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Research Article

ANTITHROMBOTIC EFFECT OF *TRIGONELLA FOENUM-GRACEUM* ON COLLAGEN/ EPINEPHRINE-INDUCED THROMBOEMBOLISM IN MICE

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ABSTRACT

Objective: This study aimed to further investigate the antiplatelet and antithrombotic activities of *Trigonella foenum-graceum* (TFG) *in vivo*.

Methods: Male mice were divided into two experimental groups (bleeding duration and survival rate). The study groups comprised vehicle controls (administered carboxymethyl cellulose [CMC]), negative controls (administered CMC), positive controls (administered aspirin), and experimental treatment groups (administered TFG extract at three doses). Bleeding duration was assessed by excising the tail vein. Survival rate was determined by inducing thrombosis through intravenous collagen/epinephrine administration.

Results: In mice treated with TFG extract for 7 days, bleeding duration was significantly increased compared with that in controls ($p<0.05$). Moreover, mice treated with TFG showed increased survival rates compared with negative controls.

Conclusion: TFG extract showed antithrombotic activity in mice by significantly increasing bleeding duration and survival rate.

Keywords: Antithrombotic, Bleeding duration, Collagen, Epinephrine, Survival rate, *Trigonella foenum-graceum*.

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INTRODUCTION

Cardiovascular diseases, including thrombosis, stroke, ischemic heart disease, and coronary heart disease, are a leading cause of mortality. In particular, thrombotic diseases represent a major cardiovascular complication affecting many patients [1]. Thrombi formed in the circulatory system due to the disruption of homeostasis can cause vascular blockage, atherothrombotic diseases, and myocardial or cerebral necrosis, which can lead to death [2]. Platelets are essential to maintain the vascular integrity and to stop bleeding through blood clot formation, but they have also been implicated to play a role in the pathological progression of arterial vascular thrombosis through adhesion to injured vessels, aggregation to form plugs and acceleration of coagulation cascades [3]. Inhibiting platelet aggregation is a strategy for preventing cardiovascular diseases.

Trigonella foenum-graceum (TFG) or fenugreek is widely distributed in Indonesia and is consumed as a lactagogue by breastfeeding mothers. A previous study has shown that fenugreek leaves possess antithrombotic activity [4]. Fenugreek seeds contain coumarin – a flavonoid with *in vitro* antithrombotic activity [5,6].

In this study, we aimed to further investigate the antiplatelet and antithrombotic activities of TFG *in vivo*. The antithrombotic activity of TFG was assessed by measuring bleeding duration and survival rate [7].

MATERIALS AND METHODS

Materials

Plant materials

Plants simplisia and aqueous extract were obtained from the Indonesian Spice and Medicinal Crops Research Institute, Bogor. Plant materials were deposited in the Center for Plant Conservation Botanic Gardens (No. B-1691/IPH.3/KS/VI/2017).

Chemicals

Ethyl alcohol (70% v/v), Aqua Dest distilled water, saline solution, ether, aspirin, and collagen were obtained from Sigma-Aldrich (Japan Corporation). CMC was purchased from Brataco (Indonesia). Epinephrine was obtained from Sigma-Aldrich (Singapore).

Experimental animals

Male DDY mice (*Mus musculus*; bodyweight 20–30 g) were used for animal studies. Before the experiments, the animals were acclimated for 1 week with food and water provided *ad libitum*. This study was approved by the Ethics Committee of Faculty of Medicine, Universitas Indonesia (No. 232/UN2.F1/ETIK/2017).

Preparation of plant extract

Dried TFG extract was dissolved in a CMC–Na suspension. TFG solutions were prepared at three dose levels (308, 616, and 1232 mg/kg) and administered orally.

Total flavonoid and phenolic contents

Total flavonoid content was determined from a working solution of plant extract. Plant extract (equivalent to 200 mg aqueous extract) was added to a round-bottom flask containing 1 mL of 0.5% hexamethylenetetramine, 20 mL of acetone, and 2 mL of 25% HCl. The suspension was refluxed for 30 min and filtered into a 100 mL volumetric flask. Then, 20 mL of filtrate was extracted with ethyl acetate 3 times, and the extract was collected and added to 50 mL of ethyl acetate in a volumetric flask. To prepare a blank solution, 10 mL of working solution was added to glacial acetic acid in a 25-mL volumetric flask. To prepare the sample solution, 10 mL of working solution was added to 1 mL of 2% $AlCl_3$ and glacial acetic acid in a 25-mL volumetric flask.

Total phenolic content was determined using the Folin–Ciocalteu test. Absorbance was measured at λ=725 nm.

Animal procedures

The *in vivo* study had two outcomes: Bleeding duration and survival rate. Bleeding duration was determined by the tail-bleeding assay. DDY mice were randomly assigned to five groups with five animals

per group: Vehicle control, positive control (aspirin), and three experimental groups receiving different doses of TFG extract. To assess survival rates, DDY mice were randomly assigned to six groups with five animals per group: Vehicle control (CMC), positive control (aspirin), negative control (CMC), and three experimental groups receiving different doses of TFG extract. Treatments were orally administered for 7 days as indicated in Tables 1 and 2.

Tail-bleeding assay

At 5 h after the last administration of extract on the last day of treatment, aspirin, or vehicle alone, animals were anesthetized using ether. A 10-mm incision was made on the tail from the tip using a surgical blade. The end of the incised tail was immediately immersed in the isotonic saline solution in a Falcon tube. Each mouse was observed for 20 min to record bleeding duration. If the bleeding was not constant, the total duration of bleeding within 20 min was summed up. The experiment was stopped after 20 min to prevent the death of the experimental animals [2].

Pulmonary thromboembolism model

Survival rates were determined using a slightly modified pulmonary thromboembolism model described by Saputri *et al*. [2]. At 24 h after the last treatment administration, experimental animals were injected with collagen-epinephrine (700 µg and 42 µg, respectively) in isotonic saline solution. The mice were observed for 15 min to assess lethal or total thrombosis. A positive control mice were given an injection with paralyzing effect. Efficacy in the three groups treated with TFG extract was calculated by comparison with the control group. Survival rates (%) were calculated using the following formula:

$$1-\left(\frac{\text{Number of dead animals}}{\text{Number of animals in the group}}\right)\times 100\%$$

RESULTS

Total flavonoid and phenolic contents of TFG extract

Both flavonoids and phenols were undetectable in TFG extract. Reportedly, TFG contains polyphenols such as apigenin-7-*O*-glycoside (1955.55 ng/mg) and luteolin-7-*O*-glycoside (725.50 ng/mg) [8]. Little flavonoids present in TFG might be difficult to detect.

Table 1: Treatment groups for the tail-bleeding assay

Groups	Treatment	
	Days 1–7	Day 7 (5 h after last administration)
Vehicle	CMC–Na	Tail-bleeding assay
ASA	Aspirin	
TFG D1	TFG extract	
TFG D2		
TFG D3		

Vehicle (CMC 0.5% volume 0.3 mL/20 g bodyweight), ASA (aspirin 0.208 mg/20 g bodyweight), TFG D1 (6.16 mg/20 g bodyweight), TFG D2 (12.32 mg/20 g bodyweight), and TFG D3 (24.64 mg/20 g bodyweight). TFG: *Trigonella foenum-graceum*, ASA: Acetylsalicylic acid, CMC: Carboxymethyl cellulose

Table 2: Treatment groups for assessment of survival rates

Groups	Treatment	
	Days 1–7	Day 8
Vehicle	CMC–Na	Isotonic saline injection
Negative	CMC–Na	
ASA	Aspirin	Collagen/epinephrine solution injection
TFG D1	TFG extract	
TFG D2		
TFG D3		

Vehicle (CMC 0.5% volume: 0.3 mL/20 g bodyweight), ASA (aspirin 0.208 mg/20 g bodyweight), TFG D1 (6.16 mg/20 g bodyweight), TFG D2 (12.32 mg/20 g bodyweight), and TFG D3 (24.64 mg/20 g bodyweight). TFG: *Trigonella foenum-graceum*, ASA: Acetylsalicylic acid, CMC: Carboxymethyl cellulose

Bleeding duration

Compared to treatment with vehicle alone, treatment for 7 days with TFG extract increased bleeding duration in mice. As shown in Table 3, bleeding duration in the positive control and TFG-treated groups was significantly different from that in the group treated with vehicle alone ($p<0.05$). The highest mean value of bleeding duration was observed in the TFG D3 group (15.99±4.51 min).

Survival rate

Compared with the negative control group, treatment for 7 days with TFG extract increased survival rate in mice. As shown in Table 4, the highest survival rate was observed in the TFG D3 group (80%).

DISCUSSION

Traditional medicines are essential, especially in developing countries. Moreover, plant-derived compounds have played an important key role in drug discovery [9]. Therefore, his study aimed to investigate the antiplatelet and antithrombotic activities of TFG. Platelet aggregation is essential for homeostasis. Antithrombotic agents have been developed to mitigate or prevent deregulated thrombosis [10]. Thrombin is the key effector enzyme of the coagulation system. The biologically important functions include activation of platelets, conversion of fibrinogen to a fibrin network, and feedback amplification of coagulation [11].

Bleeding duration

Platelets are essential in the maintenance of cardiovascular integrity and in the control of bleeding through forming blood clot [12]. In this study, TFG-treated mice showed significantly increased bleeding duration, which is in agreement with a previous *in vitro* study showing that TFG extract possessed antithrombotic activity [5]. Increased bleeding duration is due to coumarin constituents of TFG seeds that inhibit Vitamin K function, resulting in the disruption of prothrombin biosynthesis [6,13].

Survival rate

Survival rates of mice treated with all three doses of TFG extract were higher than those of negative controls. The TFG D3 group - administered

Table 3: Bleeding duration in TFG-treated and control mice

Groups	Duration of bleeding (min; mean±SD)
Vehicle	7.16±1.61
ASA	17.32±2.11*
TFG D1	15.98±4.14*
TFG D2	14.63±3.16*
TFG D3	15.99±4.51*

Vehicle (CMC 0.5% volume: 0.3 mL/20 g bodyweight), ASA (aspirin 0.208 mg/20 g bodyweight), TFG D1 (6.16 mg/20 g bodyweight), TFG D2 (12.32 mg/20 g bodyweight), and TFG D3 (24.64 mg/20 g bodyweight). *$p<0.05$ compared to the vehicle control group. SD: Standard deviation, TFG: *Trigonella foenum-graceum*, ASA: Acetylsalicylic acid, CMC: Carboxymethyl cellulose

Table 4: Result of survival rate

Groups	Survival rate (%)
Vehicle	-
Negative	0
ASA	100
TFG D1	40
TFG D2	60
TFG D3	80

Vehicle (CMC 0.5% volume: 0.3 mL/20 g bodyweight), ASA (aspirin 0.208 mg/20 g bodyweight), TFG D1 (6.16 mg/20 g bodyweight), TFG D2 (12.32 mg/20 g bodyweight), and TFG D3 (24.64 mg/20 g bodyweight). TFG: *Trigonella foenum-graceum*, ASA: Acetylsalicylic acid, CMC: Carboxymethyl cellulose

the highest dose - showed the highest survival rate (80%). Mice treated with a higher dose of TFG extract showed higher survival rates, which is consistent with a previous study showing that aqueous TFG extract inhibited coagulation in a dose-dependent manner [14].

CONCLUSION

The interaction between platelets and blood vessels is important in the development of thrombosis and cardiovascular diseases. This study showed that TFG has antithrombotic activity in male mice by significantly increasing bleeding duration and survival rate. Our results confirmed the results of previous *in vitro* studies. Further studies are warranted to determine the specific TFG constituents responsible for these effects and their mechanisms of action.

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CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Kim K, Lim KM, Kim CW, Shin HJ, Seo DB, Lee SJ, *et al*. Black soybean extract can attenuate thrombosis through inhibition of collagen-induced platelet activation. J Nutr Biochem 2011;22:964-70.
2. Saputri FC, Nabila N, Mun'im A. Combination of ginger and sappan wood extract effect on *in vivo* antithrombotic activity test observation result of bleeding time. J Young Pharma 2017;9:46-8.
3. Lee JO, Kim C, Lee S, Oak M. Antiplatelet and antithrombotic activities of *Lindera obtusiloba* extract *in vitro* and *in vivo*. Biomiol Ther 2010;18:205-10.
4. Parvizpur A, Ahmadiani A, Kamalinejad M. Probable role of spinal purinoceptors in the analgesic effect of *Trigonella foenum* (TFG) leaves extract. J Ethnopharmacol 2006;104:108-12.
5. Kontogiorgis C, Nicolotti O, Mangiatordi GF, Tognolini M, Karalaki F, Giorgio C, *et al*. Studies on the antiplatelet and antithrombotic profile of anti-inflammatory coumarin derivatives. J Enzyme Inhib Med Chem 2015;30:925-33.
6. Patil S, Jain G. Holistic approach of *Trigonella foenum-graecum* in Phytochemistry and Pharmacology- A Review. CTTS 2014;3:34-48.
7. DiMinno G, Silver MJ. Mouse antithrombotic assay: A simple method for the evaluation of antithrombotic agents *in vivo*. Potentiation of antithrombotic activity by ethyl alcohol. J Pharmacol Exp Ther 1983;225:57-60.
8. Rayyan S, Fossen T, Andersen ØM. Flavone C -glycosides from seeds of fenugreek, *Trigonella foenum-graecum* L. J Agric Food Chem 2010;58:7211-7.
9. Ekasari W, Wahyuni TS, Arwaty H, Putri NT. Determination of effective dose of antimalarial from *Cassia spectabilis* leaf ethanol extract in *Plasmodium berghei*-infected mice. Afr J Infect Dis 2018;121 Suppl:110-5.
10. Kwon SU, Lee HY, Xin M, Ji SJ, Cho HK, Kim DS, *et al*. Antithrombotic activity of *Vitis labrusca* extract on rat platelet aggregation. Blood Coagul Fibrinolysis 2016;27:141-6.
11. Ku SK, Bae JS. Antiplatelet and antithrombotic activities of purpurogallin *in vitro* and *in vivo*. BMB Rep 2014;47:376-81.
12. Klafke JZ, Arnoldi da Silva M, Fortes Rossato M, Trevisan G, Banderó Walker CI, Martins Leal CA, *et al*. Antiplatelet, antithrombotic, and fibrinolytic activities of *Campomanesia xanthocarpa*. Evid Based Complement Alternat Med 2012;2012:954748.
13. Taj Eldin IM, Abdalmutalab MM, Bikir HE. An *in vitro* anticoagulant effect of fenugreek (*Trigonella foenum-graecum*) in blood samples of normal Sudanese individuals. Sudan J Paediatr 2013;13:52-6.
14. Lee J, Kwon G, Park J, Kim JK, Choe SY, Seo Y, *et al*. An ethanol extract of Ramulus Mori improves blood circulation by inhibiting platelet aggregation. Biosci Biotechnol Biochem 2016;80:1410-5.