

Inhibitory Effect of Ethanolic Extract of *Psidium guajava* Leaves in Rat Active Cutaneous Anaphylaxis Reaction

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ABSTRACT

Psidium guajava is a plant that grows widely in some areas of Indonesia which have been proven as an antioxidant and anti-inflammatory. The aims of this study was to evaluate the effects of ethanolic extract of *Psidium guajava* (EEPG) leaves on active cutaneous anaphylaxis reaction induced by ovalbumin. This study used wistar male rats were divided into 5 groups (n = 5). Each group was induced by ovalbumin (OVA) and Al(OH) on the days of 0 and 7, and finally were challenged by ovalbumin on the day of 14 to induce active cutaneous anaphylaxis reaction. Cromolyn sodium was used as standard drug. EEPG with dose of 250mg/kgBW, 500mg/kgBW, and 750mg/kgBW were given orally at day 14. In order to determine the mast cells on the inflammation tissues, the specimens were stained with Toluidine blue. The results showed that EEPG leaves at the doses of 250, 500 and 750 mg/kg BW could inhibit the pigmentation area of vascular permeability on rats skin, significantly with control group (p<0,05), but still lower than cromolyn sodium. Histopathologically, EEPG leaves had inhibitory effect on mast cell degranulation process. It indicated that the EEPG leaves had inhibitory effect on active cutaneous anaphylaxis reaction.

Keywords: *Psidium guajava*, anaphylaxis reaction, ovalbumin

INTRODUCTION

Allergic disease is a common disease in community, and is still a health problem because of its recurrency. Allergic reactions are IgE-mediated immune response against foreign substances that enter the body¹. There were various plants which can be used as anti-allergy. The use of herbal medicine has increased in recent years. One of plants that have the potential to be developed as anti-allergy is guava plant (*Psidium guajava*). Guava contained tannins, phenolic compounds, flavonoids, volatile oil, sesquiterpenes and triterpenoids². Flavonoids and phenolic compounds contained in guava have been proven as an antioxidant and anti-inflammatory. Several studies have reported the effect of *Psidium guajava* on inflammation disease. Jahagirdar *et al.*³ reported that hydroalcohol extract of guava leaves (*Psidium guajava*) at a dose of 200 mg/ kg/ day showed antiarthritic activity. Dutta and Das⁴ observed that ethanol extract of guava leaf at a dose of 250 mg/kg and 500 mg / kg body weight significantly inhibited chronic inflammation and reduced arthritis index. Ojewole⁵ suggested that the aqueous extract of guava leaves with a dose range of 50-800 mg/kg were administered intraperitoneally had analgesic and anti-inflammatory activity in rats induced egg albumin. The aqueous extract of guava leaves (*Psidium guajava*) at a dose of 125, 250, and 500 mg/kg had anti-inflammatory activity and a decrease in rats with percentage inhibition of edema was 40.81 % , 55.45 % and 43.61 % , respectively⁶. The previous study was carried out to evaluate the effect

of *P. guajava* leaves extract in allergy disease. Han *et al.*⁷ reported that *Psidium guajava* inhibited chemokine (TARC/CCL17) expression on keratinocyte. *Psidium guajava* had suppressed IgE, TNF- α and IL-4 level on dermatitis induced 2,4-dinitrochlorobenzene (DNCB)⁸ Han *et al.*⁹, studied that ethyl acetate extract of *Psidium guajava* inhibited inflammatory cytokine production and Fc ϵ RI-dependent signaling. Guava leaves (*Psidium guajava*) had the potential to be developed as anti-allergy. The present study aimed to evaluate the *Psidium guajava* leaves in active cutaneous anaphylaxis rat model, especially with reference to the mast cell stabilizer drug, Cromolyn Sodium was used as a reference drug in this study.

MATERIAL AND METHODS

Research design

This study used a completely randomized design of unidirectional pattern. Active Cutaneous Anaphylaxis model was used to assess the anti-allergy of the ethanolic extract of *P. guajava* in rats. The research was carried out in Laboratory of Pharmaceutical Biology, Department of Pharmacy, Faculty of Health Sciences, Universitas Jenderal Soedirman and the Laboratory of Pathology and Anatomy, Medical Faculty, Gadjah Mada University, Yogyakarta.

Animals

Studies were conducted in male Wistar rats weighing 130-180 g obtained from the animal house in Bantul Yogyakarta, Indonesia. Animals were acclimatized to

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experimental conditions in cages and kept under standard environmental conditions ($22 \pm 3^\circ\text{C}$; 12/12 h light/dark cycle). Rats were allowed to feed and water ad libitum.

Plant material

Leaves were collected from Ketenger, Baturraden, Banyumas, Indonesia. Taxonomic identification of the plant was made by the Laboratory of Taxonomy, Faculty of Biology

Preparation extract

The powdered leaves (1000g) were successively extracted with ethanol ($70\text{-}80^\circ\text{C}$) for 3×24 hrs. The macerate was then evaporated in a rotary evaporator for ± 90 minutes in the temperature range of $70\text{-}80^\circ\text{C}$, then evaporated over a water bath until a thick ethanolic extract of *Psidium guajava* (EEPG) leaves was produced, and stored in a refrigerator until used in the study.

Animal experimental

Animals were randomly divided into five groups ($n=5$). The rats were Group I animals received CMC-Na 0,5% (2,5 ml/kg BW) served as control, Group II animals received Cromolyn sodium (2 mg/kg BW) served as reference standard, Group III, IV and V received EEPG leaves with a dose of 250 mg/kg BW (p.o), 500 mg/kg BW (p.o) and 750 mg/kg BW (p.o) respectively. Each group was subcutaneously sensitised by 01% ovalbumin and 10% Al(OH)₃ at the dorsal site, twice on the days of 0 and 7, and finally were challenged by ovalbumin 5,25% SC on days 14. To determine vascular permeability, the rats were injected with 1% Evans blue via the tail vein to indicate pigmentation area (blue area) on dorsal skin rats. To assess the effect of *P. gujava* leaves on active cutaneous anaphylaxis reaction, EEPG leaves given, 15 min prior to ovalbumin challenge on days 14 orally. The dorsal pigmentation areas were observed for 8 h after ovalbumin challenge.

Evaluation of the pigmentation area of vascular permeability

The pigmentation area of vascular permeability was evaluated by determination of diameter of blue area on rats dorsral skin every hour in 8 hours. The diameter of pigmentation area, were calculated on respective hour and the Area under the curve respect to untreated group was calculated on respective hours. The area under the curve of observed was calculated to evaluate the inhibitory effect.

Histological processing and assessment of mast cell on inflammation process

Rats were killed by ether anesthesia. Skin tissue were removed and fixed in 10% formaldehyde. After that the specimens were processed for paraffin embedding tissue sections (7 μm thick) and were stained with Toluidine blue. Under light microscopy, mast cells were identified as blue purple granules.

Data Analysis

The data were expressed and analyzed using SPSS software. Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by LSD test. The values $P < 0.05$ were regarded as statistically significant.

Ethical clearance

This study was accorded ethical clearance by the Commission on Research Ethics in Medicine and Health Sciences, Faculty of Medicine, Universitas Jenderal Soedirman.

RESULT

The pigmentation area developed over 8-h period and reached maximum intensity on hour 4 to 5 that indicated inflammation process on active cutaneous anaphylaxis reaction. Oral administration of EEPG leaves significantly suppressed the pigmentation area from hour 5 to hour 8 (Fig.1). Cromolyn sodium, a stabiliser of mast cell membranes, also clearly reduced the anaphylactic reaction. The effect of EEPG leaves to inhibit inflammation on active cutaneous anaphylaxis reaction was evaluated (Table 1). Cromolyn sodium had higher inhibitory effect than the EEPG leaves. The activity of Cromolyn sodium had high active in the study with the inhibition of pigmentation area of $91,35 \% \pm 1,37$ (Table 1). The effect of EEPG with dose of 250, 500 and 750 mg/kg BW on active cutaneous anaphylaxis reaction were examined with the pigmentation area inhibitory of $57,995 \pm 2,125$; $45,465 \% \pm 10,695$ and $51,3 \% \pm 6,28$, respectively. This result indicated that The EEPG leaves with dose of 250 mg/kg BW was more effective than EEPG with dose of 500 mg/kg BW and 750 mg/kg BW, but not significantly differences. The inhibitory effect of mast cells degranulation of EEPG leaves was evaluated (Fig. 2-6). The control group, which induced ovalbumin and vehicle contained only a few mast cells. Mast cells in control group also were not whole cell, that indicating degranulation process (Fig 2). After administration of EEPG leaves as well as Cromolyn sodium, mast cell on skin tissue appeared whole cell and the number of blue stained mast cells increased than control group (Fig 3-6). Representative photographs of skin sections in the study are shown, however, quantification of mast cell numbers is needed in further studies.

DISCUSSION

Cutaneous anaphylactic reaction is a type I mast cell-mediated allergy model. Present study we used ovalbumin to induce anaphylactic reaction. The inhibition of pigmentation area in anaphylaxis reaction may help to explain their protection action in allergic disease. The pigmentation area indicated inflammation process in allergic reaction. It would be reasonable to expect that plant with an anti-inflammatory effect would have effect on allergic reaction. Therefore antiallergic agent with an antiinflammatory action may be beneficial drugs for allergic disease. The present study showed that EEPG leaves potently inhibited pigmentation area on active cutaneous anaphylaxis reaction induced by ovalbumin. Previous study reported that *Psidium guajava* leaves showed antiinflammatory and antiallergy. However, we did not find any evidence that this plant inhibited allergic reaction. *Psidium guajava* extract inhibited chemokine (TARC/CCL17) expression in keratinocytes by inducing Heme Oxygenase-1 expression.⁷ *Psidium guajava* leaves also had suppressed IgE, TNF- α dan IL-4 level on

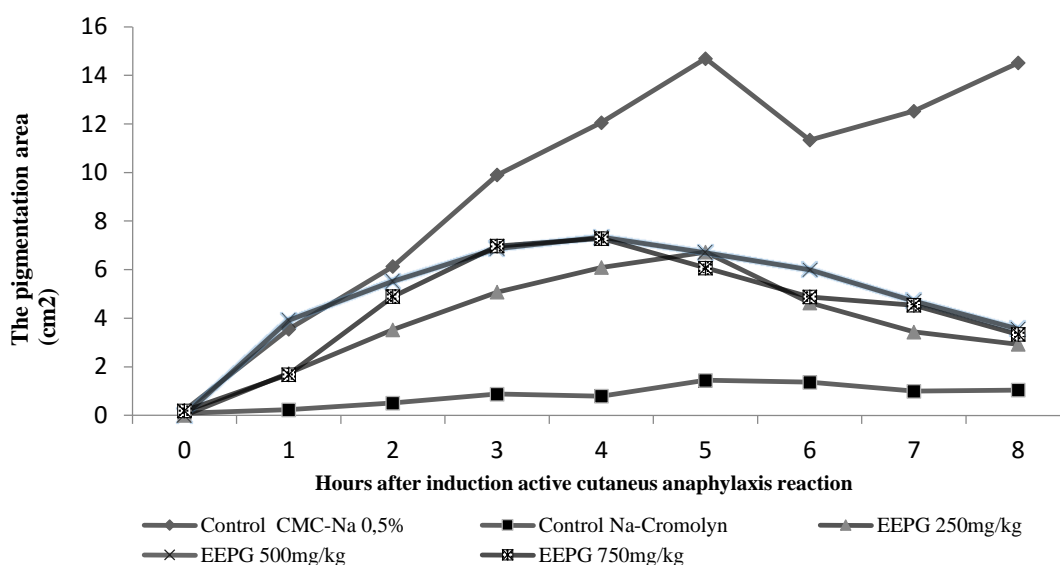


Figure 1. Effect of Ethanollic Extract of *Psidium guajava* (EEPG) leaves ovalbumin-induced active cutaneous anaphylaxis in rat dorsal skin. Data represent mean \pm SEM, and are five independent experiments.

Table 1. The inhibitory effect of Ethanollic Extract of *Psidium guajava* (EEPG) leaves ovalbumin-induced active cutaneous anaphylaxis in rat dorsal skin.

Group	AUC ₀₋₈ (cm ² .jam)	The inhibition of pigmentation area (%)
Control vehicle	77,57 \pm 4,57	-
Cromolyn Sodium	6,77 \pm 1,46	91,35 \pm 1,37
EEPG 250 mg/kg	32,68 \pm 3,57	57,995 \pm 2,125
EEPG 500 mg/kg	42,79 \pm 10,79	45,465 \pm 10,695
EEPG 750 mg/kg	38,061 \pm 7,09	51,3 \pm 6,28

The drug was administered 15 min prior to challenge with ovalbumin. Each value represents the mean \pm SEM of five independent experiments. * p <0.05, significantly different from the vehicle value.

dermatitis induced 2,4-dinitrochlorobenzene (DNCB).⁸ Ethyl acetate extract of *Psidium guajava* leaves inhibited allergy response included inflammatory cytokine production and Fc ϵ RI-dependent signaling.⁹ Most of plants showed anti-allergic activities through mechanisms related to antioxidative activities, although others display anti-allergic mechanisms unrelated to their antioxidative activity. Porwal *et al.*¹⁰ reported that *P. guajava* leaves extract contained essential oil, flavonoids, triterpenoids, vitamin C, tanins and phenolic. Quercetin is a flavonoid that recently has raised many issues and shown evidence about its action as a potential drug to allergy. Quercetin in *Psidium guajava* suppressed and inhibited NO (Nitric Oxide) production, which catalysed with inducible nitric oxide synthase (iNOS).¹¹ Chen *et al.*¹² reported that quercetin had antioxidant activity by suppressing signaling NF- κ B pathway. Quercetin inhibited eosinophil activation, especially chemokine production, and results in inhibition of the development of eosinophilic inflammatory responses.¹³ Present study showed that EEPG leaves inhibited mast cell degranulation. Mast cells are central to the pathogenesis of type I hypersensitivity. Mast cells are associated to allergic reactions, which are

initiated by the binding between antigen and immunoglobulin E / Ig E on the surface of these cells.¹⁴ Activation of mast cells occurs when there is cross-linking or bridging of Fc ϵ RI molecules by the binding of antigen and Ig E to these molecules. Leading to mast cell degranulation and release of chemical mediators like histamine (the most important mediator), SRSA (Slow Reacting Substance of Anaphylaxis), prostaglandin, ECFA (Eosinophil Chemotactic Factor of Anaphylaxis), PAF (Platelet Activating Factor), heparin and some enzymes (tryptase, chymase).¹⁴ Shaik *et al.*¹⁵ studied that quercetin blocked substances involved in allergies and able to act as an inhibitor of mast cell secretion, causes a decrease in the release of tryptase, MCP-1 and IL-6 and the down-regulation of histidine decarboxylase (HDC) mRNA from few mast cell lines. *Psidium guajava* had anti-allergic action against Th2 cell-mediated allergy.¹⁶ Histopathologically, oral administration of EEPG leaves had prevented mast cell degranulation. Therefore the EEPG leaves had inhibitory effect on active cutaneous anaphylaxis reaction through mechanism related to inhibition of mast cell degranulation. Based on the findings *Psidium guajava* leaves showed anti-allergic properties,

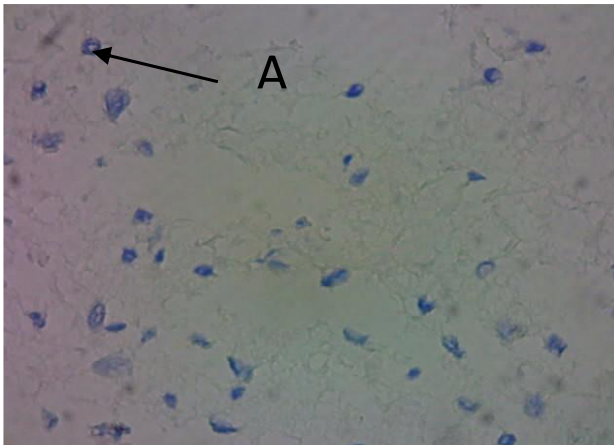


Figure 4. Histopathological representation of inflammation tissue on skin rats with Toluidine blue staining after treatment with vehicle, 10 x 40 magnification. (A) mast cell.

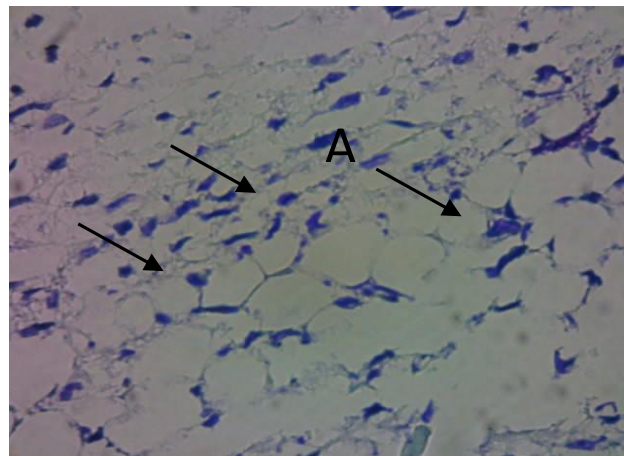


Figure 5. Histopathological representation of inflammation tissue on skin rats with Toluidine blue staining after treatment with Cromolyn sodium, 10 x 40 magnification. (A) mast cell.

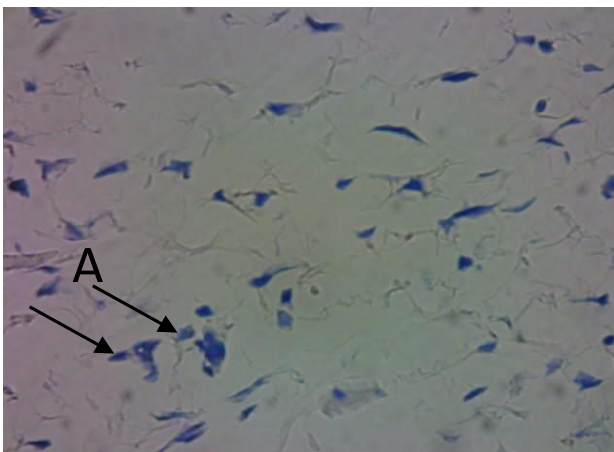


Figure 6. Histopathological representation of inflammation tissue on skin rats with Toluidine blue staining after treatment with EEPG 250 mg/kg BW , 10 x 40 magnification. (A) mast cell.

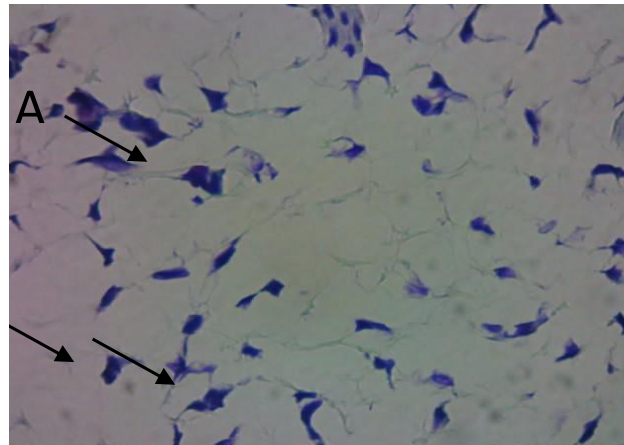


Figure 7. Histopathological representation of inflammation tissue on skin rats with Toluidine blue staining after treatment with EEPG 500 mg/kg BW, 10 x 40 magnification. (A) mast cell.

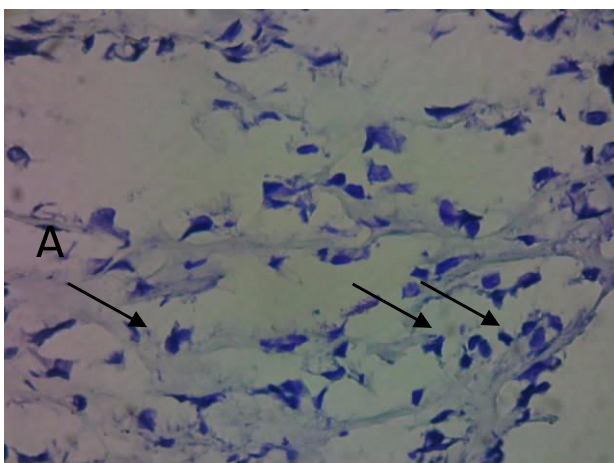


Figure 8. Histopathological representation of inflammation tissue on skin rats with Toluidine blue staining after treatment with EEPG 750 mg/kg BW , 10 x 40 magnification. (A) mast cell.

and this herbal can be a candidate for the therapeutic agent for allergy. *Psidium guajava* leaves may be used as primary therapy or in conjunction with conventional therapy. Nevertheless, further studies are required to investigate the detailed mechanisms of the anti-allergic effect of *Psidium guajava* leaves.

CONCLUSION

In conclusion, oral administration of the ethanolic extract of *Psidium guajava* (EEPG) leaves at the dose of 250, 500 dan 750 mg/kg BW had inhibitory effect on active cutaneous anaphylaxis reaction induced by ovalbumin through the suppressing of inflammation and inhibition on mast cell degranulation process.

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