

The Effect of *Oscimum sanctum* to the Thrombocytes Number on Mice

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ABSTRACT

Oscimum sanctum is herbal that was spread widely in Indonesia. *Oscimum sanctum* contains abundant of substances. One of the functions on *Oscimum sanctum* was anti-thrombocytes effect. This effect is associated with platelet function as a mechanical plug in the vascular injury during the normal homeostatic response. The disruption in thrombocytes function leads to disturb the blood clotting process. Therefore, The aims of the research were to prove the impact of *Oscimum sanctum* on the number of thrombocytes. This research used mice that divided into 3 groups, as a group I dose 250 mg/day (*Oscimum sanctum* infusion), group II dose 500 mg/day and the control group. We used clopidogrel as a positive control to determine the effectiveness of anti platelet effect. Data were analyzed by ANOVA showed that the existence of anti-thrombocyte effect in the *Oscimum sanctum* dose 500 mg/day was significantly different. This result proved that *Oscimum sanctum* has anti-thrombocytes effect by decreasing thrombocytes number.

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1. INTRODUCTION

Oscimum sanctum are found everywhere in Indonesia. *Oscimum sanctum* has many functions including as food and traditional medicine, event Indonesian people use *Oscimum sanctum* more often as food than traditional medicine. In the health sciences *Oscimum sanctum* used to cure cough, to decrease heat of the body, anti-diarrhea, to cure insect bite, to make comfort during menstruation [1].

Oscimum sanctum contains eugenol, cineol, osimen, pinene, terpinhydrate, methyl chavicol, linalool, anethole, thymol, camphor, alanine, arginine, ascorbic acid, aspartic acid, benzyl acetate, β -carotene, β -sitosterol, Caffeic acid, carbohydrate, copper, cystine, eriodictyol, aesculetin, aesculin, fat, fiber, geranyl acetate, glutamic acid, glycine, histidine, isoleucine, isoquercetin, leucine, lysine, magnesium, methionine, mersin, niacin, p-Coumaric acid, phytosterols, potassium, proline, quercetin, riboflavin, ruti, thiamine, tryptophan, tyrosine, valine, calcium, phosphorus, ferro and sulfur [2]. The substances have a function as medicine in *Oscimum sanctum* were essential oil, was found as dominant substances [3].

Currently, abundant reports were carried out to explore the substances and the function of *Oscimum sanctum*. In India, *Oscimum sanctum* were recommended to cure bronchitis, diarrhea, asthma bronchial, malaria, dysentery, dermatitis, arthritis, eye diseases, fever, insect bite, hepatoprotective, anticancer, antidiabetic, antispasmodic, antifungal, antimicrobial, analgesic, adaptogenic, antifertility, antiemetic, cardioprotective, diaphoretic [3]. Some reports were found that *Oscimum sanctum* have anti aggregation trombocytes effect [3]-[6]. The effect was associated with the function of thrombocytes as a mechanical plug

if vascular inflammatory occurred to respond normal homeostatis [7]. Disruption of thrombocytes aggregation leads to the clotting blood disturbances.

Ocimum basilicum is a family to *Oscimum sanctum*. This plant has substances similar with *Oscimum sanctum* but the concentration was different. *Oscimum sanctum* has an anti aggregation of thrombocytes effect. This effect was associated with the obstacle of thrombus generation regarding to thrombus malfunction [2],[4]. This study was conducted to prove the function of *Oscimum sanctum*, a family with *Ocimum basilicum* as resistor to the thrombocytes aggregation. How *Oscimum sanctum* interfere the number of thrombocytes was important to know to support the holistic medical.

2. RESEARCH METHOD

2.1. Grouping the subject

Sixteen male mice of swiss strain, ± 20 grams body weight, 2 months old, were divided into 3 groups to treat with *Oscimum sanctum* infusion in different dose during 10 days. The acclimatization for mice was a week. Groups 1 and 2 were treated as follows 250 mg/day, 500 mg/ day. Group 3 was positive control, treating by clopidogrel (1.35 mg/day). The blood was collected from mice tails 0.5 ml at day 0 and day 10th. The research was conducted at Animal Laboratory of Medical and Health Sciences of Universitas Muhammadiyah Yogyakarta.

2.2. Thrombocyte analyses

Thrombocytes number was detected by blood hematology analyzer at laboratorium penelitian dan pengujian terpadu (LPPT) Universitas Gadjah Mada. The calculation of thrombocyte number based on the phase-contrast methods. The calculation was repeated 5 times.

2.3. Infusion preparation

Twenty five grams *Oscimum sanctum* simplicia was heated into 150 ml water (90° C). After 15 minutes was cooled down. Water was filtered to separate from the leaf. This infusa was 250 mg/ml. The similar way to prepare 500 mg/ ml of infusa, with the weight of simplicia 50 gr.

2.4. Statistical analyses

Data are presented as mean \pm SD. Comparisons between the control and the treatment groups also statistical significance were performed by using one-way ANOVA. Statistical significance was set upon $p < 0.05$.

3. RESULTS AND ANALYSIS

There is a decreasing of thrombocytes number after treating with *Oscimum sanctum* infusion. The average of thrombocytes number at day 0 and day 10th is shown in Table 1.

Table 1. The Average of Thrombocytes Number at Day 0 and Day 10th after Treating with *Oscimum sanctum* Infusion

Groups (dose of <i>Oscimum sanctum</i>)	Thrombocyte number at day 0 (x \pm SD)	Thrombocyte number at day 10 th (x \pm SD)	Decreasing Number	P value
Groups 1 : 250 mg/day	840000 \pm 352772	828500 \pm 351771	11500	0.546
Groups 2: 500 mg/day	874250 \pm 308298	775250 \pm 335808	99000	0.07
Groups 3: clopidogrel (1,35 mg/day).	1043500 \pm 217493	595000 \pm 311683	448500	0.04*

P < 0.05, *, significant different, between day 0 and day 10th

Table 1 shows that the highest on decreasing of thrombocytes number is clopidogrel group. Dose 500mg/ day has higher effect than dose 250 mg/day. The decreasing of the thrombocyte number on dose 250 mg/ day was not significant statistically, but the *Oscimum sanctum* has an effect on the decreasing thrombocyte number. The clopidogrel was significant decreasing the thrombocytes number ($P < 0.005$). This study found that *Oscimum sanctum* infusion and clopidogrel have a function to decrease thrombocytes number. In dose more than 500mg/ day, probably has the same potentiality to clopidogrel. In this research showed that dose 500mg/day has lower potentiality to the standard dose, clopidogrel. Perhaps, the potentiality will be similar if the dose higher than 500mg/day.

Percentage on decreasing thrombocytes number is linear to the dose of *Oscimum sanctum* infusion, more dose of *Oscimum sanctum* more decrease on thrombocyte number. Table 2 shows the potentiality of infusion *Oscimum sanctum* and clopidogrel to decrease the number of thrombocyte during 10 days .

Table 2. Potentiality of Infusion *Oscimum sanctum* and Clopidogrel on the Decreasing Thrombocyte Number

Groups (dose of b <i>Oscimum sanctum</i> infusion)	Decreasing number ($\bar{x} \pm SD$)	Percentage of decreasing number (%)
Dose: 250 mg/day	11500 \pm 4796	1.52 \pm 1
Dose: 500 mg/day	99000 \pm 29944	13.84 \pm 10
Clopidogrel	448500 \pm 0	45.72 \pm 18

The dose 500 mg/day has 13,84 % and the dose 250 mg/day has much lower 1.52%. Clopidogrel as a control has 45.72%. This study used clopidogrel as positive control. Clopidogrel hydrogen sulfate, methyl (+)-(S)- α -(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-acetate hydrogen sulfate, is a novel thienopyridine derivative that irreversibly blocks adenosine diphosphate (ADP) and is important in platelet aggregation, the cross-linking of platelets by fibrin [8]. Clopidogrel as a standard medicine for anti thrombocyte was common in public markets. Beside that, clopidogrel was compare with *Oscimum sanctum* infusion because both showed the effect after 7 days [3],[5],[9].

The comparison between potentiality of *Oscimum sanctum* infusion to clopidogrel has shown in Table 3. The potentiality of infusion in 500mg/ day has 22.2% t and 250 mg/day 0.026% to clopidogrel. It was shown that the function will be significant with dose more than 500 mg/ ml. There was no meaning with dose less than 250 mg/ml.

Table 3. Comparasion of the Potentiality *Oscimum sanctum* Infusion to Clopidogrel on Decreasing Thrombocyte Number

Groups	Percentage (%) thrombocytes number
Group 1: 250mg/day / clopidigrel	2.5641
Group 2: 500mg/day/clopidogrel	22.07358

Table 3 describes that double dose of *Oscimum sanctum* infusion compare to clopidogrel result in effect around 10 times stronger on decreasing thrombocytes number. But it does not mean that 1000mg/day *Oscimum sanctum* infusion will have effect 100 times stronger than 250 mg/day.

Since Singh et al, 2007 reported that oil of *Oscimum sanctum* has the effect of anticoagulant by inhibition of TXA2 through cyclooxygenase and produces PGI3 and TXA3. Like PGI2, PGI3 have antiaggregatory property while TXA3 has much less antiaggregatory. Thus, both of substances work concurrently to contribute the anticoagulant effect on oil of *Oscimum sanctum*⁴. We use infusion because the substances of infusion more complete and the concentration higher. Eugenol in *Oscimum sanctum* was 40% [1],[3] and able to obstruct thrombocytes aggregation by inhibition on arachidonic acid, collagen, epinephrine, ADP to induce thrombocytes aggregation [10]. The inhibition on such ways leads to inhibit on the increasing of collagen, ADP and Ca^{2+} as regulator on the function of thrombocytes.

The thrombocytes regulator substances were divided into 3 groups. Collagen was the first agents that formed outside of thrombocytes and interact with receptors on thrombocyte membrane. ADP was second groups as agent that formed inside of thrombocytes and interact with receptors membrane, meanwhile the Ca^{2+} as the third group as agent that formed inside of thrombocytes and work inside [7]. In this situation if the vascular endothel damage, ADP did not involve in the increasing of thrombocytes aggregation. Nevertheless thrombocytes aggregation still occurred, thrombocyte did not produce signalling substances on fibrin formation to encourage thrombocytes plug that already available and also collagen was hampered to act as a mark on fibrin formation. With the result that thrombocytes in blood will be reduced which causes bleeding time longer.

The other report showed that *Oscimum sanctum* also contains of methanolic substances that have an antiatherogenic effect, as called Caps HT2 or HT2 blocker. Caps HT2 has a function as an antioxidant, anticoagulant, antiaggregation of thrombocytes, releasing lipoprotein lipase, anti-inflammation agent, and activate of lipolipidemic on mice [6]. *Oscimum sanctum* also have flavonoids. as shown in *Melothria maderaspatana*. Methanolic and flavonoid were extracted with hexane able to inhibit thrombocyte aggregation [11]. It was assumed that the effect of thrombocytes aggregation due to methanolic and flavonoids activity. The mechanism as antiaggregation on thrombocyte by methanolic was poor understood. Whereas the mechanism of flavonoids on the inhibition of thrombocytes was mediated with the increasing of

cyclic AMP (platelets' cyclic AMP levels) by stimulate adenylate cyclase also inhibited *cAMP phosphodiesterase* [6].

Flavonoids impede the adhesion and aggregation of thrombocytes, beside of releasing the calcium sitoplasmic to stimulate the releasing of ADP and 5HT. Serotonin (5HT) was released by thrombocyte to stimulate aggregation and vasoconstriction [10]. The inhibition on this mechanism by flavonoid, result in the inhibition of thrombocytes aggregation due to the inhibition from eugenol also. The inhibition on vasoconstriction induced to the complicity on thrombocytes plug formation, lead to the bleeding time longer. The similar effect made by eugenol. Wherefore the inhibition of thrombocyte aggregation by eugenol, methanolic and flavonoid induced in the decreasing of thrombocyte number. Perhaps, this mechanism results in the excessive of thrombus formation inhibition by the adhesion and thrombocytes aggregation. This study showed that *Oscimum sanctum* have a function as an alternative anti thrombocytes medicine [10].

4. CONCLUSION

Oscimum sanctum have a thrombocytes anti aggregations by lowering the thrombocytes number. The dose of *Oscimum sanctum* more than 500mg/day could have the similar effect to clopidogrel on the lowering of thrombocytes number. *Oscimum sanctum* could as the alternative medicine as the thrombocytes anti aggregation. We suggest for further research: (1) to explore the higher dose of *Oscimum sanctum* infusion more than 500 mg/ day to find the similar potentiality with standard dose on clopidogrel, (2) to explore the other liquid than water to extract *Oscimum sanctum* to produce the concentrated extraction, (3) the mechanism of decreasing the thrombocyte number still need explored.

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