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Original Article

Antithrombotic property of an aqueous extract from *Pseudocedrela kotschyi* and *Adenia cissampeloides*

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Abstract

Background and purpose: An aqueous extract from the root bark of *Pseudocedrela kotschyi* and aerial parts of *Adenia cissampeloides* has been proven in previous research to elicit significant anticoagulant property *in vitro*. This, therefore, indicates the potential usefulness of this extract in managing thromboembolic disease, a major global health risk. The aim of the present work was to establish the antithrombotic effect of a product made from extracts of the root bark of *P. kotschyi* and the aerial parts of *A. cissampeloides* (PAE).
Experimental approach: The effect of PAE at 500-2000 mg/kg in inhibiting tail infarction and inflammation, as well as its effect on the microthrombi count, hematological, and coagulation profiles in a carrageenan-induced thrombosis model in Sprague-Dawley rats, was studied.
Findings/Results: PAE significantly ($P \leq 0.01$-0.001) reduced length of tail infarction and inflammation (redness, swelling, pain, and temperature). Histopathological studies revealed a significant reduction ($P \leq 0.0001$) in microthrombi count in the liver and the lungs with PAE treatment. PAE treatment caused a marginal ($P \leq 0.01$) increase in prothrombin time but resulted in a significant ($P \leq 0.01$- 0.0001) dose-dependent increase in activated partial thromboplastin time, with the hematological profile being normal.
Conclusion and implications: PAE showed anticoagulant and antithrombotic effects *in vivo*, indicative of its potential benefit as a natural product, and cost-effective therapeutic option, and hence could be helpful in thromboembolic therapies.

Keywords: Activated partial thromboplastin time; Adenia cissampeloides; Antithrombotic; Prothrombin time; Pseudocedrela kotschyi; Thromboembolic disease.

INTRODUCTION

Ischemic heart disease, ischemic stroke, and venous thromboembolism, of which thrombosis is the common underlying pathology, continue to be a major cause of morbidity and mortality all over the world (1). The Global Burden of Diseases, Injuries, and Risk Factors report indicated that ischemic heart disease was the leading cause of death in 2015, with ischemic stroke accounting for 57% of all stroke deaths. The study also reported a steady rise in the numbers of reported cases and associated morbidity and mortality in many countries (2). Thromboembolic disease is often associated with a negative prognosis with the risk of death in patients experiencing pulmonary embolism 18-folds higher than in patients with deep vein thrombosis (DVT) alone (3). Pulmonary embolism-related mortality has also been estimated to be as high as 30% in studies taking into account autopsy reports (4).

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The impact of DVT and pulmonary embolism goes beyond the increased risk of mortality, for patients who survive these conditions. They sometimes will have to live with years and sometimes for the rest of their lives the dilapidating effect on their lives, such as loss or impairment of bodily function due to stroke, as seen in thromboembolic strokes. Also, survivors sometimes develop post-thrombotic syndromes such as ulcers due to months of being bedridden and impaired blood circulation resulting in gangrene (5).

Although there are several drug therapies in the management of thromboembolic disorders, considering the expensive nature and the side effects of existing therapeutic options, there is an increasing need for newer, more effective, and safer therapies. The plant kingdom continues to show huge promise in therapeutic regard, with herbal extracts being one of the most important, effective, and widely used pharmaceutical forms of medicinal herbs (6,7). Plants as sources of new anticoagulants and antithrombotic agents for the management of thromboembolic disorders have been researched over the years. The most widely used oral anticoagulant warfarin was originally discovered from plant sources i.e. sweet clove (8). In recent years, the anticoagulant effect of plants like *Allium sativum (Amaryllidaceae)* commonly known as garlic, and *Careya Arborea (Lecythidaceae)* also known as "Kumbhi" in Ayurveda just to mention a few have also been studied by numerous researchers. There is now compelling evidence that seems to suggest that, consumption of these plant materials as dietary anticoagulants may reduce or eliminate the risk of thromboembolic diseases. Such plants with anticoagulant properties have found many uses in the practice of traditional medicine, especially in the management of thromboembolic-related disorders such as numbness, varicose veins, DVT and stroke (6). Studies have confirmed an association between thrombosis and inflammation, with known anticoagulant and antithrombotic medications such as heparin and aspirin also found to possess significant anti-inflammatory properties (9,10). Likewise, plants that have been found to possess anti-inflammatory properties, in other research publications have also been reported to exhibit significant anticoagulant properties (11,12).

Pseudocedrela kotschyi (P kotschyi) and *Adenia cissampeloides (A. cissampeloides)* have been individually researched for their possible therapeutic properties, with guidance and inference from their folkloric use in traditional medicine. Previous studies by Nyansah *et al.* (13) indicated that a product made from these two plants has anticoagulant activity *in vitro* and hence could be helpful in thromboembolic therapies.

This study, therefore, sought to investigate the antithrombotic property of an aqueous extract made from the root bark of *P. kotschyi* and the aerial part of *A. cissampeloides*, utilizing the carrageenan-induced tail thrombosis model in Sprague-Dawley rats. This is the first study seeking to give scientific evidence backing the folkloric use of these two plants in the management of circulatory disorders using *in vivo* animal models.

MATERIALS AND METHODS

Plant collection and authentication

A. cissampeloides and *P. kotschyi* were collected from Tetrem near Offinso in the Afigya Kwabre District, and the Asante Mampong District in the Ashanti Region of Ghana, respectively. Plant materials were authenticated and herbarium specimen sent to the Department of Herbal Medicine, KNUST, for keeps (Specimen voucher numbers: KNUST/HM1/2015/S043 and KNUST/HM1/2015/S048, respectively).

Preparation of Pseudocedrela kotschyi and Adenia cissampeloides extract

One and a half kg of the root bark of *P. kotschyi* and 1.0 kg of the aerial part of *A. cissampeloides* were washed thoroughly with clean water and boiled in 10 L of distilled water for 45 min. The cooled preparation was then filtered using a sterile Whatman number 1 filter paper (Sigma Aldrich Co. Ltd. Irvine, UK). The filtrate obtained was then concentrated using a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) maintained at 40 °C and dried using a freeze

drier (YK-118 Vacuum Freeze Drier, True Ten Industrial Company, Taiwan). The solid mass obtained was weighed (percentage yield: 0.45%), labeled as *P. kotschyi* and *A. cissampeloides* extract (PAE) and stored in a frozen form at -17 °C from which appropriate concentrations were prepared for dosing as required to use in this study.

Drugs and chemicals

The drugs and chemicals used in this study included κ-carrageenan purchased from Sigma Aldrich Co. Ltd. Irvine, UK and heparin procured from Rotexmedica, Germany.

Animals

Adult Sprague-Dawley rats, weighing 225-300 g, were used in this study. The animals were housed in roomy cages under ambient light/dark cycle, relative humidity (60-70%), and room temperature (25 ± 3 °C). Animals were fed normal pellet chow (Agricare Ghana Ltd, Kwadaso, Ghana) and water *ad libitum*. They were allowed to acclimatize to the laboratory environment before use in experiments. All animals were kept according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee (Committee on Animal Research, Publication and Ethics (CARPE); Ethics No. FPPS/PCOL/0006/2013).

Effect of PAE on carrageenan-induced tail thrombosis

The antithrombotic activity of PAE was assessed using the Carrageenan-induced rat tail thrombosis model described by Ma *et al*. (14) with some modifications. Briefly, Sprague-Dawley rats with the tail length exceeding 13 cm, were selected and divided into six groups; A to F (n = 5). A dose of 2 mg/kg κ-carrageenan dissolved in saline was injected into the dorsal tail vein in all animals in groups B-F to induce inflammation and cause infarction in the tail. The tails were then tied with a nylon thread 13 cm from the tail tip for 10 min to induce tail thrombosis. Animals in group B (model group) were treated with normal saline (2 mL/kg) orally, and those in group C (positive control) were treated with 300 IU/kg heparin subcutaneously. Animals in groups D-F were administered PAE at oral doses of 500, 1000, and 2000 mg/kg, respectively. The frequency of infarction and the length of the infarcted region of the tail from the tip were recorded at 6, 12, 24, 48, and 72 h after carrageenan injection. Group A was made the normal control group, animals in this group were not injected with carrageenan but were administered normal saline 2 mL/kg orally.

Effect of PAE on inflammation

Cardinal signs of inflammation were assessed by the method described by Ma *et al*. (14) with slight modifications. Briefly, the rat's tail was observed for a time period of 6, 12, 24, 48, and 72 h for redness, swelling, heat, pain, and formation of thrombus. Tails were observed for redness by sight, swelling by water displacement, heat sensation by touch, and the pain was determined by means of a needle prick to the tail. The degree of inflammation was blindly scored semi-quantitatively, on a scale of 1-4 (4 being the highest), and mean scores in each group were recorded.

Coagulation analysis

After 72 h, animals from each group (A-F) previously described were sacrificed and whole blood was collected into sodium citrate vacutainer tubes (Surgfield Medicals, Meddlessex, England) for coagulation analysis.

Effect of PAE on prothrombin time in vivo

A 100 µL quantity of pre-warmed platelet-poor plasma (prepared by centrifuging test samples at 1500 g for 15 min at 37 °C) was added to an equal volume of pre-warmed thromboplastin (tissue factor)-calcium reagent (Labitec, GmbH) in a pre-warmed (37 °C) test cuvette. The time taken for clot formation was then determined automatically by a coagulation analyzer (CoaDATA 504, Labitec Germany).

Effect of PAE on activated partial thromboplastin time in vivo

A 100 µL quantity of platelet-poor plasma, (prepared by centrifuging test samples at

1500 *g* for 15 min) was added to an equal volume of partial thromboplastin reagent (phospholipid plus contact activator ellagic acid; Labitec, GmbH) in a test cuvette and warmed to 37 °C for an exact incubation time of 3 min. A 100 µL quantity of pre-warmed (37 °C) calcium chloride reagent (0.025 M) was added to this mixture to activate the coagulation cascade. Activated partial thromboplastin time (aPTT) was then determined automatically by a coagulation analyzer.

Histopathological studies

Liver, kidney, and lung tissue from each sacrificed animal were isolated, cleared of excess fat and fixed in 10% buffered formalin for histopathological analysis. Isolated organs were processed for paraffin sectioning by dehydration in varying concentrations of alcohol, cleared with xylene and embedded in paraffin blocks. Sections of about 5 µm in thickness were stained with haematoxylin and eosin (H&E) and tissues were analyzed for the micro-thrombi count.

Effect of PAE on micro-thrombi count

To assess the effect of PAE on the count of micro-thrombi in the kidney, lungs, and liver, the method described by Liu *et al*. (15) was utilized, with slight modifications. Briefly, the prepared H&E stained slides were viewed under a light microscope (Leica DM 750, Leica Microsystems AG, Switzerland). Six (16) fields were selected and the number of micro-thrombi under low magnification (x10) counted. Mean micro-thrombi count was then compared amongst groups.

Statistical analysis

Data were expressed as means ± standard error of mean (SEM). Statistical analyses were performed using both one- and two-way analysis of variance (ANOVA) followed by Sidak post-hoc test with confidence interval of 95%, with GraphPad Prism for Windows version 5.01 (GraphPad Software, San Diego, CA, USA).

RESULTS

Effect of PAE on carrageenan-induced tail thrombosis

Swelling of the tails was observed after 6 h of intravenous injection of carrageenan with tail turning auburn or dark red within 24 h, indicating the formation of a thrombus within blood vessels in the tail (Fig. 1). The degree of tail infarction was noticeably different amongst all the groups. Thrombosis was more pronounced in the normal saline-treated group (model group) compared to the heparin-treated (positive control group) and the PAE-treated groups; evidenced by a greater length of tail infarction. PAE treatment resulted in a significant ($P \leq 0.001$) dose-dependent reduction in tail infarction length compared to the model group. There was a steady reduction in tail infarction length in all groups after 48 h through to 72 h, albeit insignificant ($P > 0.05$). There were no visible signs of the formation of thrombosis in the normal control group (group A; Fig. 2).

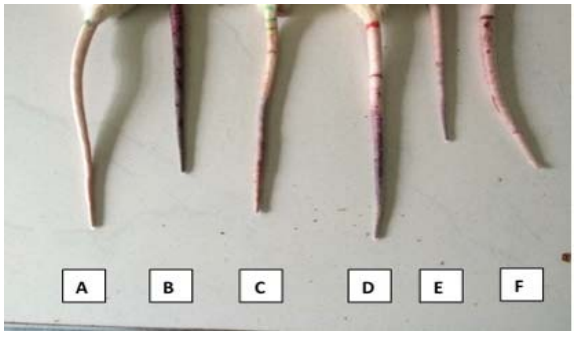

Fig. 1. Sample representation of the different lengths of infarction in the tail of Sprague-Dawley rats in the carrageenan-induced tail infarction thrombosis model. A, Normal control (no carrageenan injection); B, model group treated with 2 mL/kg normal saline after carrageenan injection; C, 300 IU/kg heparin-treated group after carrageenan injection; D-F, treated with 500, 1000, 2000 mg/kg of PAE, respectively after carrageenan injection.

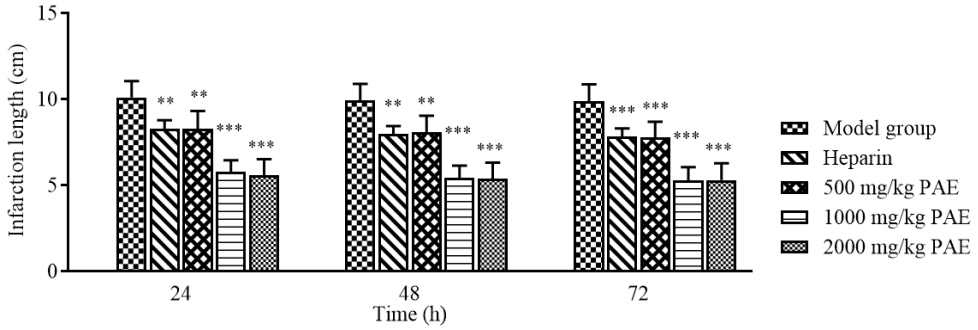

Fig. 2. Effect of PAE treatment on progression of rat tail thrombosis over time. Values plotted are means ± SEM; n = 5. ***$P \leq 0.001$ and **$P \leq 0.01$ indicate significant differences compared to the model group which was treated with normal saline and carrageenan.

Table 1. The effect of normal saline, heparin, and PAE treatment on cardinal signs of inflammation. Data are presented as means ± SEM (n = 5).

Groups	Rubor (redness)	Calor (heat)	Dolor (pain)	Tumor (swelling)
A (Normal control)	0.00 ± 0.00	3.73 ± 0.02	0.55 ± 0.34	0.00 ± 0.00
B (Model group)	3.52 ± 0.04***	1.38 ± 0.03***	2.80 ± 0.20***	2.80 ± 0.20***
C (Heparin)	1.99 ± 0.01$^{***; \dagger\dagger\dagger}$	3.48 ± 0.05 $^{ns; \dagger\dagger\dagger}$	1.82 ± 0.25$^{**; \dagger}$	1.80 ± 0.20$^{***; \dagger}$
D (PAE: 500 mg/kg)	3.28 ± 0.12$^{***; ns}$	2.40 ± 0.06$^{***; \dagger\dagger\dagger}$	2.61 ± 0.20$^{***; ns}$	2.60 ± 0.25$^{***; ns}$
E (PAE: 1000 mg/kg)	2.33 ± 0.09$^{***; \dagger\dagger\dagger}$	3.54 ± 0.05 $^{ns; \dagger\dagger\dagger}$	1.85 ± 0.18$^{**; \dagger\dagger}$	1.80 ± 0.20$^{***; \dagger}$
F (PAE: 2000 mg/ kg)	1.63 ± 0.16$^{***; \dagger\dagger\dagger}$	3.59 ± 0.07 $^{ns; \dagger\dagger\dagger}$	1.50 ± 0.22$^{*; \dagger\dagger}$	1.40 ± 0.25$^{**; \dagger\dagger}$

***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, $P > 0.05$ indicate significant differences compared with the normal control; $^{\dagger\dagger\dagger}P \leq 0.001$; $^{\dagger\dagger}P \leq 0.01$; $^{\dagger}P \leq 0.05$ versus the model control. PAE, *Pseudocedrela kotschyi* and *Adenia cissampeloides* extract.

Effect of PAE on inflammation

Blind scoring of the degree of redness, heat, pain, and swelling of the tail, revealed a relatively lower pain sensation and the absence of swelling in the normal control group. The tails were of normal coloration (i.e. no infarction) throughout the entire length of the tail even beyond the point of ligation. Tails in the normal control group were of a normal body temperature and warmth according to touch. Tails in the model group (treated with normal saline) were relatively colder to touch, of darker auburn coloration below the point of ligation and relatively more swollen compared to both the control and treatment groups. The needle prick pain sensation test revealed a heightened pain sensation in the model group compared to the other groups (control, heparin, and PAE treated groups). The heparin treated group had a relatively reduced pain sensation, decreased swelling and reduced darkening coloration of the tail below the point of ligation compared to the model group. Tails were also of relatively normal temperature and warmth according to touch compared to the model group. PAE treatment at 500 mg/kg resulted in a reduced pain sensation, decreased swelling, and reduced-auburn coloration of the tail compared to the normal control group. Tails were also relatively colder compared to the control group but less cold compared to the model group. PAE treatment at 1000 and 2000 mg/kg resulted in a similar reduction in pain sensation, swelling, and tail warmth compared to the model group. Tails of rats in the PAE at 2000 mg/kg treated group were, however less intense in auburn coloration compared to the PAE 1000 mg/kg treated group (Table 1).

Effect of PAE on prothrombin time

While the 1000 mg/kg PAE treatment resulted in a significant rise ($P \leq 0.01$) in ***prothrombin time (***PT), PAE at 500 and 2000 mg/kg and heparin treatment had no significant effect on PT in the control group. The normal saline treatment group showed a significant decrease in PT ($P \leq 0.05$) when compared to the control group (Fig. 3).

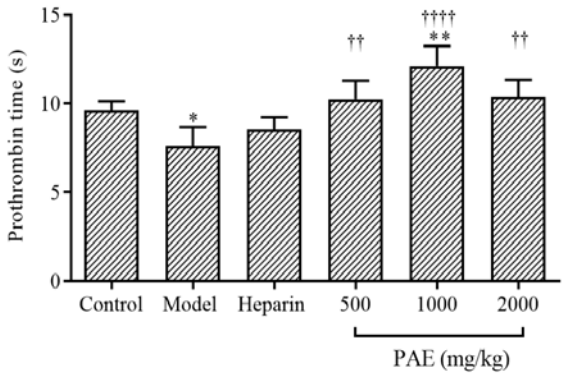

Fig. 3. Effect of normal saline, heparin, and PAE treatments on prothrombin time. Values present means ± SEM; n = 5. $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$ indicate significant differences compared with the normal control (Treated with normal saline); $^{\dagger\dagger\dagger}P \leq 0.001$ and $^{\dagger\dagger}P \leq 0.01$ against the model group (treated with normal saline and carrageenan). PAE, *Pseudocedrela kotschyi* and *Adenia cissampeloides* extract.

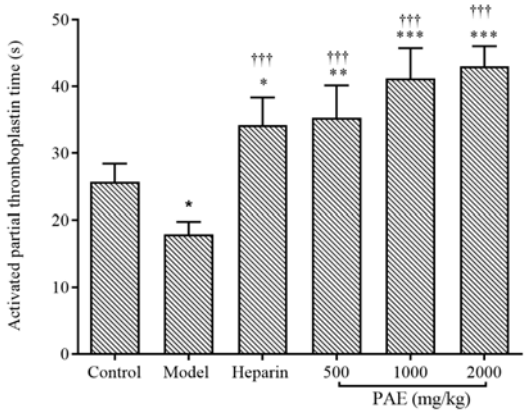

Fig. 4. Effect of normal saline, heparin, and PAE treatment on activated partial thromboplastin time. Values present means ± SEM; n = 5. $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, and $^{*}P \leq 0.05$ indicate significant differences compared with the normal control (Treated with normal saline); $^{\dagger\dagger\dagger}P \leq 0.001$ against the model group (treated with normal saline and carrageenan). PAE, *Pseudocedrela kotschyi* and *Adenia cissampeloides* extract.

The effect of PAE on aPTT

The heparin-treatment group had a significant increase ($P \leq 0.05$) in aPTT compared to the control. PAE treatment resulted in a graded increase in aPTT when compared with the control. Group B (model group) showed a significant decrease ($P \leq 0.05$) in aPTT compared with the normal control (Fig. 4).

Histopathological studies

Histopathological studies of selected tissues (kidney, lungs, and liver) revealed extensive micro-thrombi formation in the carrageenan-treated groups showing capillary hemorrhaging and neutrophil infiltration (Figs. 5-7). Histopathological analysis of tissue of the normal control group revealed significantly low ($P \leq 0.001$) counts of micro-thrombi in the lungs and liver compared to the model group. The model group had a significantly higher ($P \leq 0.001$) count of micro-thrombi especially in the lungs and liver compared to both control and all treatment groups i.e. heparin and PAE-treated groups. Histopathological studies of the kidney revealed no significant difference between the micro-thrombi count in the model group and the normal control group. Heparin-treated group (positive control) had a significant lower ($P \leq 0.001$) count of micro-thrombi in the lungs and liver compared to the model group. However, the difference in micro-thrombi count in the lungs and liver was not significant ($P > 0.05$). Similar to the model group, there was no significant difference in micro-thrombi count in the kidney of heparin-treated group. PAE treatment resulted in a significant decrease ($P \leq 0.001$) in micro-thrombi in the lungs and liver compared to the model group. However, there was no significant difference in micro-thrombi count in the kidneys of PAE-treated groups compared to both model and normal control groups (Fig. 8).

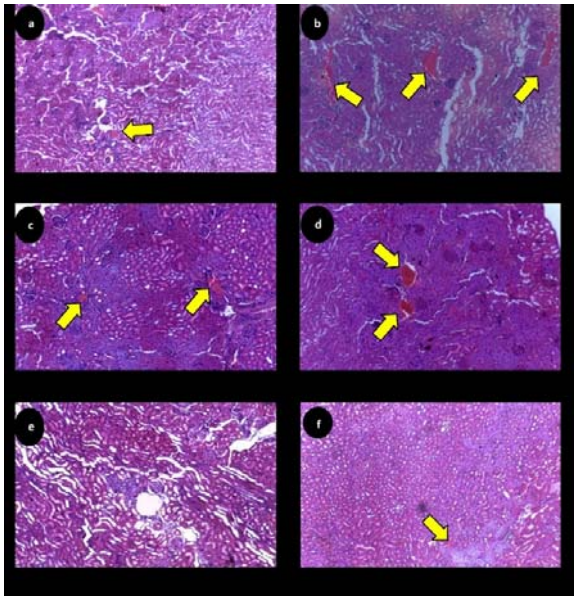

Fig. 5. Representative photomicrographs of the kidney of Sprague-Dawley rats, showing differing counts of micro-thrombi, which is indicated by the yellow arrow, for (a) normal control, as well as (b) carrageenan-treated rats treated with normal saline, (c) heparin, (d-f) 500, 1000, and 2000 mg/kg of PAE, respectively. Haematoxylin and eosin staining, objective magnification: ×10.

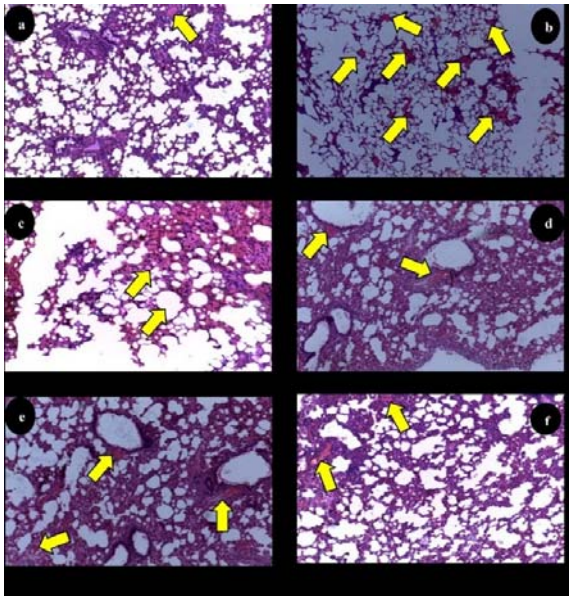

Fig. 6. Representative photomicrographs of the lung of Sprague-Dawley rats, showing differing counts of micro-thrombi, which is indicated by the yellow arrow, for (a) normal control, as well as (b) carrageenan-treated rats treated with normal saline, (c) heparin, (d-f) 500, 1000, and 2000 mg/kg of PAE, respectively. Haematoxylin and eosin staining, objective magnification: ×10.

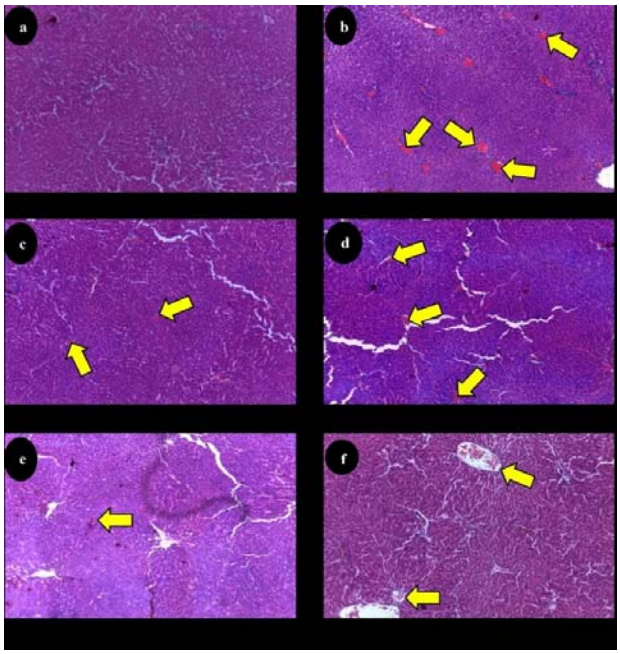

Fig. 7. Representative photomicrographs of the liver of Sprague-Dawley rats, showing differing counts of micro-thrombi, which is indicated by the yellow arrow, for (a) normal control, as well as (b) carrageenan-treated rats treated with normal saline, (c) heparin, (d-f) 500, 1000, and 2000 mg/kg of PAE, respectively. Haematoxylin and eosin staining, objective magnification: ×10.

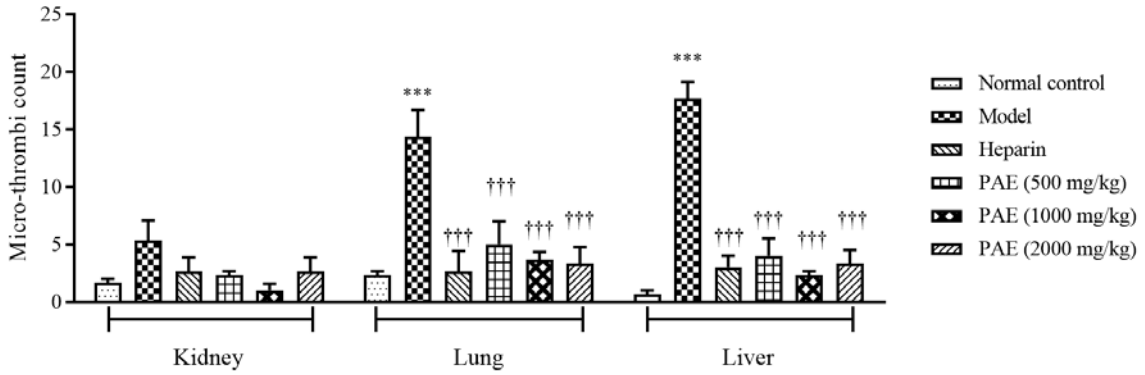

Fig. 8. Effect of Normal saline, heparin, and PAE treatment on the micro-thrombi count in highly perfused organs. Values present means ± SEM; n = 5. *** $P \leq 0.001$ indicates significant differences compared with the normal control (Treated with normal saline); ††† $P \leq 0.001$ against the model group (treated with normal saline and carrageenan). PAE, *Pseudocedrela kotschyi* and *Adenia cissampeloides* extract.

DISCUSSION

Evaluation of the *in vivo*-effects of potential anticoagulant and antithrombotic agents by means of animal models is essential in confirming the activity and efficacy of agents, the establishment of dose-activity relationship and possible translation to use in human subjects (16). The carrageenan-induced-tail thrombosis model was chosen amongst the many models for investigating the *in vivo* antithrombotic properties of drugs because it enables the observation and measurement of the progression of thrombosis visually, continuously, and accurately. Most importantly, it is non-invasive and easily performed in small laboratory animals with a highly reduced risk of mortality (17).

From the model, PAE was found to prevent thrombosis as evidenced by the significant reduction in tail infarction length and micro-thrombi counts in the liver and lungs. PAE was also found to significantly reduce the extent of inflammation associated with thrombosis. Amongst the many physiological effects of carrageenan are its inflammation and thrombosis-inducing properties. Carrageenan is thought to induce thrombosis by causing inflammation of local blood vessels and injury to endothelial cells through the release of pro-inflammatory mediators (14). In this model of thrombosis, the thrombogenic potential of carrageenan was increased as a result of the tail ligation. This reduced the clearance of the carrageenan from the tail into systemic circulation by slowing blood circulation in the tail (stasis), allowing the increased effect of the drug in the tail thereby increasing the frequencies of thrombosis recorded in the various groups (18).

The relatively shorter length of tail infarction in the PAE-treated groups (similar to heparin effect) could possibly be a result of PAE reducing the vascular damaging effect of carrageenan which would have increased platelet adhesion and activated the coagulation cascade. The lack of any significant difference in the gradual decline of the length of tail infarction in the treatment group compared to the model group could also mean PAE has no fibrinolytic properties and as such could not dissolve the already formed clot. This gives an insight also into the possible mechanism of action of PAE, which could be the prevention of thrombosis and growth of an already-formed clot.

Also, the pro-inflammatory effects of carrageenan could explain the pronounced cardinal signs of inflammation (pain, swelling, warmth, and redness) in the model group, which was relatively reduced in the heparin and PAE-treated groups. Apart from the anticoagulant and antithrombotic effect of heparin and its derivatives, the anti-inflammatory effect of heparin is also well-known. Amongst the many proposed mechanisms of anti-inflammatory action of heparin is the inhibition of adhesion of leukocytes and neutrophils to endothelial cells by binding to p-selectin and induction of

apoptosis by modulation of the activity of tumor necrosis factor alpha and nuclear factor kappa B (19). This could explain the reduced inflammation observed in the heparin-treated group. PAE could be acting in a similar way to reduce the inflammation caused by carrageenan injection.

There has been research into the anti-inflammatory and antinociceptive properties of both *P. kotschyi* and *A. cissampeloides*. Musa *et al*. (20) reported the ability of an ethyl acetate extract of the leaves of *P. kotschyi* to reduce inflammation and pain in rats and mice, in a raw egg albumin-induced inflammation model and acetic acid-induced writhing model, respectively. An aqueous extract of *A. cissampeloides* has also been reported to modulate pain in mice. This antinociceptive action of *A. cissampeloides* is thought to be both centrally and peripherally mediated (21**).**

The increased liver and lung micro-thrombi count in the model group compared to the PAE and heparin-treated groups could be due to an increased-hypercoagulable state in the model group. Amongst the many systemic effects of carrageenan is disseminated intravascular coagulation (DIC). Hypercoagulable states such as DIC which could be due to sepsis, snake envenomation, etc. result in extensive activation of the coagulation system and have been associated with an increased count of micro-thrombi in blood vessels and well-vascularized tissues like the lungs, kidney, and liver. Anticoagulants such as heparin and its derivatives have been a standard treatment in the management of DIC over the years (22). The significantly reduced micro-thrombi count as a result of PAE treatment could therefore be due to its profound *in vivo* anticoagulant properties similar to what is seen with heparin treatment. This could give an indication of the potential of PAE in the management of DIC.

PAE had a similar effect to heparin on coagulation profile, resulting in no significant effect on PT but prolonging aPTT. The *in vivo*-effect of PAE was similar to what has been observed *in vitro* studies (13). Heparin by its anticoagulant mechanism of action has a limited effect on the extrinsic pathway of the coagulation cascade hence less likely to affect PT unless at very high doses. The lack of any significant effect of PAE on PT could be due to PAE also not acting *via* the extrinsic pathway. However, the slight elevation of PT at high doses of PAE could be similar to that observed for high doses of heparin. PAE just like heparin, could thus be binding to antithrombin III and resulting in antithrombin III activation thereby inactivating thrombin and factor Xa. This could give an indication of the sight of action of PAE on the coagulation cascade, which is the intrinsic or common pathway.

The significantly decreased PT in the model group could be due to a hypercoagulable state in the model group. A shortened aPTT is often associated with an increased risk of thromboembolic disease. As such treatments that are able to prolong aPTT are considered integral in the management of thromboembolic disease (23). Hematological analysis after 72 h also revealed no significant difference in hematological parameters in the model in heparin and PAE-treated groups compared to the control group, although platelet count was comparatively lower in the model group compared with the treatment groups. Several researchers have identified low platelet counts in patients with established cases of DVT and DIC. In DIC overstimulated coagulation cascade, results in a mop-up and exhaustion of free circulating platelets, hence the low platelet count observed. However, thrombotic risk has been found not to be a function of platelet count and thrombocytopenia itself has not been associated with a decreased risk of thromboembolic disease (24). The antithrombotic effect of PAE could therefore have reduced the extent of thrombosis in the PAE-treated group and hence the comparatively normal platelet count in the PAE-treated group compared to the model group. This effect was similar to that observed for heparin.

In the previous study, the aqueous extract of the root bark of *P. kotschyi* and the aerial parts of *A. cissampeloides* has been found to contain the secondary metabolites flavonoids, tannins, and coumarins (13). The confirmed presence of flavonoids and coumarins in PAE could thus explain the antithrombotic effect of PAE with flavonoids thought to exert an anticoagulant and subsequent antithrombotic effect by

interfering with primary hemostasis (platelet aggregation). The proposed mechanisms of antiplatelet action of flavonoids are inhibitory effects on enzymes involved in cellular signaling pathways such as phosphodiesterases, cyclooxygenase, and lypooxygenases (25). On the other hand, coumarins are vitamin K antagonists and are thought to exhibit their anticoagulant action through the inhibition of γ-carboxylation of glutamate residues in factors VII, IX, X, prothrombin, and endogenous protein C. Coumarins are also thought to inhibit vitamin K conversion cycle, resulting in hepatic production of partially carboxylated and decarboxylated proteins with reduced procoagulant properties (26).

The anti-inflammatory and antinociceptive properties of flavonoids and tannins are well-documented and could explain the effects demonstrated by PAE (27). Phytochemicals present in PAE would offer antioxidant property and this could have the ability to cause fibrinolysis; another mechanism of antithrombotic effect (28).

CONCLUSION

The aqueous extract made from the root bark of *P. kotschyi* and the aerial parts of *A. cissampeloides* prolongs aPTT, with minimal effect on PT reduced tail infarction length, cardinal signs of inflammation and micro-thrombi counts in highly perfused tissues indicating anticoagulant and antithrombotic properties *in vivo*. It thus justifies the folkloric use of the extract in the management of symptoms of circulatory disorders such as numbness and subsequently could be useful in the management of deep vein thrombosis and disseminated intravascular coagulation. Considering the relatively high cost of conventional therapies used in the management of thromboembolic conditions, herbal remedies such as PAE could prove a viable, effective, and cost-effective option.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

W.B. Nyansah conducted all the research work. G.A. Kuffuor as the supervisor assisted in the scoping of the research, ideation process, research design, and manuscript writing. L. Gyanfosu, I.O. Ben, and B.E. Ehigiator contributed to the preparation of the plant extract and experimental procedures. All authors approved the manuscript.

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