolate flavor has made the demand for cocoa bean continuously increase, with 3% increment per year[1]. Some people have exceptional appreciation of chocolate that the terms of 'chocoholics' and 'chocolate-craver' exist[2,3]. However, with the emerging trend of healthy eating, public perception of chocolate and cocoa products may change from 'enjoyable' to 'threatening'. High fat and sugar content attributed to cocoa products have induced guilty feelings after eating chocolate.

Attempts to enhance public perception of cocoa have been focused on health benefit, and accordingly numbers of cocoa products have entered the market with various claims. Popular health properties introduced to consumers are for cardiovascular protection[4], antitussive properties[5] and skin perfection[6]. Two bioactive classes of compounds were documented, namely polyphenols and alkaloids. Cocoa polyphenols mainly come from the flavonoid class among which (+)-catechin and (–)-epicatechin are dominant compounds. In the other hand, cocoa also contains alkaloids (theobromine and caffeine) that have been extensively explored.

Our previous work reported that cocoa extract inhibited growth of *Escherichia coli*[7] and *Shigella dysenteriae*[8]. In subsequent research, it was observed that effective doses for in vivo testing were much lower than the theoretical dose derived from in vitro evaluation, suggesting cocoa extract may induce the immune response in animals rather than directly counteract bacteria cells. This study aimed to explore immunomodulatory properties of cocoa extract, with a focus on the non-specific immune response exerted by macrophages in a mouse model.

2. Methods

Cococa extract (CE) was prepared from freshly dried cocoa powder (fat removed, unroasted, unfermented) extracted using ethanol overnight. The liquid was filtered and was concentrated by using a vacuum evaporator to result in CE powder containing 14% catechin (assayed by Folin-Ciocalteu's reagent[9]) and 0.6% caffeine (assayed using acid-base chromatographic column[10]). The positive control was syrup of *Phyllanthus niruri* Linn. extract (Stimuno®, DexaMedica). Male Swiss-Webster mice (12 weeks, 30-35 g) were purchased from a local breeder. A *Staphylococcus epidermidis* culture (5 x 10^5 CFU/ml), chloroform, immersion oil, phosphate buffered saline, and Giemsa stain were provided by the Microbiology Laboratory, Faculty of Medicine, Jember University.

Mice were acclimatized for at least one week while receiving feed and water *ad libitum.* Twenty mice were distributed into 5 groups, 3 groups of treatment, positive control and negative control group. Treatment groups receiving different doses of CE, i.e. 7.14 mg/30 g body weight (BW) (CE1), 14.28 mg/30 g BW (CE2), and 28.56/30 g BW (CE3). The positive control group received 17.55 mg/30 g BW of *Phyllanthus niruri* Linn. extract (PN) and negative control consumed sterile water (SW). Test samples were delivered through oral administration once a day for 7 days. On day 8, *S. epidermidis* culture was injected intraperitoneally, and after one hour of settlement mice were sacrificed. Intraperitoneal fluid was obtained and processed for fixation and staining prior to microscopic observation. This study has been approved for ethical clearance by the Committee from Faculty of Medicine, Jember University.

Data analysis employed *Minitab 14* and *SPSS 22 for Windows* statistical software for testing normal distribution, homogeneity and one-way analysis of variance (ANOVA). Significant difference between groups was evaluated using *post-hoc Duncan's* test to determine *the p value* with $\alpha = 0, 05$.

3. Results and discussion

The effect of oral administration of SW, PN and CE in Swiss-Webster male mice for seven consecutive days was evaluated from the number of active macrophages per hundred total macrophages (%). The negative control group consuming sterile water (SW) had 46 ± 5% active macrophages. In the other hand, the PN group had a greater number of active macrophages, 80 ± 4%. PN extract has been studied for immunomodulatory properties, particularly in stimulating macrophage activity and T-lymphocyte proliferation[11].

After consumption of CE1, the active macrophage number was improved to 73 ± 3%. Higher concentration in CE2 and CE3 resulted in proportions of active macrophages of 76 ± 3% and 85 ± 12%, respectively, which were not significantly different compared to CE1. This indicates improvement of CE in macrophage activation did not occur in a dose-dependent manner at these (high) doses. However, since CE1 did not show a significantly different result