Hibiscus Rosa-Sinensis Linn Leaf Extract Increased Platelet Counts and Fibrin Density: in Vivo Study

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Abstract

Failure of hemostasis post-tooth extraction procedures can cause secondary bleeding and interfere wound healing process. Essential factors for the hemostasis process were platelet counts and fibrin density. Hibiscus rosa-sinensis Linn extract has been widely studied and proven increased the wound healing process.

This study aimed to explore the effect of Hibiscus rosa-sinensis Linn in the post-tooth extraction during the hemostasis process by examining platelet counts and fibrin density.

In vivo experimental study with five groups post-extraction socket of Rattus norvegicus. Two control groups without treatment and 3% CMC-Na gel-based group. The treatment groups received treatment with Hibiscus rosa-sinensis Linn leaf extract gel (HrsLE) with different concentrations, namely 10% HrsLE, 20% HrsLE, and 40% HrsLE. Blood clots from the extraction socket were evaluated for platelet counts and fibrin density using histopathological examination under a light microscope. Platelet counts were considered manually using Giemsa staining and fibrin density examination using Masson's trichrome staining. Data were analysed statistically using ANOVA for platelet counts and Kruskal Wallis for fibrin density, with a significance level of $p<0.05$ considered significant.

The highest platelet counts and fibrin density were found in the 40% HrsLE group. All treatment groups were significantly higher for platelet and fibrin density than control ($p<0.001$ and $p<0.0001$). Among all, 40% HrsLE the highest count for platelet and fibrin density ($p<0.0001$).

40% HrsLE promote the hemostasis process post-tooth extraction by increase the platelet counts and fibrin density in socket.

Experimental article (J Int Dent Med Res 2023; 16(3): 1086-1090)

Keywords: Hibiscus rosa-sinensis Linn; Affordable Medicines; Hemostasis Agent; Platelet Counts; Fibrin Density.

Received date: 04 June 2023 **Accept date:** 24 August 2023

Introduction

Hemostasis is the first and most crucial phase in wound healing post-tooth extraction sockets. When a blood vessel is injured, hemostasis occurs by spasm of the blood vessel, formation of a platelet plug, formation of a blood clot, and growth of connective tissue in the blood clot to close the hole in a blood vessel. Platelets and fibrin are essential factors in the process of hemostasis. Platelets produce growth factors such as Platelet-Derived Growth Factor (PDGF), which attract neutrophils and macrophages and influence fibroblast mitogenesis and transforming growth factor beta (TGF-β), as well as cytokines involved in angiogenesis. Platelet aggregation will initiate the activity of coagulation factors. The coagulation process will result in the formation of a fibrin matrix. Fibrin is the main structural protein in blood clot, which stop bleeding and serve as scaffolds to promote wound repair.[1-3]

Humans have used natural ingredients in medicine since ancient times, and there are still many studies conducted using natural sources available many studies are conducted using natural sources in each country. The advantages

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of using natural materials include the availability of raw materials available in nature so that they are more economical and affordable medicine. Still, it is essential to research to find formulations of natural ingredients and procedures for making and using them so that raw materials can be used with optimal and safe benefits in medical treatment.[4]

Hibiscus rosa-sinensis Linn is a herbaceous plant that grows in the tropics in Indonesia. Several studies have reported that secondary metabolites derived from flower extracts, leaf extracts, and root extracts contain, among others: tannins, saponins, flavonoids, terpenoids, and polyphenols, which can provide benefits including: as an antibiotic, antifungal, anti-inflammatory, antioxidant, anticoagulant, antidiabetic.[5-7] One of the studies on the hemostatic effect of Hibiscus rosa-sinensis Linn leaf extract (HrsLE) conducted by Gunawan et al. (2016), by administering to wounds on rats' tails, showed faster bleeding times and blood clotting times.[8] This study aimed to explore the effect of Hibiscus rosa-sinensis Linn in the post-tooth extraction during the hemostasis process by examining platelet counts and fibrin density.

Materials and methods

HrsLE Extract and Gel Making

HRSL leaf extract was performed with maceration method using 70% ethanol, according to the protocol at UPT Materia Medica Batu Malang, Indonesia with extract certificate number 074/120C/102.7/2018.

HrsL leaves were washed with running water, dried at room temperature and cut into small pieces of about 1-2 mm. Then 60 grams of small pieces leaves were extracted using 70% ethanol solvent as much as 1000 ml in an extractor. The solution was shaken for 2x24 hours with a shaker and evaporated with an evaporator at a temperature of 60^0C. The extract was then prepared into a gel form using a 3% CMC Na gel base material with composition describe in table 1.

Post-extraction Model

This research was an in vivo experimental study on Wistar rats *(Rattus norvegicus)* with a post-test-only control group design. The total sample in this study was 25 Wistar rats weighing 200-300 grams. All animals were adapted to cage conditions for seven days. During the adaptation period, animals were fed food and water regularly. Treatment of these experimental animals was carried out according to the protocol at Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga. The protocol was approved by the Ethical Clearance of Health Research Ethics Commission, Faculty of Dental Medicine, Universitas Airlangga, Surabaya under registered-number 146/ HRECC.FODM/ VII/ 2018.

Name	Composition (g)		Final weight (g)
	3% CMA-Na	HrsL extract	
3% CMC-Na	10	-	10
10% HrsLE	9	1	10
20% HrsLE	8	2	10
40% HrsLE	6	4	10

Table 1. The HrsLE gel composition.

The post-extraction model was created on mandibular incisors tooth were extracted by luxation technique. During the procedure, all animals were anaesthetized with 10% ketamine and 2% xylazine via intramuscular injection. After tooth extraction, all the sockets were immediately treated with 10% HrsLE, 20% HrsLE, 40% HrsLE (treatment group) and 3% CMC-Na-based -gel and left empty socket (control groups) by inserting 0.1 ml into the tooth socket with a syringe. Sampling was carried out 24 hours after treatment, by euthanizing rats using lethal dose of ether by inhalation.

Platelet Counts

The platelet was counted using blood from the socket as a sample. The socket blood was dripped into the slide glass object and fixed with methanol. After drying, the object glass was immersed in Giemsa solution for 15 minutes and rinsed with running water twice. The platelet was counted in nine different areas under a light microscope with 400x magnification by a single oral pathologist.

Fibrin Density Counts

The fibrin density was counted using socket tissue. The socket tissue was decalcification using 10% EDTA at least one month and continued with the staining procedure using Masson's Trichome (MT) . Fibrin density was calculated using a predetermined scoring method as follows: 0 = absence of fibrin, 1 = thinly diffused fibrin density, 2 = thinly aggregated fibrin density, 3 = thick diffused fibrin density, 4 = thick clumped fibrin. The platelet was counted in nine different areas under a light

microscope with 400x magnification by a single oral pathologist.

Data Analysis

Statistical data analysis in this study used IBM SPSS Statistics 21 software. Platelet count data were statistical tests using One Way ANOVA and the Post-hoc Tukey test to determine the significance of differences in platelet counts between groups. Fibrin density data were statistical tests using Kruskal Wallis and using the Mann-Whitney test to see the significance of differences in fibrin density between groups.

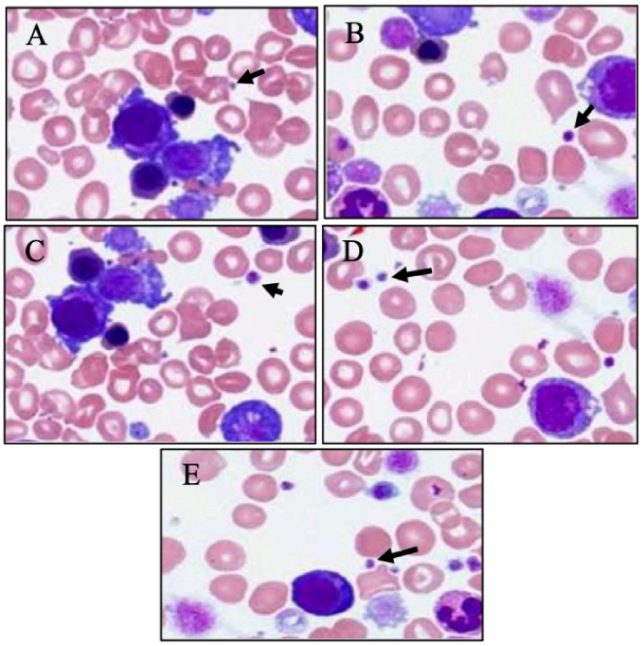

Figure 1. Histologic examination image of platelet counts. Using Giemsa stain, viewed through a 400x magnification light microscope. Black arrows point to platelets that appear purplish red in color: A. without gel group, B. CMC Na 3% base gel group, C. HRS-L 10% group, D. HRS-L 20% group, E. HRS-L 40% group.

Results

Platelet Counts

The platelets look like oval-shaped cells with the smallest size among other blood cells. Platelets are blue-purple in colour and have granules (Figure 1). The platelet count was observed with a higher number in all HrsLE groups compared control groups. The 40% HrsLE groups showed the highest number of platelets ($p<0.001$). While the 20% HrsLE and 10% HrsLE showed a higher number of platelets compared to all control groups ($p<0.001$ and $p<0.01$) (Figure 2A).

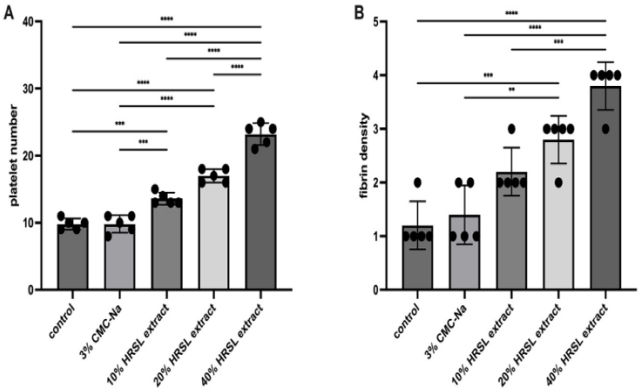

Figure 2. The number of platelet **(A)** and fibrin density **(B)** in post-extraction tooth after treatment with HrsLE. **p<0.05; ***p<0.01; ****p<0.001.

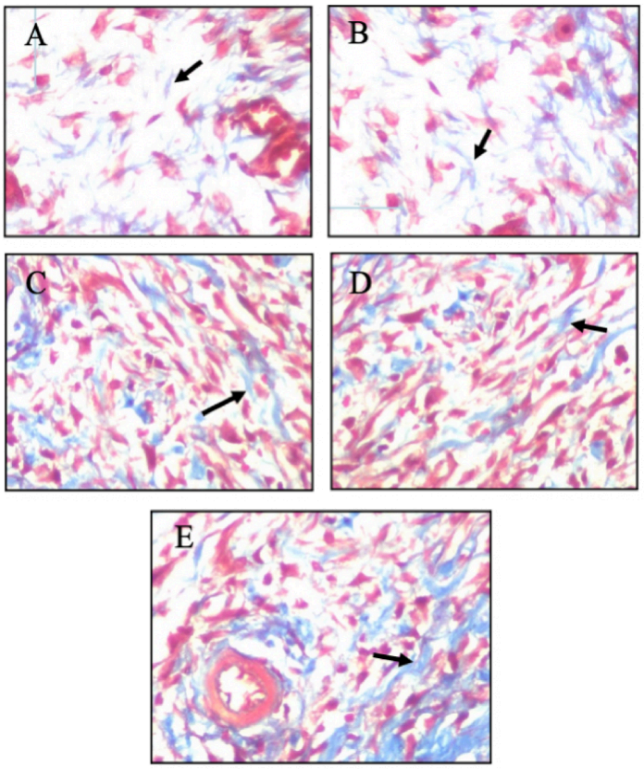

Figure 3. Histologic examination image of fibrin with Masson trichrome staining, viewed through a 400x magnification under light microscope. Black arrows point to fibrin like blue fibers in the preparation: A. without gel group, B. CMC Na 3% base gel group, C. HRS-L 10% group, D. HRS-L

20% group, E. HRS-L 40% group.

Fibrin Density

Fibrin appears as blue fibres (Figure 3). The fibrin density was observed with a higher number in all HrsLE groups compared control groups. The 40% HrsLE groups showed the highest fibrin density ($p<0.001$ and $p<0.01$). While the 20% HrsLE showed a higher fibrin density compared to all control groups ($p<0.01$ and $p<0.05$) (Figure 2B).

Discussion

The phytochemical screening of the Hibiscus rosa-sinensis plant contained alkaloids, flavonoids, tannins, saponins, and terpenoids. Tannins are plant polyphenols that can bind and precipitate proteins.[9] The use of HrsLE in post-extraction tooth socket in this study turned out to be able to strengthen the nature of tannins in Hibiscus rosa-sinensis Linn because tannin has the property of binding proteins and precipitates them so that its effect in the oral cavity can precipitate proteins in saliva making become stringy. Tannins can also react with bacterial cell walls, polysaccharides, carbohydrates and enzymes in the oral cavity. The astringent effect in the oral cavity is caused by the interaction between proteins in saliva and mucous membrane so that lubrication is reduced and the surface tissues contract.[10]

This study showed that platelet counts and fibrin density were higher in groups using HrsLE than in the control. This happens because the tannin content in Hibiscus leaves can precipitate the blood protein, which is albumin. This protein production process will induce the synthesis of thromboxane A2 to increase platelet aggregation, resulting in the formation of platelets in the hemostasis phase. A research report by Tedjasulaksana et al. (2017) on the effect of Piper betle Linn leaf extract gel on wounds after deciduous tooth extraction. Tannins in ethanol extract from Piper betle Linn leaves can shorten bleeding time on hemostasis because tannins work as vasoconstrictors due to their astringent effects. Hemostasis occurs through the process of vasoconstriction, formation of a platelet plug and coagulation. Two things that show an increased hemostatic effect are evidence of differences in platelet counts during the platelet plug formation phase and fibrin density during the coagulation phase. This happens because platelet aggregation will initiate the activity of coagulation factors. The coagulation process will result in the formation of a fibrin matrix. A study by Gunawan et al. (2016) concluded that HrsLE on a rat's tail wound could also accelerate bleeding time.[8,11,12]

Platelet counts were higher at higher concentrations, and the difference in platelet counts from each concentration was significant. The fibrin density score results also found that dense and clumped fibrin density was only found in the 40% HrsLE group. Although a thick diffused fibrin density was also found in the 40% HrsLE, the difference in fibrin density in the 40% HrsLE was significantly more significant than the other groups. This result supports the evidence that the role of increasing platelet counts and fibrin density is HrsLE given to tooth extraction socket and is also influenced by the concentration used. The higher the concentration of HrsLE, the more it supports the hemostasis process by increasing platelet counts and fibrin density. The study of the effect of Sesbania grandiflora Linn flower ethanol extract ointment containing tannins oleanolic acid on the tensile strength of wound incison, also showed better results at higher concentration.[13]

The group without extract, without the treatment group and with 3% CMC-Na gel group showed the same results in platelet counts. However, the standard deviation was higher in the 3% CMC Na group, but the significance test showed no significant difference between the without-treatment group and the 3% CMC Na gel group. It was also found in the fibrin density score result that the density of thin diffuse and thin fibrin was found in some samples without the treatment group and the 3% CMC Na group. This result suggested that exposure of platelets to a "foreign" surface, namely 3% CMC Na as a gel base material applied in the socket wound, promoted coagulation. Thus, this platelet activity was called "platelet factor III availability".[14] The 3% CMC Na group data showed higher platelet counts and fibrin density than without the treatment group. Still, the difference is insignificant, so it can be concluded that 3% CMC Na as a gel base material does not affect the platelet counts and fibrin density.

Conclusions

In this study, it can be concluded that

40% HrsLE promotes hemostasis process in the post-tooth extraction by increasing the platelet counts and fibrin density in the socket.

Acknowledgements

This work was supported by the Research Group Grant of Faculty of Dental Medicine Universitas Airlangga, fiscal year 2022.

Declaration of Interest

The authors report no conflict of interest.

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