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Potential of aloe vera gel as an alternative inductor in platelet aggregation test

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Abstract: Platelet aggregation tests are crucial for evaluating platelet function, with inductors being substances that stimulate the aggregation process. The use of ADP (adenosine diphosphate) as an inductor has the disadvantage of being costly and difficult to obtain. Aloe vera, a plant native to Africa, has been widely used for wound healing and therefore has the potential to serve as an alternative inductor agent. This study aims to evaluate the effectiveness of Aloe vera gel as a platelet inductor compared to the ADP reagent using the Velaskar method. This research began with the preparation of fresh Aloe vera to obtain Aloe vera gel. Venous blood was then collected from 16 participants for the platelet aggregation test. The test was divided into two groups: the ADP group (addition of ADP) and the Aloe vera group (addition of Aloe vera gel). The final step involved analyzing the results of platelet aggregation statistically using the Independent Sample T-test. The average percentage of platelet aggregation with ADP and Aloe vera gel was 88.2 ± 5.6% and 85.7 ± 5.4%, respectively. Independent Sample T-test analysis showed no significant difference between the percentage of platelet aggregation with ADP and Aloe vera gel. In conclusion, Aloe vera gel has the potential to be an alternative platelet inductor. Some benefits of Aloe vera gel include its ability to induce platelets similarly to ADP, its application not affecting the morphology of erythrocytes, and its economical and practical nature (as it does not require elaborate preparation). However, a disadvantage is that pure Aloe vera gel contains several components that may affect its performance as an inductor.

Keywords: Aloe vera, Adenosine Diphosphate, Alternative inductor, platelet aggregation

INTRODUCTION

Hemostasis is the process of stopping bleeding spontaneously from blood vessels that have acquired vascular injury[1]. Platelets play a major role in hemostasis through formation and stabilization of the platelet plug[2]. Disruption of platelet function can cause myocardial infarction or commonly known as a heart attack[3]. According to the World Health Organization (WHO), cardiovascular disease is the leading cause of mortality worldwide, accounted for 17.3 million deaths in 2013. It is predicted to reach 23.6 million by 2030[4]. The highest prevalence of acute myocardial infarction in Indonesia is East Nusa Tenggara (4.4%), while in Central Java it reaches 0.5%[5].

Platelet aggregation test is one of the tests to evaluate platelet function. Several methods were used for the test, one of them was the method introduced by Velaskar DS and Chitre in 1982. The Veslaskar method was applied using a peripheral blood smear, with the principle of aggregation visible when the smear is formed, free platelets and aggregated platelets can be counted separately on smear[6].

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Platelet aggregation test is very sensitive assay because influenced by several factors, such as the concentration of an inductor addition[7]. Inductors are substances that stimulate the aggregation process. The strength of the inductor influences platelet responsiveness. Weak inductors are adenosine diphosphate (ADP) and epinephrine, moderate inductors are thromboxane A 2 (TxA2), while powerful inductors are thrombin and collagen. ADP is the most commonly used inductor for the Velaskar method[8].

Aloe vera is a plant from Africa which has been widely used for wound healing[9]. This plant is divided into two parts, namely the mucilage gel and the exudate (mucus) part, which is composed of yellow sap (yellow mucus) and colorless mucin gel. Aloe vera contains several substances such as tannins, flavonoids, saponins, and anthraquinones that play a role in the blood clotting coagulation[10].

Aloe vera contains several substances that play a role in blood coagulation, including tannins, flavonoids, saponins, and anthraquinones[10]. According to Sukeksi and Rizqy (2021), the tannin present in the ethanol extract of betel leaves has the potential to substitute ADP since it has astringent effects. The astringent effect is the ability to form complexes with macromolecules, particularly proteins[11].

The use of the platelet aggregation test Velascar method plays an important role as a screening test prior to examining platelet aggregation using the Aggregometry method. Furthermore, the platelet aggregation test Velascar method also serves as an assessment of platelet aggregation function in small to medium-sized laboratories or health centers that do not have facilities for testing platelet aggregation function using the Agregometric method. Providing the significance of this test, the use of ADP reagents becomes critical. The use of ADP in the laboratory has affordability and availability limitations[12,13]. On the other hand, *Aloe vera* gel shows potential as a natural alternative inductor agent. The use of *Aloe vera* gel in platelet aggregation tests has never been reported. The objective of this study is to investigate the potential of *Aloe vera* gel as a new platelet inductor.

MATERIAL AND METHOD

Materials

Fresh *Aloe vera* leaves, ADP reagent (Helena laboratories), 70% ethanol (Merck), methanol (Merck), and Wright-Geimsa stain (Merck), Analytical balances (O'hauss), centrifuge (Gemmy PLC03), blender (Philips), glass object (Sail brand), micropipette (Human), white-tip and yellow-tip (Axygen), filter paper (Whatman), glass funnel (Herma), microscope (Olympus CX23), Syringe 3 mL (BD).

Aloe vera Gel Preparation

Fresh *Aloe vera* leaves are collected from plants 8 months old and 40-70 cm long. Remove the outer skin of the aloe vera leaf and thoroughly wash it with water. After peeling the skin, the visible aloe vera gel is cut into 2×3 cm sizes, then put in a mixed until smooth and filtered. Filtered Aloe vera gel is ready to be used for platelet aggregation test.

Samples for Analysis

All samples were taken from the 16 subjects (normal invidious). A clean venipuncture was used to collect 0.9 ml of blood into a syringe containing 0.1 ml of 3.8% sodium citrate, mixed and delivered into a sil-iconized glass tube. (Note: One drop of sample was transferred to a glass slide, spread to produce a thin smear, and immediately dried in air to measure the so-called initial aggregation (no inductor addition).

Platelet aggregation test

Inductor agent addition

A amount of the sample (0.2 mL) was transferred to a new siliconized glass tube. 0.02 mL of the appropriate agent was added to the sample. ADP group (ADP addition) and *Aloe vera* group (addition of *Aloe vera* gel). Simultaneously, a stop watch was started. For 10 seconds, the tube was vigorously agitated. (No more agitation was provided to the tube until the test was completed).

Smears preparation

The smears were made at various time intervals, as shown below. ADP group: thin smears were made from 0.01 ml of mixed blood with inductor at the exact 180 second after addition of ADP. *Aloe vera* group: thin smears were made from 0.01 ml of mixed blood with inductor at the exact 60, 120, 180, 240, 300 second after addition of aloe vera gel. All smears were rapidly dried in air and labeled.

Fixation dan staining

The smears were fixed in methanol for 10 minutes and stained by the Wright-Geimsa stain for 20 minutes.

Examination

The smears were observed using an ordinary light microscope with an oil-immersion objective lens after being washed and dried. All platelets encountered in such a linear examination (lateral zone) were counted differentially: the number of free platelets against the number of platelets in aggregation. Both these numbers were recorded and added together. The platelet aggregation percentage was calculated and rounded to the nearest whole value.

Data analysis

The data of platelet aggregation percentages were analyzed using the Statistical Package for the Social Sciences (SPSS) software 16.0. The data of platelet aggregation percentages were analyzed using the Statistical Package for the Social Sciences (SPSS) software 16.0. The Mann-Whitney test was used to analyze data normality, and the Independent Sample T-test was used to compare means. P-value smaller than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The potential of *Aloe vera* gel as a new inductor agent was evaluated using the percentage of platelet aggregation in citrate blood samples with the addition of ADP and *Aloe vera* gel (Graph 1). visible image of the aggregation smears using ADP and *Aloe vera* gel is shown in Figure 2.

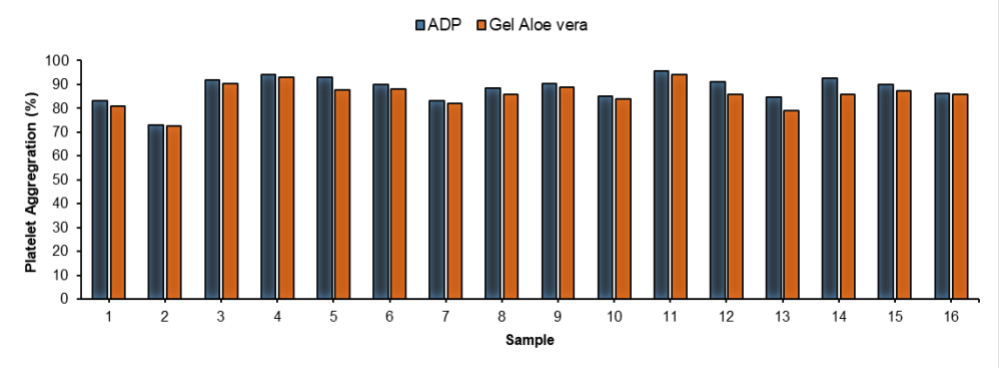

Figure 1. Comparison of platelet aggregation percentage in 16 samples using adenosine diphosphate (ADP) and *Aloe vera* gel

The percentage of platelet aggregation with ADP was higher than *Aloe vera* gel with a mean of 88.2 ± 5.6% and 85.7 ± 5.4%, respectively. The highest percentage of platelet aggregation was found in platelet aggregation smears with

ADP, at 95.6%, while the lowest value was found in platelet aggregation smears with *Aloe vera* gel, at 72.4%. statistical analysis of the Independent Sample T-test (p-value > 0.05) showed no significant difference between the percentage of platelet aggregation with ADP and *Aloe vera* gel. Microscopic observations also showed a similar pattern. Platelet aggregation smears with *Aloe vera* gel produced the same microscopic appearance as the ADP. Platelets aggregated are clearly seen, not covered by latex with normal erythrocyte morphology surrounding.

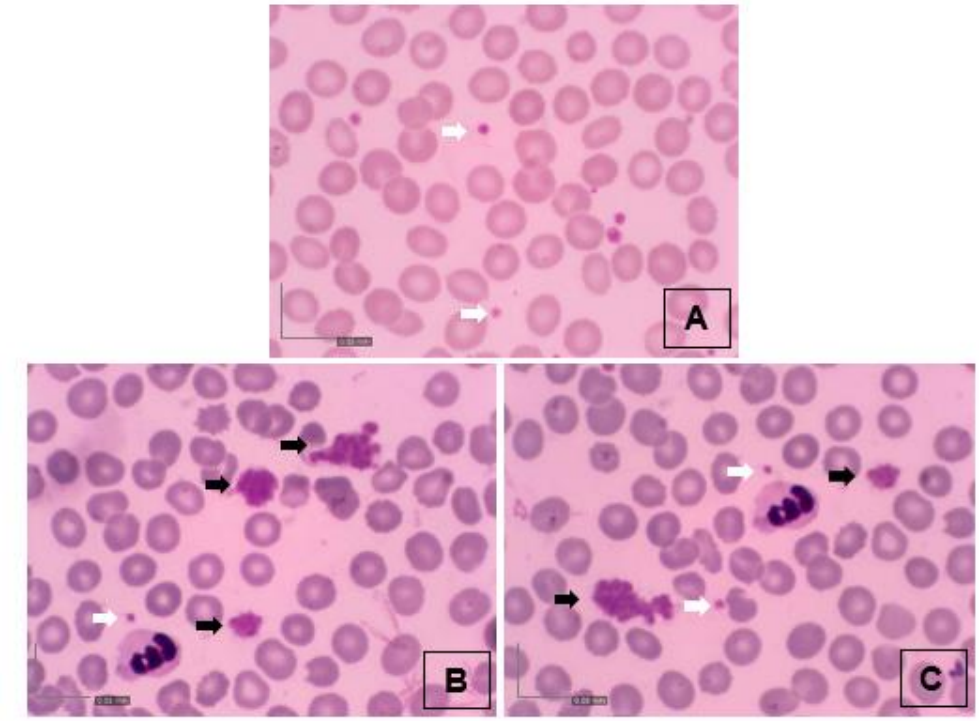

Figure 2. Microscopic observation of platelet aggregation smears using Giemsa staining (1000× magnification); (A) Initial aggregation, (B) ADP inductor, (C) *Aloe vera* gel, black arrow: platelets aggregate; white arrow: free platelets.

Both of these results showed that aloe vera gel has the same potential as ADP to stimulate platelets and aggregation formation. The concentration of 10L of *Aloe vera* gel added is equivalent to the concentration of 10L of ADP, where 10L of the ADP reagent has been determined in the procedure. The ability of *Aloe vera* gel as an inductor does not affect the morphology of erythrocytes, thus, making Aloe vera gel as an ideal inductor agent.

Aloe vera gel contains arachidonic acid, an unsaturated fatty acid that can stimulate platelets to aggregate through the Cyclooxygenase enzyme[14,15,16,17]. The cyclooxygenase-1 enzyme catalyzes the transformation of arachidonic acid into the intermediate product Prostaglandin H2 (PGH2). PGH2 is further metabolized to Thromboxane A2 (TxA2), which is found in platelets. Thromboxane A2 is a potent inductor platelet that stimulates platelet aggregation. Thromboxane A2 not only stimulates platelet aggregation but also shows strong vasoconstrictive effects[18,19]. *Aloe vera* gel contains the amino acid tryptophan which is the precursor of serotonin. Serotonin or 5-hydroxytryptamine (5-HT) is a substance released by platelets that play a role in hemostasis by enhancing the effect of contraction by norepinephrine, histamine, or angiotensin II. This effect is expected to enhance platelet function during hemostasis[20].

Platelet aggregation is the process when platelets adhere to one another. Platelet aggregation is divided into two stages: primary aggregation (reversible aggregation) and secondary aggregation (irreversible aggregation). The primary aggregation phase occurs when thromboxane A2 (TXA2) synthesis increases, leading to platelet aggregation and vasoconstriction. Besides TXA2, ADP is also

an aggregation inducer which with the P2Y12 receptor then induces changes in the shape of platelets from discs to oval pseudopods[21,22].

Furthermore, ADP promotes the expression of the calcium-GPIIb/IIIa complex on the platelet surface and binds to fibrinogen to create bridge-like linkages. This binding will facilitate platelet aggregation in the primary aggregation phase. The secondary aggregation phase occurs when levels of ADP, serotonin, and epinephrine increase, causing irreversible platelet aggregation[21,23].

A previous study on alternative inductors was conducted using betel leaf ethanol extract. There was a difference in platelet aggregation ability between ADP and betel leaf ethanol extract. The mean value of the betel leaf extract was higher than that of the ADP, indicating that the ethanol extract of betel leaf has the potential to be an alternative inductor agent, although the concentration added is not equivalent to that of the ADP reagent[11].

The response of the aggregating platelets to *Aloe vera* gel was evaluated at various time intervals, including 60 seconds, 120 seconds, 180 seconds, 240 seconds, and 300 seconds after the addition of aloe vera gel (Figure 3). The maximum percentage of platelet aggregation was seen 180 seconds after the addition of *Aloe vera* gel, reaching 85.7±2.3%. The percentage of platelet aggregation rose to about 1.36% after the addition of *Aloe vera* gel from 60 seconds to 120 seconds, reaching a maximum percentage at 180 seconds. There is a gradual decrease after reaching the maximum percentage at 240 seconds and 300 seconds. These results indicate that the optimal response of platelets to *Aloe vera* gel is 180 minutes after reacting it with citrate blood.

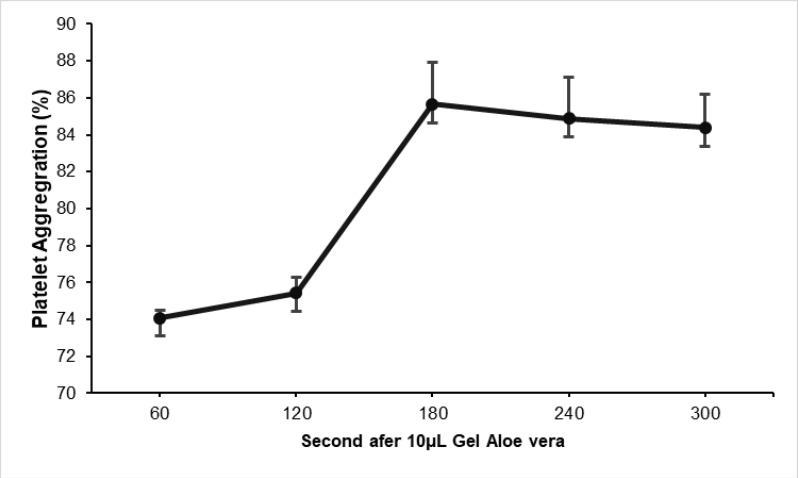

Figure 3. The percentage of platelet aggregation after the addition of 10µL *Aloe vera* gel at various time intervals (all result expressed as mean value ± 1SD)

A comparison of the percentage of platelet aggregation with the addition of different inductors reported using the Light transmission platelet aggregometry method with platelet-rich plasma samples. The results indicate the addition of different inductors to stimulate different platelet activation pathways. ADP (low dose) induces both primary and secondary waves of aggregation (biphasic curve). ADP < 1 µmol induces only a reversible form of platelet aggregation (primary aggregation), without thromboxane synthesis or intraplatelet release of ADP. However, increasing the ADP dosage may produce more significant irreversible aggregation (secondary aggregation)[24,25,26,27].

Arachidonic acid, collagen, and ristocetin induce one wave of aggregation (monophasic curve). Arachidonic acid will induce a change in platelet shape followed by maximum aggregation at 180 seconds then will be constant from 240 seconds to 300 seconds. These results are contrasted with the response of aggregating platelets to *Aloe vera* gel because *Aloe vera* gel used in the study still contains other substances. One of them is the flavonoid which can inhibit the

metabolism of arachidonic acid, reducing the level of aggregating platelets[28]. This is a limitation in this study, the pure aloe vera gel used includes several active components which can affect the performance of aloe vera gel as an inductor[29,30,31].

AUTHORS' CONTRIBUTIONS
All authors contributed equally to this work.

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DATA AVAILABILITY STATEMENT
The utilized data to contribute in this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT
The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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