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# Interleukin-6 Level In Vivo And In Vitro Anti Inflammatory and Anti-Pyretic Evaluation of Aloe Vera Ethanolic Extract in Sprague Dawley

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## ABSTRACT

Aloe vera has been extensively used in cosmetic applications. *Aloe vera* has anti-inflammatory, antipyretic and antioxidant effects.

**Objective**: to correlate the anti-inflammatory & antipyretic effect of Aloe vera ethanolic extract (AVEE) in vivo and in vitro models in rats in the interleukin-6 level.

**Methods**: a true experimental design with randomized approach included 25 male Sprague-Dawley rats. Separation and purification of each secondary metabolite of AVEE by phytochemical screening at the University of Indonesia laboratory. Rat paw edema was induced with cotton pellet-induced granuloma formation and IL-6 level. Efficacy of aloe vera was analyzed using ANOVA by SPSS v.27.0. the p-value for statistical significance was defined as $p<0.01$

**Results**: AVEE effective reduced granuloma tissue formation and protected granuloma through inflammation induced by cotton pellets and histological examination at 200 mg/kg BW. The effective concentration that effect IL6 concentration is 200 mg/kgBW AVEE and statistically significant($P<0.01$).

**Conclusion:** Aloe Vera Ethanolic Extract (AVEE) has anti-inflammatory and antipyretic activity in rats, altrough no significant to activity antipyretic in rats.

**Keyword:** *Aloe Vera extract, cotton pellet-induced, granuloma formation, Interleukin-6*

## 1. INTRODUCTION

Aloe vera, a member of the Alliaceae family has been extensively used in cosmetic applications(Sánchez et al. 2020). *A*nti-inflammatory effect in rats of *Aloe vera* gel at doses of 100-300 mg/kg BW, by inhibiting carrageenan-induced edema formation and reducing Il-6 expression (Ikebunwa et al. 2023).

Aloe vera pathway of anti-inflammatory activity by inhibiting the arachidonic acid pathway, hindering the migration of neutrophils, by suppressing prostaglandin biosynthesis in male Wistar rats in vitro. *Aloe vera'*s ability to inhibit prostaglandin production can reduce pain, which ultimately reduces the body temperature during inflammation (Kothari and Kumar 2015)

There is no scientific data of *Aloe vera* ethanolic extract (AVEE) anti-inflammatory and antipyretic effect in rats in Indonesia. Further research is needed to investigate these aspects and to understand the potential molecular mechanisms underlying AVEE's effects on inflammation and fever induced by these agents.

Interleukin-6 (IL-6) is plays a pivotal role in the acute phase response to promotes pro-inflammatory effects such as inducing intercellular adhesion molecules and recruiting leukocytes. IL-6 acts as a crucial mediator of endotoxin-induced fever in both central and peripheral organs, further stimulating the central thermoregulatory organ, the hypothalamus (Kanashiro et al. 2018).

## 2. MATERIALS AND METHODS

### Animal preparation

a true experimental design with randomized approach included 25 male Sprague-Dawley rats were categorized into five groups, aged around 2-4 months with an average body weight of 150-200 grams, and placed in plastic cages with temperature modulated at approximately 24 ± 1°C and 12 hours of dark/light cycle. They were allowed to acclimatize for 14 days prior to the experiment.

### Plant extract

The ethanolic extracts were washed and rinds manually separated from the inner succulent flesh using, air-dried in a shaded area until completely desiccated. Once devoid of moisture, the dehydrated substance was crushed into a fine powder using a blender. A total of 500 grams of the coarse powder was tightly packed into a Soxhlet apparatus and subjected to extraction with 70% ethanol at a ratio of 1:6 for 72 hours, with periodic shaking maintained at 60°C throughout the extraction process. The resulting extract was then concentrated to its original volume through evaporation, yielding 72.3379 grams.

### Experimental protocol

The independent variables were positive control (Aspirin 100 mg/kg BW), negative control (normal saline =NaCl 0.9%), ethanolic extract of Aloe vera ethanolic (100, 200, and 300 mg/kg BW)

Granulomatous lesions will be established by subcutaneously implanting a sterile cotton pellet (10 ± 0.5 mg) in the dorsal area of the rats. Cotton pellets were subcutaneously injected into the thorax and left leg of rats over a period of ten (10) days. Histological assessment of granuloma pouch appearances was conducted using a scoring system (Table 1)

**Table 1. Granuloma histology scoring**

| No. | Variable | Explanation | Scoring |
|---|---|---|---|
| 1 | No granuloma tissue | dense connective tissue, no inflammation, and no bleeding | 0 |
| 2 | Mild granuloma | little connective tissue and mild inflammation, no bleeding. | 1 |
| 3 | Moderate granuloma | moderate connective tissue with the number of inflammatory cells is quite a lot, light bleeding. | 2 |
| 4 | Heavy granuloma | thick connective tissue, lots of inflammation and still a lot of bleeding. | 3 |

Aloe vera ethanolic extract (AVEE) given orally will occur at doses of 0, 100, 200, and 300 mg/kg body weight, alongside 0.9% NaCl, once a day for ten consecutive days. On the 11th day, the rats will be anesthetized, and the pellets surrounded by granulomatous tissue will be carefully excised and weighed. The average weights of the pellets from each treatment group will be calculated and compared to the control group using the designated formula. Meanwhile, mean CP pre is the average of cotton pellet pre-implanted (mg), and mean CP post is the average of cotton pellet post-implanted (mg).

### Bioassay blood serum assessment of IL-6 level

Following the protocol provided by the manufacturer, interleukin-6 (IL-6) was assessed using enzyme-linked immunosorbent assay (ELISA) kits. Briefly, diluted standards or samples (100 µl of each supernatant) were introduced into 96-well plates pre-coated with IL-6-specific affinity-purified polyclonal antibodies for rats. Enzyme-linked polyclonal antibodies were then inserted to each well and incubated at 37°C for 60 minutes, followed by washing the plates five times. Subsequently, substrate solutions were added, and the optical densities were measured at 450 nm. The strength of the readings was directly related to the concentration of IL-6 generated. (Geng et al. 2017).

### Statistical Analysis

The data were expressed as mean ± standard error o mean (SEM). Differences between control and treatment groups were analyzed using one-way analysis of variance (ANOVA) with SPSS software version 21, followed by the Duncan Multiple Range Test (DMRT) at a confidence level of 99% ($p < 0.01$). Prior to conducting the ANOVA test, all data tested normality and homogeneity.

## 3. RESULTS

Anti-inflammatory effect of AVEE in vivo was effective in reducing granuloma tissue formation, protected granulomas through inflammation induced by cotton pellets and histological examination at 200 mg/kg BW. There was no significant difference in the scores between rats treated with AVEE and NaCl ($P > 0.01$) based on the histological scoring of granulomas. Induction of cotton pellet significantly affect formation of granuloma pouches ($P < 0.01$) (Table 2&3).

**Table 2. Mean Histological Scoring of Granuloma Pouch.**

| Treatment | Granuloma Pouch (mean ± std) um | Scoring (mean ± std) |
|---|---|---|
| NaCl | 221.2 ± 10.36 | 3 ± 0.45 |
| 100 mg | 155.4 ± 17.24[b] | 2.6 ± 0.55 |
| 200 mg | 162.4 ± 13.41[b] | 1.4 ± 0.55 |
| 300 mg | 152.5 ± 22.40[b] | 1.6 ± 0.55 |

**Explanation:** Different superscripts in the same column indicate significant differences ($P < 0.01$)

**Table 3. ANOVA test of AVEE anti-inflammatory effect on granuloma pouch.**

| Parameter | Sig | α = 1 % |
|---|---|---|
| Granuloma pouch | 0.000 | 0.01 |

Histopathological examination of the tissue surrounding the granuloma pouch induced by cotton pellet after 10 days revealed treated with AVEE (100, 200, and 300 mg/kg BW) exhibited only mild inflammation. (Figure 1)

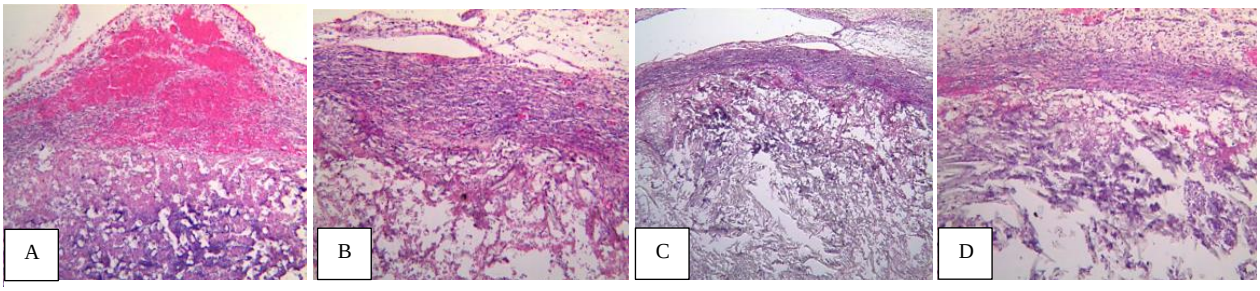


**Figure 1. Histopathological examination of the tissue surrounding the granuloma pouch induced by cotton pellet treated with AVEE. A. Negative control (NaCl), B. *Aloe vera* 100 mg, C. *Aloe vera* 200 mg, D. *Aloe vera* 300 mg**

The changes in cotton pellet weights before and after treatment with AVEE (100, 200, and 300 mg/kg BW) and NaCl over the 10-day period (Table 4). There was no significant difference in the weights of cotton pellets before and after treatment with AVEE and NaCl, significant differences were noted in the weights of cotton pellets after implantation and in the formation of granulomas (Table 5) .

**Table 4: Cotton pellet weight before and after AVEE administration.**

| Treatment | Weight Cotton Granuloma Pouch | | | Percentage protection (%) |
|---|---|---|---|---|
| | Pre (gr± std) mg | Post (gr ± std) mg | Granuloma formation (gr ± std) mg | |
| NaCl | 10.048 ± 0.09 | 19.956 ± 0.93 | 9.908 ± 0.87 | 0.000 ± 0.00 |

| | | | | |
|---|---|---|---|---|
| 100 mg | 10.072 ± 0.10 | 19.548 ± 1.54[a] | 9.476 ± 1.54[a] | 4.36 ± 1.45[a] |
| 200 mg | 10.096 ± 0.12 | 14.264 ± 0.54[b] | 4.150 ±0.48[b] | 58.11 ± 4.86[b] |
| 300 mg | 10.048 ± 0.11 | 14.160 ± 0.76[b] | 4.112 ± 0.74[b] | 58.50 ± 7.43[b] |

**Explanation:** Different superscripts in the same column indicate significant differences (P < 0.01),

**Table 5. ANOVA test of AVEE anti-inflammatory effect on cotton pellet weight in granuloma pouch.**

| Parameter | Sig | α = 1 % |
|---|---|---|
| Cotton pellet weight pre | 0.872 | 0.01 |
| Cotton pellet weight post | 0.000 | 0.01 |
| Cotton pellet weight delta | 0.000 | 0.01 |

The Duncan's Multiple Range Test (DMRT) indicated significant differences in cotton pellet weights (post-implantation and granuloma formation) following administration of AVEE and NaCl treatments (P < 0.01). The weight of cotton pellets after treatment with 100 mg/kg BW AVEE before implantation differed significantly from those treated with 200 mg/kg BW AVEE (P < 0.01). The effective dose that significantly affected granuloma pouch formation was 200 mg/kg BW AVEE.

**Table 6. ANOVA test of AVEE anti-inflammatory effect on percentage protection.**

| Parameter | Sig | α = 1 % |
|---|---|---|
| Percentage protection | 0.000 | 0.01 |

AVEE and NaCl treatments influenced the percentage protection of the granuloma pouch. The protective percentage in the NaCl treatment group was lower compared to AVEE treatments. The percentage protection in rats treated with NaCl did not significantly differ from those treated with 100 mg/kg BW AVEE (P > 0.01). The effective dose influencing the percentage protection of the granuloma pouch is 200 mg/kg BW AVEE.

Cytokine assay especially interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA) kits. IL-6 concentrations decreased along with the increase in AVEE doses were 176.13 ± 6.47 mmol/L in the 100 mg/kg BW, 123.30 ± 4.95 mmol/L in the 200 mg/kg BW, and 97.51 ± 3.72 mmol/L in the 300 mg/kg BW. AVEE administrations influenced on the IL-6 concentrations but is not significantly different (P > 0.01) (Figure 2, Table 7). NaCl and Aspirin treatments showed significantly different with AVEE (P < 0.01). The effective concentration that affects the Interleukin-6 concentration is 200 mg/kg BW AVEE.

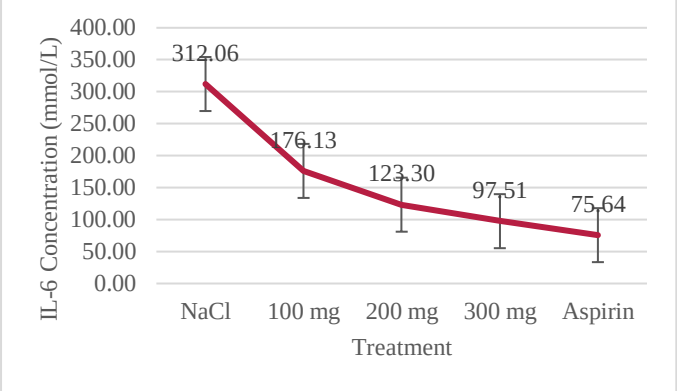


**Figure 2. Interleukin-6 concentrations have same effect on IL-6 concentrations in rats at 200 mg/kg of BW dose.**

**Table 7. Comparison of the anti-inflammatory effects of AVEE with aspirin**

| Treatments | *Aloe vera* ethanolic extract (AVEE) | Aspirin |
|---|---|---|
| IL-6 concentration | $123.30 \pm 4.95^{bc}$ | $75.64 \pm 1.66^{b}$ |

**Explanation:** Different superscripts in the same column indicate significant differences ($P < 0.01$),

There was no significant difference in the scores between rats treated with AVEE and NaCl on histological score. Rats treated with NaCl and 100 mg/kg BW AVEE exhibited a histological score of 3 heavy granuloma formation. In contrast rats treated with 200 mg/kg BW AVEE showed a histological score of 1 and rats treated with 300 mg/kg BW AVEE had a histological score of 2, indicating moderate granuloma formation, moderate connective tissue, a substantial number of inflammatory cells, and minimal bleeding.

Granuloma pouch formation in rats induced by cotton pellet after treatment with AVEE and NaCl, induction of cotton pellet significantly influenced the formation of granuloma pouches ($P < 0.01$).The granuloma pouch formation in rats treated with NaCl was significantly different compared to those treated with AVEE ($P < 0.01$) (Table 8) .

**Table 8. Mean Histological Scoring of Granuloma Pouch.**

| Treatment | Granuloma Pouch (mean ± std) um | Scoring (mean ± std) |
|---|---|---|
| NaCl | $221.2 \pm 10.36$ | $3 \pm 0.45$ |
| 100 mg | $155.4 \pm 17.24^{b}$ | $2.6 \pm 0.55$ |
| 200 mg | $162.4 \pm 13.41^{b}$ | $1.4 \pm 0.55$ |
| 300 mg | $152.5 \pm 22.40^{b}$ | $1.6 \pm 0.55$ |

**Explanation:** Different superscripts in the same column indicate significant differences ($P < 0.01$)

## 4. DISCUSSION

The goal of this study was to assess interleukin-6 level of Granuloma pouch model induced by cotton pellets in sprague dawley models. Sprague dawley models have been suggested as a study model because of their high availability, simplicity of handling and treatment, and habitation

In this study, the phytochemical evaluation on Aloe vera leaves. The qualitative analysis of secondary metabolites in *Aloe vera* ethanolic extract (AVEE) revealed positive results for alkaloids as a strongest. (Sánchez et al. 2020). Martin et al reported dominant alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycosides, with steroids absent (Martins and Terblanche 2003).

The cotton-pellet induced granuloma pouch formation test is widely recognized for assessing chronic anti-inflammatory properties (Chitsaz et al. 2023). This model evaluates the capacity of agents to mitigate leukocyte infiltration and suppress granuloma formation within the inflamed site. It is utilized to examine both the transudative and proliferative aspects of chronic inflammation.

Granuloma refers to a cluster of white blood cells and surrounding tissue characterized by macrophages that transform into epithelioid cells, encircled by mononuclear leukocytes primarily composed of lymphocytes and occasionally plasma cells (Mahdani, 2013).

The inflammatory process involves the proliferation of macrophages, neutrophils, and fibroblasts, which are fundamental contributors to granuloma formation. Quinine, a principal mediator influencing granuloma formation, induces vasodilation and increases blood vessel permeability. During the repair phase of inflammation, fibroblast proliferation and neovascularization initiate. These proliferative cells infiltrate and lead to the production of exudates, forming a highly vascularized and reddened mass known as granulation tissue (Alhajj and Goyal 2022).

The exudate was absorbed by the implanted cotton pellets, where the dry weight of these pellets correlates with the extent of granulomatous tissue formation (Zakaria et al. 2008). According to observations, the weight of cotton pellets decreased with

increasing doses of *Aloe vera* ethanolic extract (AVEE). Furthermore, the study results indicated that the weight of cotton pellets in the AVEE-treated groups was lower compared to those in the NaCl group, suggesting that AVEE administration may suppress granuloma formation or the proliferation phase relative to NaCl administration. The suppressive effect of AVEE on granuloma formation was observed to be 4.36% at 100 mg/kg, 58.11% at 200 mg/kg, and 58.50% at 300 mg/kg doses. These findings showed AVEE's potential to inhibit chronic inflammation induced by cotton pellets. Agung V, Mappiase A, Wahab S, Alam G, Cangara M.H (2016) research showed *Aloe vera* extract reduced granuloma and edema formation in mice after carrageenan induction.

Other studies indicate that *Aloe vera* gel exhibits potent properties in mitigating leukocyte and fibroblast migration during inflammation, thereby significantly attenuating the formation of exudates and granulomas (Afsar et al. 2013). Specifically, *Aloe vera* gel demonstrates efficacy In both the exudative and proliferative stages of inflammation, the administration of Aloe vera gel leads to a decrease in transudate and granuloma formation. This reduction is associated with fewer fibroblasts and lower levels of collagen and mucopolysaccharides, which are essential for granuloma tissue development. Additionally, these suppressive effects on exudates and granulomas might be due to increased levels of anti-inflammatory cytokines and decreased production of pro-inflammatory mediators such as myeloperoxidase, nitric oxide, and interleukins through inhibition of lysosomal or membrane-stabilizing enzymes (Afsar et al. 2013).

The anti inflammatory effects of Aloe vera gel are mediated through the inhibition of bradykinin activity, thromboxane B2, prostaglandins, and magnesium lactate. This study underscores how the secondary metabolites in *Aloe vera* ethanolic extract (AVEE) influence the inhibition of exudate and granuloma formation. Flavonoids, known for their anti-inflammatory properties, effectively suppress the proliferation of fibroblasts, collagen synthesis, and mucopolysaccharide production in granuloma tissue models (Venkataramana et al. 2013).

Flavonoids mitigate granuloma size by inhibiting granulocyte infiltration and inhibit enzymes involved in inflammation induction, such as lipoxygenase and cyclooxygenase. Tannins also modulate inflammatory responses by scavenging free radicals and inhibiting inducible nitric oxide species in macrophages. Steroid compounds found in AVEE are believed to exert anti-inflammatory effects through glucocorticoid receptor activation, which regulates the transcription of genes involved in inflammation. Furthermore, the glycoprotein Aloctin within *Aloe vera* gel inhibits the production of Prostaglandin E2 (Osifo et al. 2022) .

In this study we also measure the differences of the interleukin 6 level after AAVE aplication between the four groups at days to explain their effect on inflammation and wound healing. Interleukin-6 (IL-6) acts as a crucial mediator of endotoxin-induced fever in both central and peripheral organs, further stimulating the central thermoregulatory organ, the hypothalamus (Kanashiro et al. 2018).

In the present study IL-6 level were observed to increase following cotton pellet induction. AVEE effectively inhibited IL-6 concentrations, with a significant reduction observed at higher doses. Interestingly, the group treated with the standard drug aspirin at 100 mg/kg BW exhibited a sharp decline in IL-6 concentrations, comparable to the effect seen with AVEE at 200 mg/kg BW. In contrast, IL-6 concentrations in the negative control group receiving NaCl remained elevated compared to other groups. (Prabjone et al. 2016) Active ingredients in *Aloe vera* inhibit or suppress pro-inflammatory cytokines such as TNF-α and IL-6, thereby modulating leukocyte-endothelium interactions. Blocking the activity of IL-6, a key pro-inflammatory cytokine, effectively reduces inflammation and suppresses pathways that activate T cells (Dinarello, 2013).

The steroid compounds present in AVEE are observed to inhibit the production of cytokines such as IL-6, IL-12, and TNF-α. Consequently, IL-6 concentrations in rats decreased following administration of AVEE after LPS injections, with reductions correlating with increasing doses. This effect aligns with findings by indicating that blocking IL-6 and its signaling pathway is effective for preventing and treating inflammatory diseases (Gabay 2016).

**LIMITATIONS**

This study focuses on a subset of inflammatory mediators, specifically IL-6, and does not analyze other important factors such as histamine, bradykinin, prostaglandins, and COX enzymes. As a result, the research is limited in scope and would benefit from further exploration and development to comprehensively understand the broader inflammatory pathways involved.

## 5. CONCLUSIONS

Aloe Vera Ethanolic Extract (AVEE) has anti-inflammatory and antipyretic activity in rats, altrough no significant to activity antipyretic in rats. AVEE effectively inhibited granuloma tissue formation induced by cotton pellets over 11 days at the same dose. In vitro of AVEE showed significantly decreased IL-6 production in LPS-induced rats at a dose of 200 mg/kg body weight. AVEE exhibits potent anti-inflammatory and anti-pyretic activities in rats, suggesting its potential as a therapeutic agent for inflammatory and febrile conditions.

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