EVALUATION OF THE ANTICOAGULANT EFFECT OF SAPHIA ALKALI K90 IN AN EXPERIMENTAL THROMBOSIS MODEL

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The study aimed to investigate the anticoagulant effects of Saphia Alkali K90 in a rat model of lipopolysaccharide-induced thrombosis. Saphia Alkali K90 and rivaroxaban were administered orally for seven consecutive days. Two hours after the final administration, LPS (3 mg/kg) was injected via the tail vein to induce thrombotic changes. Blood samples were collected four hours later to assess coagulation. The results showed that Saphia Alkali K90 at 54 ml/kg/day produced a significant anticoagulant effect, evidenced by increased platelet count and fibrinogen concentration, as well as a significant prolongation of the activated partial thromboplastin time compared to the vehicle-treated group ($p < 0.05$). In contrast, Saphia Alkali K90 at 27 ml/kg/day showed a non-significant tendency toward similar changes ($p > 0.05$). In conclusion, SAK90 at 54 ml/kg/day exerted an anticoagulant effect in rats with LPS-induced thrombosis.

Keywords: Saphia Alkali K90, anticoagulant effect, lipopolysaccharide, *Wistar* rats.

I. INTRODUCTION

Thrombosis remains a major contributor to global morbidity and mortality, underlying a wide range of cardiovascular and cerebrovascular disorders such as myocardial infarction, ischemic stroke, and pulmonary embolism.[1] The incidence of thromboembolic diseases continues to rise worldwide, imposing a substantial burden on healthcare systems.[2] Despite considerable advances in antithrombotic therapy, the management of thromboembolic diseases remains a significant challenge due to the delicate balance between preventing thrombosis and avoiding excessive bleeding. Conventional anticoagulants, including heparins, vitamin K antagonists, and direct oral anticoagulants, are effective in clinical practice but are limited by adverse bleeding risks, food or drug interactions, high treatment costs, and the need for continuous +monitoring.[3,4] These limitations highlight an ongoing need for safer, more affordable, and accessible therapeutic alternatives.

In recent years, extensive research has focused on identifying novel antithrombotic agents with improved efficacy and safety profiles. Natural products and herbal formulations have attracted increasing attention as promising sources of such compounds.[2] Traditional medicines used to promote blood circulation and prevent blood stasis often contain bioactive compounds that modulate platelet function, thrombin activity, and fibrinolysis. Many of these natural preparations demonstrate multi-target effects and favourable safety profiles compared with synthetic drugs.[2,5] Therefore, identifying and characterising such agents may contribute to the development of

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safe, integrative strategies for the prevention and management of thrombosis.

Functional food Saphia Alkali K90, a multi-herbal formulation developed by Kiem Saphia Joint Stock Company, is a natural preparation composed of selected medicinal plants and mineral elements traditionally used to promote blood circulation, reduce blood stasis, and improve vascular health. The formulation integrates several herbs, including *Pseuderanthemum palatiferum*, *Phyllanthus urinaria*, Psidium guajava, *Achyranthes aspera*, and *Crinum latifolium*, many of which have been reported to possess anticoagulant or fibrinolytic activities. These herbal ingredients also contain bioactive compounds that may modulate platelet function, inhibit thrombin generation, and enhance fibrinolysis through multiple mechanisms.[6-10] Despite its traditional use for improving circulation and preventing blood stasis, scientific evidence supporting its anticoagulant efficacy and mechanism of action remains limited. To address this knowledge gap, the present study investigated the anticoagulant effects of Saphia Alkali K90 in a lipopolysaccharide-induced thrombosis rat model.

II. MATERIALS AND METHODS

1. Materials

Saphia Alkali K90 product

Kiem Saphia Joint Stock Company Saphia Alkali K90 product (abbreviated as SAK90). Each 100 mL of the product contains *Celastrus hindsii* 3.0 g; *Pseuderanthemum palatiferum* 2.5 g; *Hedyotis diffusa* 2.0 g; *Scutellaria barbata* 2.0 g; *Phyllanthus urinaria* 2.0 g; *Eclipta prostrata* 2.0 g; Guava (*Psidium guajava* L.) Leaves 2.0 g; *Perilla frutescens* 2.0 g; *Solanum procumbens* 2 g; *Mentha arvensis* 1.5 g; *Wedelia chinensis* 1.5 g; *Lactuca indica* 1.5 g; *Ilex kaushue* 1.5 g; *Amaranthus spinosus* 1.5 g; *Achyranthes aspera* 1.5 g; *Adenosma caeruleum* 1.5 g; *Crinum latifolium* 1.5 g; Microelements are activated from rare earths. Additional ingredients: Pure water enough for 100 mL.

The estimated human dose of SAK90 was 60-75 mL/day divided into three doses. SAK90 was diluted in distilled water and administered to the mice by oral gavage at 10 mL per kg b.w./day, based on the conversion (dose extrapolation factor of 6) from an equivalent dose of 75 mL/day for patients weighing approximately 50 kg in the clinic. The doses were prepared fresh daily and administered one hour before meals to optimize absorption.

Instruments and Chemicals

Lipopolysaccharides from Escherichia coli O55:B5 (L2880-25MG) were obtained from Sigma-Aldrich (USA).

Thromborel® S (containing thromboplastin and calcium) and Dade® Actin® FSL Activated PTT Reagent (containing phospholipids) were purchased from Siemens Healthineers, Germany, and imported by Sysmex Vietnam Co., Ltd. A 0.025 mol/L calcium chloride solution (Siemens, Germany) was also supplied by Sysmex Vietnam Co., Ltd.

Coagulation parameters were measured using a Sysmex CA-50 semi-automatic coagulation analyzer (Sysmex Corporation, Japan).

Haematological parameters were analyzed using Horiba Medical reagents and the ABX Micros 60 ES haematology analyzer (Horiba Medical, France).

Experimental animals

A total of 50 adult *Wistar* rats (weighing 180 ± 20 g) of both genders were used for the experiment. The animals were acclimated

for seven days in the animal facility under controlled environmental conditions, including a temperature of 25 ± 1°C, appropriate humidity, and a 12-hour light/dark cycle. Rats had free access to water and were housed under standard laboratory conditions at the Department of Pharmacology, Hanoi Medical University.

2. Methods

In the lipopolysaccharide (LPS)-induced thrombosis model, coagulation was experimentally triggered in *Wistar* rats by intravenously injecting LPS at a dose of 3 mg/kg, administered slowly over 3 minutes via the tail vein.[11,12]

The rats were randomly divided into five groups (n = 10 per group) as follows:

- Group 1 (Sham): received distilled water orally and physiological saline intravenously;

- Group 2 (Vehicle-treated LPS-induced thrombosis): received distilled water orally and LPS 3 mg/kg intravenously;

- Group 3 (Rivaroxaban-treated LPS-induced thrombosis): received rivaroxaban 3 mg/kg orally for seven consecutive days, followed by an intravenous injection of LPS 3 mg/kg;

- Group 4 (SAK90-treated LPS-induced thrombosis): received SAK90 at 27 ml/kg/day (*three times the equivalent human dose, calculated using an interspecies conversion factor of 6*) orally for seven consecutive days, followed by an injection of LPS at 3 mg/kg;

- Group 5 (SAK90-treated LPS-induced thrombosis): received SAK90 at 54 ml/kg/day (*six times the equivalent human dose, calculated using an interspecies conversion factor of 6*) orally for seven consecutive days, followed by an injection of LPS at 3 mg/kg.

All rats received the test product or distilled water daily for seven days before LPS administration to induce thrombosis. On the seventh day, two hours after the final oral dose, rats in Group 1 were injected intravenously with physiological saline, while rats in Groups 2–5 received LPS (3 mg/kg) via slow tail vein injection over 3 minutes.

Blood samples were collected four hours after LPS injection to assess coagulation, hematological, and biochemical parameters, including platelet count; prothrombin time (PT), prothrombin activity (PT%), and PT-INR; activated partial thromboplastin time (aPTT) and aPTT ratio (sample/control); fibrinogen concentration. All parameters were compared across the experimental groups.

3. Data analysis

Data were analyzed using SigmaPlot 12.0 (SYSTA Software Inc., Richmond, CA, USA). All data are expressed as the mean ± S.D. and analyzed by Student's t-test or one-way ANOVA followed by the post hoc Student-Newman-Keuls test. $p < 0.05$ was considered statistically significant.

III. RESULTS

The anticoagulant effect of SAK90 in rats with LPS-induced thrombosis was assessed using coagulation parameters, including platelet count, fibrinogen concentration, PT, and aPTT.

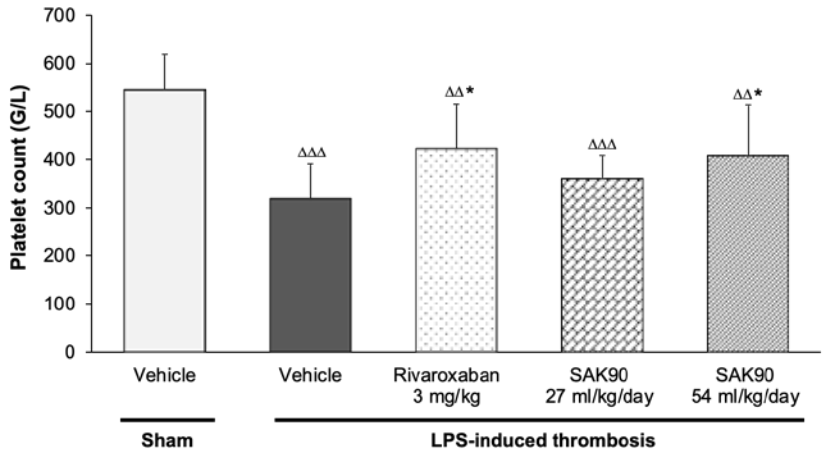

Figure 1. Effect of SAK90 on platelet count in the LPS-induced thrombosis model

*Data are represented as mean ± S.D. (n = 10). $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.001$ vs. sham rats; *p < 0.05 vs. vehicle-treated thrombosis in rats.*

In the LPS-induced thrombosis model, platelet count in the vehicle-treated group was significantly lower than in the sham group ($p < 0.001$). Rats treated with rivaroxaban (3 mg/kg/day) showed a significant increase in platelet count compared with the vehicle-treated thrombosis group ($p < 0.05$). Rats receiving SAK90 at 27 mL/kg/day showed a tendency toward increased platelet count compared with the model group, although the difference was not statistically significant ($p > 0.05$). However, SAK90 at 54 mL/kg/day significantly increased platelet count compared with the vehicle-treated thrombosis group ($p < 0.05$) (Fig. 1).

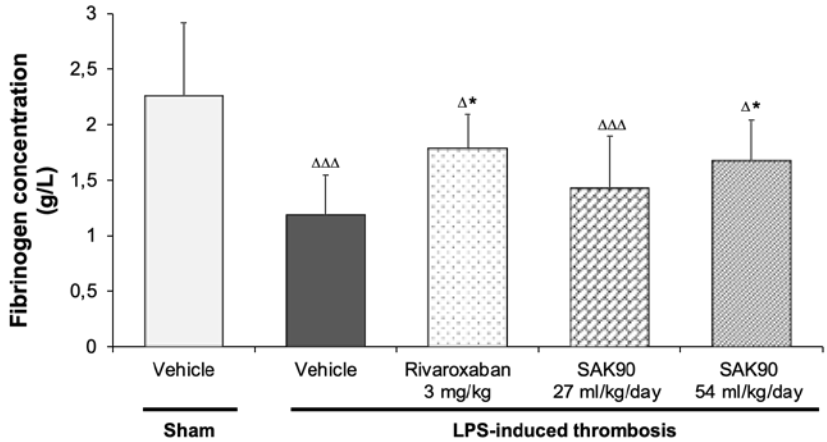

Figure 2. Effect of SAK90 on fibrinogen concentration

*Data are represented as mean ± S.D. (n = 10). $^{\Delta}p < 0.05$, $^{\Delta\Delta\Delta}p < 0.001$ vs. sham rats; *p < 0.05 vs. vehicle-treated thrombosis in rats.*

As shown in Figure 2, fibrinogen concentration in the vehicle-treated LPS group was significantly decreased compared with the sham group ($p < 0.001$). Rats treated with rivaroxaban (3 mg/kg/day) exhibited a significant increase in fibrinogen concentration compared with the vehicle-treated thrombosis group ($p < 0.05$). Rats receiving SAK90 at 27 mL/kg/day showed a tendency toward increased fibrinogen concentration compared with the model group, although the difference was not statistically significant ($p > 0.05$). However, SAK90 at 54 mL/kg/day significantly increased fibrinogen concentration compared with the vehicle-treated thrombosis group ($p < 0.05$).

Table 1. Effect of SAK90 on PT, PT%, and PT-INR

Operation	Treatment	PT (seconds)	PT%	PT-INR
Sham	Vehicle	15.42 ± 3.79	69.01 ± 29.86	1.25 ± 0.39
LPS-induced thrombosis	Vehicle	19.86 ± 1.93$^{\Delta\Delta}$	42.57 ± 5.68$^{\Delta\Delta}$	1.71 ± 0.17$^{\Delta\Delta}$
	Rivaroxaban	23.43 ± 3.20 $^{\Delta\Delta\Delta}$*	34.83 ± 5.06 $^{\Delta\Delta\Delta}$*	2.02 ± 0.28 $^{\Delta\Delta\Delta}$*
	SAK90 27 mL/kg/day	19.24 ± 2.56$^{\Delta\Delta}$	45.08 ± 8.33$^{\Delta\Delta}$	1.66 ± 0.22$^{\Delta\Delta}$
	SAK90 54 mL/kg/day	20.72 ± 3.73$^{\Delta\Delta}$	42.26 ± 11.99$^{\Delta\Delta}$	1.79 ± 0.32$^{\Delta\Delta}$

*Data were represented as mean ± S.D. (n = 10). $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.001$ vs. sham rats; *$p < 0.05$ vs. vehicle-treated thrombosis in rats.*

As shown in Table 1, rats in the vehicle-treated LPS group exhibited a significant prolongation of PT, an increase in PT-INR, and a decrease in PT% compared with the sham group ($p < 0.01$). The rivaroxaban-treated group (3 mg/kg/day) showed prolonged PT, increased PT-INR, and reduced PT% compared with the vehicle-treated thrombosis group ($p < 0.05$). SAK90 at 27 and 54 mL/kg/day showed no significant difference in PT, PT-INR, or PT% compared with the vehicle-treated thrombosis group ($p > 0.05$).

Table 2. Effect of SAK90 on aPTT and aPTT ratio

Operation	Treatment	aPTT (seconds)	aPTT ratio (sample/control)
Sham	Vehicle	19.81 ± 2.82	0.73 ± 0.10
LPS-induced thrombosis	Vehicle	24.40 ± 4.49$^{\Delta}$	090 ± 0.17$^{\Delta}$
	Rivaroxaban	29.47 ± 4.35$^{\Delta\Delta\Delta}$*	109 ± 0.16$^{\Delta\Delta\Delta}$*
	SAK90 27 mL/kg/day	26.17 ± 2.79$^{\Delta\Delta}$	097 ± 0.10$^{\Delta\Delta}$
	SAK90 54 mL/kg/day	28.91 ± 5.28$^{\Delta\Delta\Delta}$*	1.07 ± 0.20$^{\Delta\Delta\Delta}$*

*Data were represented as mean ± S.D. (n = 10). $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.001$ vs. sham rats; *p < 0.05 vs. vehicle-treated thrombosis in rats.*

According to the data presented in Table 2, rats in the vehicle-treated LPS group exhibited a significant prolongation of aPTT and an increase in the aPTT ratio (sample/control) compared with the sham group ($p < 0.05$). Treatment with rivaroxaban (3 mg/kg/day) prolonged aPTT and increased the aPTT ratio compared with the vehicle-treated thrombosis group ($p < 0.05$). SAK90 at 27 mL/kg/day showed a tendency toward prolonged aPTT and increased aPTT ratio compared with the model group, although the differences were not statistically significant ($p > 0.05$). In contrast, SAK90 at 54 mL/kg/day significantly prolonged aPTT and increased the aPTT ratio relative to the vehicle-treated thrombosis group ($p < 0.05$).

IV. DISCUSSION

Thrombosis is a complex pathological process involving the activation of the coagulation cascade, platelet aggregation, and subsequent fibrinolytic responses.[1] Lipopolysaccharides (LPS), a key component of the outer membrane of Gram-negative bacteria, are widely used to induce systemic inflammation and hypercoagulability in experimental models. LPS stimulates the release of pro-inflammatory cytokines such as TNF-α and IL-6, which activate both the extrinsic and intrinsic coagulation pathways, leading to excessive thrombin generation and platelet and coagulation factor consumption. Consequently, PT and aPTT are typically prolonged, while platelet count and fibrinogen concentration decrease due to their consumption during clot formation. Additionally, secondary activation of fibrinolysis further promotes the degradation of fibrin and fibrinogen, resulting in a state of consumptive coagulopathy.[11,13] In the present study, LPS demonstrated significantly prolonged PT and aPTT compared with the sham rats, confirming that the LPS-induced thrombosis model was successfully established.

Rivaroxaban, a selective direct factor Xa inhibitor, has been extensively employed for the prevention and treatment of venous thromboembolism and stroke associated with atrial fibrillation.[3] In this study, administration of rivaroxaban effectively attenuated the LPS-induced thrombosis, as evidenced by increased platelet count and fibrinogen concentration, and significant prolongation of PT and aPTT compared with the model group. These results demonstrate the antithrombotic effects of rivaroxaban in this experimental setting, consistent with the findings of Perzborn et al., who characterized its mechanism and efficacy as an oral, direct factor Xa inhibitor.[14]

The present study aimed to evaluate the anticoagulant effects of SAK90 in an LPS-induced thrombosis model. Administration of SAK90 at a dose of 54 mL/kg/day produced a significant anticoagulant effect, reflected by increased platelet count and fibrinogen concentration, together with a significant prolongation of aPTT. In contrast, the 27 mL/kg/day dose showed only a non-significant trend toward similar changes. These findings suggest that SAK90 exhibits a dose-dependent anticoagulant effect in this experimental thrombosis model, likely resulting from the synergistic actions of its active herbal and biological components.

Among the herbal constituents of SAK90, *P. palatiferum* appears to play a notable role in its anticoagulant profile. Polyphenolic–polysaccharide conjugates extracted from *P. palatiferum* leaves have been shown to exert marked anticoagulant activity. Experimental results demonstrated that these conjugates could prolong plasma clotting time, indicating

an inhibitory effect on the intrinsic coagulation pathway. The anticoagulant effect was attributed to their ability to interfere with fibrin formation and modulate coagulation factors, thereby delaying clot generation.[6] *P. urinaria* may also contribute to its anticoagulant and antithrombotic properties. Corilagin, a bioactive constituent isolated from *P. urinaria*, has been reported to inhibit fibrin formation and platelet aggregation.[7] A corilagin-rich fraction from *P. urinaria* exhibited significant antithrombotic activity by reducing thrombus formation in both arterial and venous models and inhibiting platelet–neutrophil adhesion. It prolonged kaolin partial thromboplastin time and shortened euglobulin lysis time, indicating modulation of the intrinsic coagulation pathway and enhanced fibrinolysis.[15]

In addition, *P. guajava* (guava leaves) may contribute to its anticoagulant potential. Experimental findings have demonstrated that guava leaf extracts and their active phenolic compounds-such as ferulic acid, gallic acid, and quercetin-exhibit significant anticoagulant activity. These extracts effectively prevented the shortening of thrombin clotting time and preserved antithrombin III activity, indicating inhibition of thrombin generation and maintenance of normal coagulation balance. The phenolic constituents are believed to act through antioxidant and protein-protective mechanisms, stabilising coagulation factors and preventing excessive fibrin formation.[8,16] *A. aspera* also exhibits notable antithrombotic and anticoagulant activities. Experimental studies comparing its methanolic and aqueous extracts demonstrated that the methanolic extract produced a significant thrombolytic effect, achieving approximately 75% clot lysis at 1000 µg/mL, which was markedly higher than the aqueous extract (about 51%). This effect is attributed to the high levels of phenolics, saponins, tannins, alkaloids, and flavonoids, which are known to facilitate fibrin degradation and modulate coagulation pathways. By dissolving preformed clots and regulating thrombin activity, *A. aspera* improves blood flow and prevents thrombosis without causing excessive bleeding.[9] *C. latifolium* has recently been reported to exhibit thrombolytic activity, supporting its potential contribution to the formulation's overall antithrombotic effect. In an *in vitro* study, the crude methanolic extract of *C. latifolium* leaves demonstrated moderate fibrinolytic activity, achieving 21.78 ± 1.04%, 28.43 ± 0.98%, and 33.84 ± 1.75% clot dissolution at concentrations of 6, 8, and 10 mg/mL, respectively, compared with 47.27 ± 2.00% for the reference drug streptokinase. These results suggest that *C. latifolium* contains bioactive compounds that promote fibrinolysis and may enhance thrombus resolution.[10]

Collectively, these findings suggest that SAK90 acts through multiple mechanisms to inhibit hypercoagulability and reduce thrombus formation, highlighting the synergistic interactions among its active constituents that contribute to its overall anticoagulant effect. Further studies are needed to clarify its underlying mechanisms, isolate and characterise the major bioactive compounds responsible for its anticoagulant and thrombolytic activities, and assess its safety and therapeutic efficacy in in vivo and clinical investigations. Such research will provide a more comprehensive understanding of SAK90 as a potential natural agent for the prevention and management of thrombotic disorders.

V. CONCLUSION

In the LPS-induced thrombosis model in rats, Saphia Alkali K90 at 27 mL/kg/day showed a tendency toward increased platelet count and fibrinogen levels, as well as prolonged aPTT, although these differences were not statistically

significant. The 54 mL/kg/day dose exhibited a significant anticoagulant effect, characterized by increased platelet count and fibrinogen concentration, together with prolonged aPTT compared with the model group.

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