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Research Article

Antiplatelet, anticoagulant, and thrombolytic effect of *Carica papaya* L. leaf ethanol extract in the thrombosis mouse model

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ABSTRACT

Cardiovascular diseases such as stroke and chronic ischemia are among the leading causes of death worldwide. The one factor that causes this disease is thrombosis. A thrombus blocks blood flow, reduces oxygen supply, and damages endothelial tissue and myocytes. Various anti-thrombotic drugs that have been used so far have several side effects, such as bleeding, thrombocytopenia, pain, and others. *Carica papaya* L. is a plant that affects antiplatelet, anticoagulant, and thrombolytic *in vitro*. This study aimed to determine the effect of antiplatelet, anticoagulant, and thrombolytic agents *in vivo* using the thrombosis mouse model. The research subjects were 24 mice divided into eight groups: normal control, negative control (thrombosis), positive control (clopidogrel, warfarin, and natto-kinase), *Carica papaya* L leaf ethanol extract (CPEE) 2, 20, and 200 mg/kg body weight. The treatment was given for 24 hours after thrombosis induction. The blood sample was taken to determine platelet aggregation inhibition, PT, APTT, and percent of thrombolysis. The study showed that the ethanol extract of *Carica papaya* leaf at 20 and 200 mg/kg BW significantly ($p<0.05$) differed from the negative control. Ethanol extract of *Carica pap*aya L leaves at a dose of 200 mg/kg BW is the best dose to inhibit platelet aggregation, normalize PT and APTT after thrombosis induction, increase the percentage of thrombolysis and D-Dimer level in the thrombosis mouse model.

Keywords:
Anticoagulant; Antiplatelet; *Carica papaya* L; Leaf; Thrombolytic

1. INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death throughout the world. According to WHO data in 2019, the estimated death rate due to cardiovascular diseases accounts for 17.9 million or 32% of deaths globally, mainly heart attacks and strokes [1]. Stroke and ischemia accounted for 2 out of 10 Disability Adjusted Life Years (DALYs) or disease burden in Indonesia [2]. One of the main factors in the occurrence of CVD is the presence of a thrombus (blockage) originating from a blood clot in the lesion of atherosclerosis, and its spread causes disturbances in heart function [3]. A thrombus is a blockage often associated with organ dysfunction, important in the body, especially heart function disorders such as atherosclerosis, embolism, Myocardial Infarction, and Ischemic Stroke [4]. A thrombus is a blockage in the form of blood clots due to hemostasis disorders, including platelet aggregation, coagulation, and fibrinolysis. Hemostasis disorders leading to blockage of blood vessels can occur due to hyperaggregation, hypercoagulation, and disorders of fibrinolysis. Thrombus treatment is necessary to prevent blockages that lead to cardiovascular problems [5].

Thrombus formation in the hemostasis process is related to the activity of platelets, coagulation, and fibrinolysis. Platelet hyper-reactivity factors can cause thrombosis, which is preceded by vasoconstriction of blood vessels, adhesions on the endothelial wall, activation,

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and aggregation. The aggregate will be continued in the cascade coagulation [6]. Platelet hyperreactivity is influenced by genetic factors, platelet reactivity, hormonal disorders, such as epinephrine, and high plasma fibrinogen due to oxidative stress and inflammatory factors [7]. Factor hypercoagulation and hypo-thrombolysis also increase the incidence of thrombosis. The factors that cause hypercoagulation and hypo-thrombolysis, among other disorders, include genetics, hormonal disorders, obesity, inflammation, immune disorders, malignancies (cancer), liver cirrhosis, and other factors that increase and decrease the activity of coagulation factors and fibrinolysis factors [8].

Anti-blood clotting drugs include antiplatelets, anticoagulants, and thrombolytics, which cardiovascular disease patients widely use to reduce blockages and more severe heart organ dysfunction. Antiplatelets work on the hemostasis system to prevent platelet aggregation, preventing blood clots and thrombi. Aspirin and clopidogrel are some of the types of antiplatelets that are often used. Aspirin inhibits the COX (cyclooxygenase) enzyme, thereby inhibiting the production of TXA2 (Thromboxane A2), and the aggregation of platelets can be inhibited [9]. Clopidogrel works competitively and irreversibly inhibits Adenosine Diphosphate (ADP) receptors via P2Y12 [10]. Anticoagulants such as heparin work by inhibiting FXa so that they can prevent coagulation [11]. Streptokinase works by increasing plasminogen activator (tPA) activity [12].

Various studies show that several anti-blood clotting drugs have side effects on the body. Side effects of antiplatelet, anticoagulant, and thrombolytic drugs include bleeding [13], headache, fever, nausea, and diarrhea [14], neutropenia [15], thrombocytopenic purpura [16], hypersensitivity [17] as well as tachycardia and arrhythmia [18]. Based on these problems, it is necessary to develop anti-clotting treatments and safer blood for patients with cardiovascular disease, especially those with thrombus or blockage due to blood clots, one of which is through drug development based on natural ingredients and local Indonesian resources.

Currently, medicines are developed based on natural ingredients from local Indonesian resources, which are being intensively carried out to encourage national independence, especially in the pharmaceutical sector. Armed with abundant natural resources, we have confidence in diverse biological resources with the potential for treatment. One of the local plants in Indonesia that is widely researched for treatment is the *Carica papaya* L. The *Carica papaya* L. are widely distributed in tropical regions such as Indonesia. It contains metabolite compounds that have the potential to be medicinal ingredients.

Empirically, papaya leaves are widely used for treating digestive disorders and as an anthelmintic. As research progresses, Papaya leaves are known to have several pharmacological effects, including treating fever and bleeding, anti-malarial, and anti-diabetic [19], antihypertensive, immunomodulator [20], hyperlipidemia, antibacterial, gastroprotective, and anti-inflammatory [21]. Papaya leaves have antiplatelet, anticoagulant, and thrombolytic effects *in vitro* [22].

Papaya leaves contain secondary metabolites that have been proven to have pharmacological effects. One of them is from the alkaloid compounds group. Papaya leaf alkaloids are proven to have the activity to prevent platelet aggregation and prolong CT, PT, APTT, and increase clot retraction [23]. Carpaine is a compound in the leading alkaloid group contained in papaya leaves at a level of 0.93 g/kg [24]. In silico study of Carpaine showed that Carpaine can bind to P2Y12, potentially inhibiting its binding to ADP [23]. This study aims to determine the effect of antiplatelet, anticoagulant, and thrombolytic properties of *Carica papaya* L. ethanol extract in mice with deep vein thrombosis induction (*in vivo).*

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this research were *Carica papaya* L. obtained from Tawangmangu area of Central Java, ethanol (Emsure, Merck, Germany), Ethyl acetate (Emsure, Merck, Germany), chloroform (Emsure, Merck, Germany), HCL (Emsure, Merck, Germany), NH_4OH (Emsure, Merck, Germany), Mayer (Nitra Kimia Indonesia), Dragendorff (Emsure, Merck, Germany), Wagner (Nitra Kimia Indonesia), Mg (Emsure, Merck, Germany), FeCl3 (Emsure, Merck, Germany), Kappa-Carrageenan (Merck), Clopidogrel Bisulfate (Dexa Medica), Warfarin Sodium Clathrate (Novell), Natto-kinase (Lapi), Adenosine Diphosphate (ADP) (Biotop Medical Belanda), Prothrombine Time (PT) Reagen (Teclot, Rusia), Activated Partial Thromboplastin Time (APTT) Reagen (Teclot, Rusia) and D-dimer ELISA Kit (Reed Biotech). The equipment used in this research was a rotary evaporator (RV8, IKA), an Oven (UN30, Memmert), a Spectrophotometer UV-Vis (Genesys 10S, Thermo Scientific), and a water bath (Health, Korea).

2.2. Ethical clearance and group of treatment

Mus musculus (Male, 20-30 g, n=24) was used in this pre-clinical research. The research received an ethics certificate from the ACUC, Faculty of Veterinary Medicine, Universitas Airlangga (No: 3.KEH.102.07.2024). Mice were induced by Deep Vein Thrombosis using Kappa carrageenan (1,000 µg/ml) in NaCl 0.9% until thrombosis in the tail. This study consisted of (1) normal control, (2) negative control (deep vein thrombosis), (3) Positive control Clopidogrel (9.75 mg/kg body weight), (4)

Warfarin (1.3 mg/kg body weight), (5) Natto-kinase (13 mg/kg body weight), (6) *Carica papaya* L leaf ethanol extract (CPEE) 2 mg/kg body weight, (7) CPEE 20 mg/kg body weight, (8) CPEE 200 mg/kg body weight. The parameters were the percent of platelet aggregation inhibition, PT APTT, and the percent of thrombolytic.

2.3. *Carica papaya* L. leaf extraction

500 g of dried papaya leaf powder was macerated with 96% ethanol solvent for 24 hours, 3 times. The filtrate from maceration is evaporated using a solvent rotary evaporator to obtain a thick ethanol extract of papaya leaves [22]. We prepared 2, 20, and 200 mg/kg body weight of papaya ethanol extract.

2.4. Phytochemical assay

Phytochemical tests were carried out to qualitatively investigate the *Carica papaya* L. content, including flavonoids, alkaloids, polyphenols, saponins, tannins, and steroids. The alkaloid test was carried out using Mayer, Dragendorff, and Wagner. Flavonoid test using Mg powder and concentrated HCl. Test Saponification was carried out using HCl reagent 1 N. Meanwhile, the tannin test used the $FeCl_3$ reagent at 10%.

2.5. Deep-Vein thrombosis induction

This research used the Deep-Vein Thrombosis Mice Model using Kappa-Carrageenan (KCG). KCG was dissolved in normal saline at 1,000 µg/ml. For each experiment, we prepared the KCG solution using the powder immediately before use. KCG (80 µg/g body) was administered intravenously through the tails of mice. Thrombi were observed shortly after exposure to Kappa-carrageenan [25].

2.6. Animal preparation

Male mice weighing 20 – 30 g were treated with Good Laboratory Practice (GLP) principles. All protocols in this study were submitted for review and approval from the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universitas Airlangga. Experimental animals were placed in drums with a capacity of 3 per cage conditions, air exhaust 10%, humidity 60 ± 5%, air temperature 25 ± 30^{0}C and a 12:12 hour light-dark cycle. Experimental animals were given food ad libitum [26].

2.7. Treatment

All test preparations were administered as a suspension in agar 0.2% orally. Mice were induced to thrombosis using κ-carrageenan through the back of the tail for the groups. After 3 hours, we administered 8 group treatments. After 24 hours, we observed the thrombus length and took the blood samples to observe various test parameters with blood serum samples [25].

2.8. Platelet aggregation test

Antiplatelet activity tests were carried out by observing the inhibition of platelet aggregation that occurs when blood plasma is treated with Adenosine Diphosphate (ADP). Platelet aggregates formed were assessed by comparing plasma absorbance before and after being given ADP using a UV-Vis spectrophotometer. The more significant the decrease in plasma platelet uptake, the larger the aggregates formed. Platelet Rich Plasma (500 µl) was added with 125 µl of the test solution and then incubated at 37% in a water bath for 20 minutes. After incubation, the absorption of PRP was measured using a UV-VIS spectrophotometer with Platelet Poor Plasma (PPP) as a blank. PRP that has been calculated. ADP was then increased by as much as 10 µL, which was then incubated in a water bath at 37 °C for 20 minutes, and then the absorption was measured again [22]. The percent inhibition of platelet aggregation was calculated using the formula [27]

% Aggregation inhibition = (1- B)/A x 100%.....[27]
B: Absorbance after the addition of ADP
A: Absorbance before adding ADP

The following formula calculates the percentage (%) aggregation inhibition relative to the control negative:

% Relative aggregation inhibition = (A-B)/A x 100%..[27]
A: Percentage of aggregation inhibition of negative control
B: Percentage of treatment aggregation inhibition

2.9. Prothrombin time (PT) test

PT reagent incubated and ethanol extract of Papaya leaf at 37˚C for 10 minutes, pipette 25 µl of sample, incubate at 37˚C for 1-2 minutes, add 50 µl of PT reagent, which has been added with 25 µl of ethanol extract of Papaya leaf, and start the stopwatch, note the freezing time. The normal PT value is 11-18 seconds (Manual Lab of PT Assay).

2.10. Activated partial thromboplastin time (APTT) test

$CaCl_2$ incubated at 37˚C for 10 minutes, a pipette 25 µl of plasma sample incubated at 37˚C for 1-2 minutes, 25 µl of Papaya leaf ethanol extract and 25 µl of APTT reagent are mixed and incubated at 37˚C for 3 minutes in a water bath, 25 µl of $CaCl_2$ was added which had been incubated at 37˚C, then pipette the mixture into the sample, stopwatch and record the

Table 1. Qualitative phytochemical screening of *Carica papaya* L. ethanol extract

Group of Compounds	Ethanol Extract
Alkaloid	
Mayer	+
Dragendorff	+
Wagner	+
Flavonoid	+
Saponin	+
Tanin	+
Steroid	+

freezing time. The normal value of APTT is between 25 - 35 seconds (Manual Lab of PT Assay).

2.11. Thrombolytic test

The thrombolytic percentage was measured by comparing the difference in thrombus length before and after treatment in the mice's tails.

2.12. Enzyme-linked immunosorbent assay (ELISA)

D-Dimer detection was performed using the competitive ELISA method (Reed Biotech). Standard and sample (50 µl) is added by 50 µl biotinylated Ab working solution and incubated for 30 min at 370C, after that we aspirated and washed the plate 3 times. HRP conjugate working solution was added (100 µl) into the well and incubated the sample for 30 min at 370C, after that we aspirated and washed the plate 3 times again. Substrate reagent (100 µl) was added to the well and incubated for 15 min at 37^0C. Stop solution (50 µl) was added to the sample. D-Dimer level was calculated from the absorbance value from the ELISA reader at 450 nm.

2.13. Data analysis

The data obtained from the results of antiplatelet, anticoagulant, and thrombolytic tests were then analyzed statistically using one-way analysis of variance (ANOVA) followed by the Tukey test. For the correlation and influence between doses and parameters, we used Pearson Correlation and Linear Regression Test. The results of statistical significance analysis can be seen in the differences between groups at the significance level ($p<0.05$).

3. RESULTS

3.1. Phytochemical screening

Based on the results of extraction (re-maceration) using 96% ethanol, the yield was 12.2%. The yield value of the extraction results was declared good because it was not more than 10%. The water content of the papaya leaf extract obtained was 2.29%. Based on the results of measuring the water content of papaya leaf ethanol extract, this study showed that the extract was declared good. The results of organoleptic observations of the ethanol extract of papaya leaves were that it had a typical odor, was blackish brown, and had a thick form.

Based on the qualitative phytochemical screening, it was found that CPEE and CPCF contained secondary metabolites (see Table 1).

3.2. Antiplatelet activity

The percentage of aggregation inhibition is carried out to observe the inhibition of platelet aggregation that occurs when blood plasma is treated with induction with Adenosine Diphosphate (ADP). The platelet aggregates formed are assessed by comparing plasma absorption before and after being given ADP using a UV Vis spectrophotometer. The more significant the decrease in plasma platelet absorption, the greater the aggregate formed. Platelet Rich Plasma (PRP) as much as 0.1 mL was added with 25 µl of test solution and PZ solution, then incubated at 37% in a water bath for 20 minutes. After incubation, the PRP absorbance was measured using a UV-Vis spectrophotometer and Platelet-Poor Plasma (PPP) as a blank. PRP that has been measured for its absorbance is then added with 2 µL of ADP and then incubated in a water bath at 37 °C for 20 minutes, and then its absorbance is measured again [29]. The results of the antiplatelet activity test by observing the percentage value of platelet aggregation inhibition can be seen in Table 2 and Figure 1.

The results of statistical tests using Analysis of Variance (ANOVA) show that there was a significant difference between each treatment as indicated by the $F_{test} > F_{table}$ with a significance value of 0.000 ($p<0.05$). The highest percentage of platelet aggregation inhibition was shown by clopidogrel at 72%. Based on the results of Tukey's test, *Carica papaya* L. leaf ethanol extract (CPEE) 2 mg/kg body weight was not significantly different from the negative control. However, CPEE 20 and 200 mg/kg body weight significantly increased platelet aggregation inhibition successively at 13% and 58%. The correlation test show

Table 2. Result of platelet aggregation inhibition observation

Treatment Groups	Percent of Platelet Aggregation Inhibition (%) ± SD
Normal Control	$2^{ab} \pm 0$
Negative Control (Deep Vein Thrombolysis + Placebo)	$0^{a} \pm 0$
Positive Control (Deep Vein Thrombolysis + Clopidogrel 9.75 mg/kg body weight	$72^{e} \pm 2{,}52$
CPEE 2 mg/kg body weight	$4^{b} \pm 0{,}58$
CPEE 20 mg/kg body weight	$13^{c} \pm 1{,}15$
CPEE 200 mg/kg body weight	$58^{d} \pm 2$

showed a positive correlation in dose-dependent effect with a correlation coefficient $R^2 = 0.993$. The regression test showed that ethanol extract concentration (p=0.000, test> table) significantly affected platelet aggregation inhibition with the regression equation y=5.852 + 0.262x. This study showed that platelet aggregation inhibition has an antiplatelet effect on CPEE 20 and 200 mg/kg body weight.

3.3. Anticoagulant activity

Anticoagulant activity was calculated by the Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT). The PT and APTT test results can be seen in Table 3 and Figure 2.

The results of statistical tests using Analysis of Variance (ANOVA) show that there was a significant difference between each treatment as indicated by the $F_{test} > F_{table}$ with a significance value of 0.000 (p<0.05). Tukey's further tests showed that normal controls, positive controls with administration of warfarin, administration of 200 mg/kg body weight ethanol extract, and administration of alkaloid fractions had normal PT values between 10 – 15 seconds. Based on the results of Tukey's test, *Carica papaya* L. leaf ethanol extract (CPEE) 2 mg/kg body weight was not significantly different from the negative control, and CPEE 20 mg/kg body weight was not substantially different from CPEE 2 mg/kg body weight. The PT level of CPEE 200 mg/kg body weight was not significantly different from that of normal conditions. This result showed that CPEE 200 mg/kg body weight can return PT to normal after thrombosis. The correlation test showed a positive correlation in dose-dependent effect with a correlation coefficient $R^2 = 0.812$. The regression test showed a significant effect of ethanol extract concentration (p=0.001, test> table) at PT improvement with regression equation y=7.907 + 0.021x. This study showed that CPEE 200 mg/kg has an anticoagulant effect by prothrombin time (PT) or coagulation time improvement after thrombosis.

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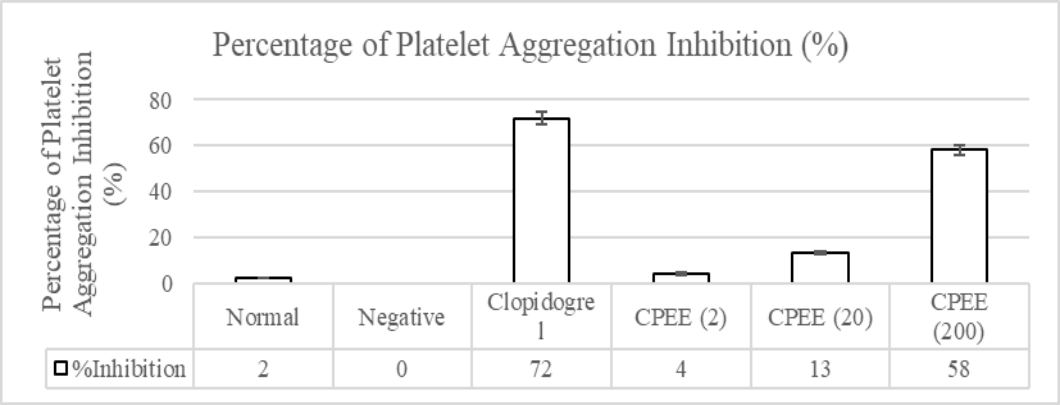

Figure 1. Platelet aggregation inhibition percentage graph

Table 3. Result of PT and APTT

Treatment Groups	PT (second) ± SD	APTT (second) ± SD
Normal Control	14^{c} ± 1.53	34^{d} ± 1.53
Negative Control (Deep Vein Thrombolysis + Placebo)	5^{a} ± 1.15	16^{a} ± 1
Positive Control (Deep Vein Thrombolysis + Warfarin 1.3 mg/kg body weight	14^{d} ± 0.58	32^{d} ± 2
CPEE 2 mg/kg body weight	7^{ab} ± 0.58	19^{a} ± 1.15
CPEE 20 mg/kg body weight	9^{b} ± 1	23^{b} ± 1.15
CPEE 200 mg/kg weight	12^{c} ± 1	28^{c} ± 1.53

improvement with regression equation y=20.87 + 0.035x. This study showed that CPEE 200 mg/kg has an anticoagulant effect by activating partial thromboplastin time (APTT) or improving coagulation time after thrombosis.

The results of statistical tests using Analysis of Variance (ANOVA) show that there was a significant difference between each treatment as indicated by the $F_{test} > F_{table}$ with a significance value of 0.000 ($p<0.05$). Tukey's further tests showed that normal controls, positive controls with administration of warfarin, administration of 200 mg/kg body weight ethanol extract, and administration of alkaloid fractions had normal PT values between 10 – 15 seconds. Based on the results of Tukey's test, *Carica papaya* L. leaf ethanol extract (CPEE) 2 mg/kg body weight was not significantly different from the negative control, and CPEE 20 mg/kg body weight was not substantially different from CPEE 2 mg/kg body weight. The PT level of CPEE 200 mg/kg body weight was not significantly different from that of normal conditions. This result showed that CPEE 200 mg/kg body weight can return PT to normal after thrombosis. The correlation test showed a positive correlation in dose-dependent effect with a correlation coefficient $R^2 = 0.812$. The regression test showed a significant effect of ethanol extract concentration (p=0.001, test> table) at PT improvement with regression equation y=7.907 + 0.021x. This study showed that CPEE 200 mg/kg has an anticoagulant effect by prothrombin time (PT) or coagulation time improvement after thrombosis.

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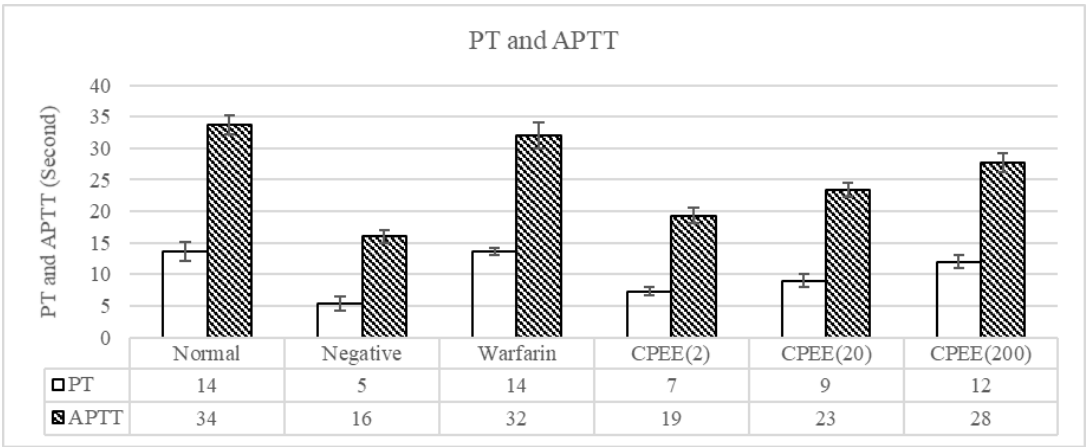

Figure 2. PT and APTT Graph

Table 4. Result of platelet thrombolysis presentation

Treatment Groups	Percent of Platelet Thrombolytic (%) ± SD
Normal Control	$100^{d} \pm 0$
Negative Control (Deep Vein Thrombolysis + Placebo)	$8^{a} \pm 1.92$
Positive Control (Deep Vein Thrombolysis + Natto-kinase 9.75 mg/kg body weight	$51^{c} \pm 2.99$
CPEE 2 mg/kg body weight	$14^{b} \pm 2.82$
CPEE 20 mg/kg body weight	$19^{b} \pm 1.05$
CPEE 200 mg/kg body weight	$50^{c} \pm 1.72$

3.4. Thrombolytic activity

Thrombolysis activity can be seen from the percentage of thrombolysis and the D-dimer level. The results of the thrombolysis activity test can be seen in Table 4 and Figure 3.

The results of statistical tests using Analysis of Variance (ANOVA) show that there was a significant difference between each treatment as indicated by the $F_{test} > F_{table}$ with a significance value of 0.000 ($p<0.05$). Based on the results of Tukey's test, *Carica papaya* L. leaf ethanol extract (CPEE) 200 mg/kg body weight is the best dose because there was no significant difference with the positive control (Natto-kinase 9.75 mg/kg body weight). The correlation test showed a positive correlation in dose-dependent effect with a correlation coefficient $R^2 = 0.987$. The regression test showed that ethanol extract concentration ($p=0.000$, Ttest> Ttable) significantly affected platelet aggregation inhibition with regression equation $y=14.87 + 0.174x$. This study showed that thrombolysis stimulation has a thrombolytic effect of 200 mg/kg.

The biomolecular effects of the ethanol extract of *Carica papaya* L leaves on the thrombolytic process can also be seen from the levels of D-dimer. The results of the study showed that the ethanol extract of *Carica papaya* L increased D-dimer levels. D-dimer level data is shown in the graph in Figure 4.

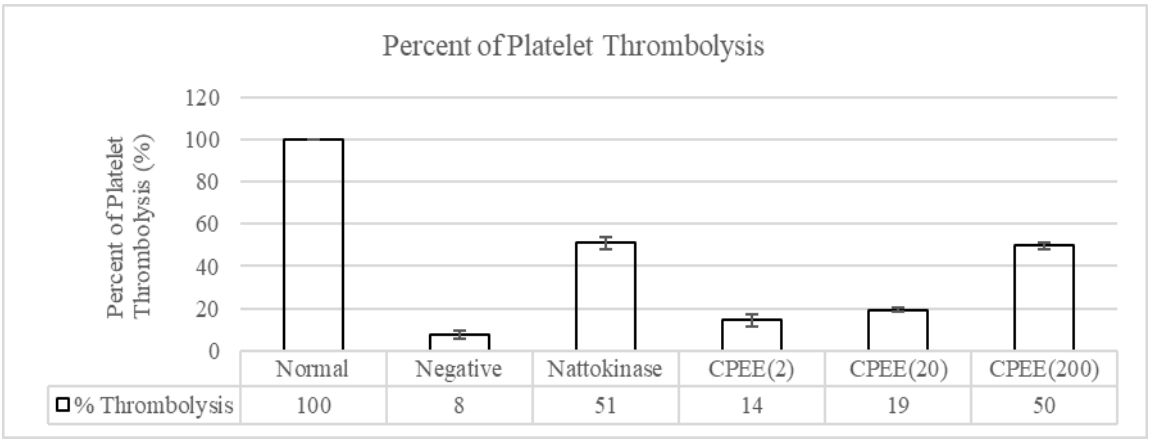

Figure 3. Platelet Thrombolysis Graph

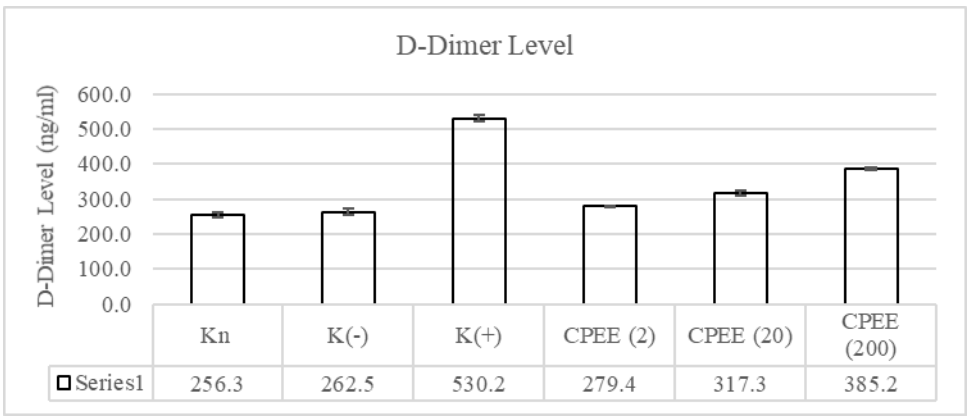

Figure 4. D-Dimer Level Graph

4. DISCUSSION

Based on the phytochemical analysis, it was known that the ethanol extract of papaya leaves had several secondary metabolite contents, including alkaloids, flavonoids, saponins, and tannins. Based on previous studies, it was known that the secondary metabolite content of papaya leaves consists of alkaloids (1.11 ± 0.02%), saponins (0.53 ± 0.04%), tannins (0.43 ± 0.4%), flavonoids (0.67 ± 0.01%), and phenols (0.91 ± 0.03%). The literature study shows that papaya leaves' alkaloid content is more dominant than other groups of compounds [28].

This research used deep vein thrombosis mice to determine papaya leaf extract's antiplatelet, anticoagulant, and thrombolytic activity in thrombotic or pathologic conditions. We used Kappa-carrageenan as an inducer to obtain a mouse model of deep-vein thrombosis in the tail. We used 1000 µg/mL Kappa-carrageenan concentration in normal saline to induce deep-vein thrombosis, administered intravenously through the tails of mice (80 µg/g body weight). Kappa-carrageenan induces platelet aggregation with α-granule secretion, activated integrin αIIbβ3, and phosphatidylserine exposure. KCG induces platelet activation via CLEC-2 [25]Carrageenan in carboxymethyl kappa-carrageenan also increased platelet adhesion and activation, percentage of coagulation in whole blood clotting, and fibrinogen adsorption [30].

This research showed that 200 mg/kg body weight ethanol extract of *Carica papaya* L. (CPEE) had antiplatelet, anticoagulant, and thrombolytic effects. Table 1 and Figure 2 show that CPEE 200 mg/kg body weight significantly increased the percentage of platelet aggregation inhibition in deep-vein thrombolysis mice. Ethanol extract (200 mg/ kg body weight) increased platelet aggregation inhibition to 53%, whereas the negative control had no platelet aggregation inhibition. Clopidogrel, as a positive control, inhibits platelet aggregation by 72%. Previous research showed (*in vitro* study) that 2 mg/ml chloroform fraction of *Carica papaya* L leaf had an antiplatelet effect by platelet aggregation inhibition until 2.74% [22]. In silico study also showed that some of the alkaloids contained in papaya leaf, such as carpaine, pseudocarpaine, dehydrocarpaine I, dehydrocarpaine II, and emetin, can bind to P2Y12 receptor [23]. The active P2Y12 receptor was central to platelet aggregation [31]. Emetin had the best docking score (-142.75 kcal/mol). It was better than the docking score of clopidogrel. Another study showed that *Carica papaya* leaf extract had platelet aggregation inhibition during dengue infection in an *in vitro* study [32]. This research shows that 200 mg/kg body weight ethanol extract of *Carica papaya* L. leaf had an antiplatelet effect by platelet aggregation inhibition in mice thrombosis conditions induced by kappa-carrageenan.

Carica papaya L. leaf was also a potential active anticoagulant. Data in Table 3 and Figure 2 showed that the ethanol extract of *Carica papaya* L leaf (200 mg/kg body weight) had an anticoagulant effect. The normal range of PT was 10-15 seconds, and APTT was 25 – 35 seconds. The PT of normal control was 14 seconds, and the APTT of normal control was 34 seconds. Kappa carrageenan shortened PT (5 seconds) and APTT (16 seconds) in the negative control group. It showed that Kappa carrageenan increases blood coagulation. The ethanol extract of *Carica papaya* L. leaf at 2 and 20 mg/kg body weight did not significantly normalize PT and APTT. The ethanol extract of *Carica papaya* L leaf (200 mg/kg body weight) had significantly normalized PT and APTT in the normal range. Warfarin as a positive control (1,3 mg/kg body weight) normalizes PT (14 seconds) and APTT (32 seconds) in the normal range. In vitro, the previous study showed that the chloroform fraction of *Carica papaya* L. normalizes PT and APTT in the normal range [22]. Another study showed that aqueous extract of *Carica papaya* leaves at concentrations of 400 and 800 mg/kg body weight induced thrombocytopenia, which can normalize the clotting time and increase the platelets in circulation.[33] This research shows that 200 mg/kg body weight ethanol extract of *Carica papaya* L. leaf had an anticoagulant effect by PT and APTT normalization in mice thrombosis conditions induced by kappa-carrageenan.

Carica papaya L. leaf was also a potential active thrombolytic. Based on the data in Table 4 and Figure 3, CPEE 200 mg/ kg body weight increased thrombolytic activity by 50%. The negative control only had thrombolysis activity of 8%. Natto-kinase, as a positive control, had a thrombolysis activity of 51%. Based on statistical analysis, CPEE 2, 20, and 200 mg/kg body weight significantly differed from the negative control, but CPEE 200 mg/kg was the best dose. Previous research showed (*in vitro* study) that 2 mg/ml chloroform fraction of *Carica papaya* L leaf had a thrombolysis effect of 65.4%, and natto-kinase had a thrombolysis effect of 66.3% [22]. Another study showed that the aqueous extract of *Carica papaya* leaf had a thrombolytic effect of 23.67% [34]. The ELISA test showed that the ethanol extract of *Carica papaya* L. increased the D-dimer level. During thrombosis after kappa-carrageenan induction, D-dimer levels were lower than normal. Nattokinase and ethanol extract of *Carica papaya* L can increase D-dimer levels. D-dimer is a blood clotting product from a breakdown process that can be measured via analysis of a blood sample. D-dimer is released when a blood clot begins to break down [35]. D-dimer level increases after thrombolytic therapy due to the breakdown of blood clots. D-dimer becomes a sign of successful clot dissolution and is used to monitor the effectiveness of thrombolysis [36].

5. CONCLUSIONS

The ethanol extract of *Carica papaya* leaf at 200 mg/kg body weight significantly increased platelet aggregation inhibition, normalized PT and APTT after thrombosis induction, increased the percentage of thrombolysis, and increased D-dimer level in the thrombosis mice model induced by kappa carrageenan. This study showed that the ethanol extract of *Carica papaya* leaf at 200 mg/kg body weight has antiplatelet, anticoagulant, and thrombolytic effects in mice with thrombosis conditions.

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Author contribution

MKR: Conceptualization, writing original draft, collecting and analyzing the samples. AH: Ensure the chemical method, AO: Ensure hemostatic analysis.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The research received an ethics certificate from the ACUC, Faculty of Veterinary Medicine, Universitas Airlangga (No: 3.KEH.102.07.2024).

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