ANTITHROMBOTIC ACTIVITY OF TAMARINDUS INDICA L. IN MICE

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ABSTRACT

Objective: This study aimed to investigate the antithrombotic activity of Tamarindus indica L. extract (TIE) in mouse models (in vivo).

Methods: TIE was orally administered to mice at three different doses for 7 days. TIE-treated mice were used in two experiments of antithrombotic activity: An examination of bleeding time following tail cutting and an examination of survival rate after collagen-epinephrine-induced thromboembolism. The TIE groups were observed after 7 days of treatment and compared to an aspirin-treated group and a control group.

Results: Treatment with TIE led to a significant increase in bleeding time compared with that in the control group. TIE treatment also protected mice from thromboembolic death, significantly increasing survival rates in a dose-dependent manner.

Conclusion: TIE has the potential as an antithrombotic agent against platelet thromboembolism.

Keywords: Antithrombotic, Bleeding time, Survival rate, Tamarindus indica L.

INTRODUCTION

Thrombosis, as one of the risk factors of cardiovascular disease, is the formation of a blood clot in an artery or a vein which starts with platelet aggregation [1,2]. Deep vein thrombosis (DVT) refers to the formation of one or more blood clots in one of the major blood vessels of the body, most commonly in the lower limbs. The most serious complication that can arise from DVT is a pulmonary embolism (PE), which occurs in more than one-third of patients with DVT and often causes sudden death [3-5]. Acetylsalicylic acid (ASA), commonly known as aspirin, is often used as a platelet aggregation inhibitor agent. ASA inhibits platelet aggregation by inhibiting the cyclo-oxygenase (COX) enzyme within the COX pathway, thus preventing thrombus formation [6].

In addition to chemical drugs, herbal medicines have been studied for their potential as the antithrombotic agent. Tamarind (Tamarindus indica L.), a flowering plant within the family Fabaceae, is a potential antithrombotic agent due to its active compound dotriacontanoic acid [7]. This compound is one component of D-003, a natural compound comprising triacontanoic, dotriacontanoic, and tetradecanoic acid [8,9]. Compound D-003 has been tested in vitro on a venous thrombosis model; a dose of 200 mg/kg has shown to increase the number of prostacyclin and reduce thromboxane A2. In vivo experiments with D-003 have demonstrated that this compound can reduce the formation of venous thrombus at a dose of 400 mg/kg in collagen-induced and epinephrine-induced mice [8]. In this study, the effects of T. indica L. extract (TIE) were tested in vivo on mice using bleeding time and survival rate as metrics of antithrombotic activity.

MATERIALS AND METHODS

Materials

Ethanolic TIE was obtained from the Indonesian Spice and Medicinal Crops Research Institute, Bogor. This study used aspirin (Medifarma, Indonesia) as a positive control. Carboxymethyl cellulose (CMC) (0.5%; Brataco, Indonesia) and saline (Euro-Med, Indonesia) were used as a vehicle group (normal and negative control group). Voucher specimens were deposited at the Center for Plant Conservation Botanic Gardens (No. B-1693/IPH.3./KS/VI/2017).

Total flavonoid and phenolic content assays

Total flavonoid content was measured using the aluminum chloride colorimetry method, with absorbance measured at λ=510 nm [10]. The total phenolic compound content was measured using the Folin–Cioclatelu method, with absorbance measured at λ=725 nm [11].

Animals

Male mice (Mus musculus, ddY strain) weighing between 20 and 30 g were obtained from the Institute Pertanian Bogor. The use of animals in this study was approved by the Ethics Committee of Faculty of Medicine, Universitas Indonesia (Number: B.232/UN2.1/F/ETIK/2017).

Antithrombotic activity tests

Bleeding time test

The treatment of bleeding time was determined according to protocols described by Saputri et al. (2017) with the following modifications. Mice were divided into the treatment groups shown in Table 1 and acclimatized for 1 week. Experimental treatment doses were given to mice through oral administration for 7 days. The dose of aspirin applied was 80 mg/day [12-14]. Experimental TIE doses were 14, 28, and 56 mg/20 g mice. Antithrombotic activity was evaluated by a bleeding time of 5 h after experimental day 7. The animals were anesthetized with ether by inhalation and placed in a horizontal position. An approximately 10-mm segment of the tail was amputated from each animal using a scalpel. The end of each severed tail was immediately immersed in a 15 mL Falcon tube containing isotonic saline. The remaining portion of the tail was vertically placed and then horizontally placed approximately 2 cm below the body. Bleeding observations were conducted for 20 min [12]. Differences in bleeding time with TIE treatment were obtained by comparing each experimental dosage group to the vehicle group.

Survival rate test

Mice used for survival rate tests were divided into treatment groups as shown in Table 2. The survival rate tests were performed 24 h after experimental day 7. To induce pulmonary thrombosis, experimental mice were injected with a mixture of collagen (700 µg) and epinephrine (42 µg)
Table 1: Treatment groups used for tests of bleeding time in mice

| Group         | Number of mice (n) | Dosage (mg/20 g mice) | Bleeding time treatment |
|---------------|--------------------|-----------------------|-------------------------|
| Normal        | 5                  |                       | CMC 0.5%                |
| ASA           | 5                  | 0.208                 | Tail bleeding assay     |
| Dose 1        | 5                  | 14                    | Aspirin                 |
| Dose 2        | 5                  | 28                    | Tamarind extract (TIE)  |
| Dose 3        | 5                  | 56                    |                         |

CMC: Carboxymethyl cellulose, ASA: Acetylsalicylic acid

Table 2: Treatment of survival rate in mice

| Group         | Number of mice (n) | Dosage (mg/20 g mice) | Survival rate treatment |
|---------------|--------------------|-----------------------|-------------------------|
| Normal        | 5                  |                       | CMC 0.5%                |
| Negative      | 5                  | 0.01                  | Saline injection        |
| ASA           | 5                  | 0.208                 | CMC 0.5%                |
| Dose 1        | 5                  | 14                    | Aspirin                 |
| Dose 2        | 5                  | 28                    | Tamarind extract (TIE)  |
| Dose 3        | 5                  | 56                    |                         |

CMC: Carboxymethyl cellulose, ASA: Acetylsalicylic acid

Discussion

Analysis of total flavonoid and phenolic content

Tamarind extract has a low flavonoid content. The sample used in this study had no detectable flavonoid content. Flavonoid compounds play a role as antioxidants and also function as antithrombotic agents by reducing adenosine diphosphate (ADP)-induced aggregation and thrombin. Specifically, flavonoids act as TXA2 receptor antagonists, reducing TXA2 which then indirectly inhibits COX-1. In addition, flavonoid compounds increase the production of nitric oxide, which is important for the inhibition of platelet aggregation [16].

Analysis of bleeding time

The antithrombotic effect of TIE was evaluated by comparing the bleeding time of treated animals to animals in the vehicle group. Treatments with TIE, which contains the D-003 compound dotriacontanoic acid, resulted in significant, dose-dependent increases in bleeding time. However, the bleeding times of the TIE-treated animals were not significantly different from those of ASA-treated animals, indicating that the antithrombotic effect was the same for both groups. The largest bleeding time effect was seen in the experimental group given the highest dose of TIE (56 mg/20 g mice).

Inhibition of platelet aggregation includes the reduction of TXA2 formation and prostacyclin (PgI2) enhancement. TXA2 is a potent agonist causing the activation of platelets and thrombus formation. TXA2 causes irreversible platelet aggregation, vasoconstriction, and proliferation of smooth muscle cells [17]. PgI2 is synthesized by PgI2-synthase in endothelial cells and has an effect as an aggregation inhibitor and vasodilator. PgI2 acts to inhibit vasoconstriction and dilate blood vessels [8]. When the formation of the thrombus is inhibited, blood flow is smooth and bleeding times increase.

In this study, the antithrombotic effects of TIE treatments were similar to that of the aspirin treatment. Aspirin inhibits collagen-induced platelet aggregation at optimal doses of 81–162 mg/day [18]. Aspirin works as an antithrombotic agent by inhibiting the enzyme COX and non-COX inhibition. Inhibition of COX-1 will inhibit TXA2 formation and stimulate platelet aggregation. In non-COX enzymes, aspirin will alter glycoprotein IIB/IIIa receptor function and affect the permeability of clots. In addition, aspirin also inhibits acetylchylase, prothrombin, antithrombin, fibrinogen, and factor XIII [18].
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The proportional increase in bleeding time across experimental treatment groups, as compared with the normal control group.

**Table 3: Results of bleeding time tests**

| Group | Bleeding time (mean±SD) |
|-------|-------------------------|
| Normal | 7.32±1.10 |
| ASA    | 16.50±3.24* |
| Dose 1 | 14.56±3.02* |
| Dose 2 | 15.92±2.01* |
| Dose 3 | 16.68±3.21* |

Normal (CMC 0.5% volume: 0.3 ml/20 g BW), ASA (aspirin 0.208 mg/20 g BW), Dose 1 (14 mg/20 g BW), Dose 2 (28 mg/20g BW), Dose 3 (56 mg/20 g BW). *p≤0.05, compared with the normal control group.

**Table 4: Survival rates within experimental treatment groups**

| Group | Survival rate (%) |
|-------|-------------------|
| Normal | - |
| Negative | 0 |
| ASA | 100 |
| Dose 1 | 80 |
| Dose 2 | 100 |
| Dose 3 | 100 |

Normal (CMC 0.5% volume: 0.3 ml/20 g BW), ASA (aspirin 0.208 mg/20 g BW), Dose 1 (14 mg/20 g BW), Dose 2 (28 mg/20 g BW), Dose 3 (56 mg/20 g BW). CMC: Carboxymethyl cellulose, ASA: Acetylsalicylic acid, BW: Bodyweight, TIE: Tamarindus indica L. extract.

**Analysis of survival rate**

A combination of collagen and epinephrine was used to induce thrombosis in the experimental animals. Collagen is a major activator of platelet aggregation induced by ADP. Epinephrine is an β2-adrenergic agonist which causes a disruption of the exchange between potassium ions and sodium and calcium ions, leading to hypokalemia [20,21]. Hypokalemia causes stimulation of the muscle membrane to be disrupted which can lead to paralysis [22]. The injections of collagen and epinephrine caused the deadly effects in mice through thromboembolism or vasoconstriction by increased TxA2 and Pgs from platelets [17].

The active antithrombotic compound within TIE, the D-003 compound dotriacontanoic acid, can inhibit platelet aggregation induced by collagen-epinephrine. The D-003 compound inhibits platelet aggregation induced by collagen, arachidonic acid, serotonin, and ADP [23,24]. In addition, the D-003 compound also inhibits platelet aggregation induced by a combination of collagen and epinephrine [8]. Prior studies have found that treatment with D-003 inhibits platelet aggregation by 55% at a dose of 400 mg/kg BW of mice [8]. The dotriacontanoic acid compound (D-003) can also significantly increase the survival rate in a dose-dependent manner. TIE is more effective at inhibiting platelet aggregation induced by collagen-epinephrine than aggregation that is induced by ADP. A prior study, involving treatment with the D-003 compound at a dose of 200 mg/kg BW of mice, documented that D-003 resulted in a 33% inhibition of ADP-induced platelet aggregation and a 39% inhibition of aggregation induced by collagen-epinephrine [8].

**CONCLUSION**

Using in vivo experiments, we demonstrated that TIE has antithrombogenic potency evidenced by increasing bleeding times and survival rates in TIE-treated experimental animals. The greatest antithrombogenic potency was found at a dose of 56 mg/20 g mice.

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**CONFLICTS OF INTEREST**

All authors have none to declare.

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