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*Research Article*

# Ferulenol, a Prenylated Coumarin, Suppresses Collagen-Induced Platelet Activation via PLCγ2-Mediated cPLA2 Signaling in Humans

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## Abstract

**Background:** Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide and are strongly associated with atherothrombotic events. Thrombus formation results from the interplay between platelet activation and the coagulation cascade. While anticoagulant systems are essential for maintaining hemostasis, these systems can also increase the risk of bleeding complications. Coumarins led to the development of clinically important oral anticoagulants such as warfarin, which inhibits vitamin K epoxide reductase complex subunit 1 (VKORC1), a key enzyme in vitamin K recycling and normal blood clotting. Ferulenol, a prenylated coumarin derived from *Ferula communis*, has been reported to inhibit VKORC1, indicating a similar anticoagulant mechanism. Indeed, the close interplay between coagulation and platelet activation suggests that ferulenol may also modulate platelet function. However,the role of ferulenol in platelet activation has not yet been fully clarified. **Methods:** In this study, washed platelets obtained from healthy human donors were used to examine whether ferulenol suppresses platelet activation. To further clarify the underlying mechanisms, this study performed immunoblotting and confocal microscopy. In addition, this study evaluated the *in vivo* antithrombotic and hemostatic effects of ferulenol using a vascular thrombosis model and a tail bleeding time assay in mice. **Results:** Collagen (2 μg/mL)-induced platelet aggregation was reduced by ferulenol in a concentration-dependent manner from 10 to 40 μM, with near-complete inhibition at 40 μM. Ferulenol produced only moderate inhibition of arachidonic acid (AA, 60 μM)-induced aggregation at 80 μM and did not significantly affect by thrombin (0.02 U/mL)-induced platelet responses. Moreover, ferulenol markedly reduced collagen-induced platelet activation markers, including P-selectin expression, intracellular $Ca^{2+}$ mobilization, and adenosine triphosphate (ATP) release. These inhibitory effects were accompanied by reduced phosphorylation of phospholipase Cγ2 (PLCγ2), cytosolic phospholipase A2 (cPLA2), and mitogen-activated protein kinase (MAPK) family members, including extracellular signal-regulated kinase, p38 MAPK, and c-Jun N-terminal kinase. Ferulenol also suppressed activation of the phosphoinositide 3-kinase (PI3K)/Akt/glycogen synthase kinase-3β (GSK3β) signaling pathway. In mice, ferulenol extended the time to thrombotic platelet plug formation, without significantly increasing bleeding time. **Conclusions:** This study, provides the first evidence that ferulenol inhibits platelet activation. Mechanistically, ferulenol may inhibit collagen-induced platelet activation, at least in part, by suppressing PLCγ2 activation, thereby reducing phosphorylation of cPLA2, MAPKs, and PI3K/Akt/GSK3β signaling proteins. These inhibitory effects were reflected by reduced P-selectin exposure on the platelet surface, suggesting suppression of α-granule release. In addition, ferulenol reduced dense-granule ATP release and limited the rise in intracellular $Ca^{2+}$ levels, thereby contributing to the inhibition of platelet aggregation. Therefore, ferulenol may be a promising candidate for preventing thromboembolic events associated with CVDs.

**Keywords:** ferulenol; phospholipase C gamma 2; human platelets; thrombosis; coumarins

## 1. Introduction

Atherothrombotic events are major contributors to the global burden of cardiovascular diseases (CVDs), which remain among the principal causes of illness and death worldwide. Atherosclerosis developmentinvolves progressive lipid accumulation, endothelial dysfunction, and chronic inflammation within the vascular wall, ultimately leading to plaque formation. Upon plaque disruption or erosion, exposure of subendothelial components, such as collagen, triggers platelet aggregation and initiates arterial thrombosis and the coagulation cascade [1,2]. This synergistic process results in fibrin formation, thereby leading to an occlusive thrombus [2]. Crucially, under pathophysiological conditions, thrombus formation is tightly regulated by endogenous anticoagulant mechanisms that maintain a balance between coagulation and anticoagulation. Disruption of this balance can lead to excessive thrombus formation, thereby contributing to the development and progression of cerebrovascular diseases and CVDs [2].

The anticoagulant system is essential for maintaining hemostatic balance; however, excessive anticoagulant activity may increase the risk of bleeding complications. Major endogenous anticoagulant mechanisms, such as antithrombin, the tissue factor pathway inhibitor (TFPI), and protein C/protein S system limit overactivation of the coagulation cascade [3]. In addition to its key role in fibrin generation, thrombin functions as a major platelet agonist that activates platelets through protease-activated receptors, leading to platelet aggregation, granule secretion, and integrin activation [2,4]. Conversely, activated platelets expose negatively charged phospholipids, such as phosphatidylserine, providing a procoagulant surface that supports the assembly of coagulation complexes, including tenase and prothrombinase, which further amplify thrombin generation [5]. This interaction creates a positive feedback loop between coagulation and platelet activation, highlighting their coordinated roles in thrombosis [4].

Given the central role of platelets in arterial thrombi, a detailed understanding of platelet activation is essential. Platelets are anucleate cell fragments derived from megakaryocytes that circulate in a resting state under normal conditions but rapidly activate upon vascular injury [6]. When a blood vessel wall is damaged, exposure of subendothelial matrix proteins, especially collagen, allows platelets to adhere via specific collagen receptors, triggering platelet activation and aggregation. This activation involves intracellular signaling cascades leading to shape changes, granule secretion, and activation of integrin $\alpha_{IIb}\beta_3$, which binds fibrinogen to link platelets together and form aggregates [6]. Secondary mediators, such as ADP and thromboxane $A_2$ ($TXA_2$), are generated or released during platelet activation and amplify platelet recruitment and aggregation through receptor-mediated signaling. Platelet activation is further regulated by intracellular calcium mobilization and kinase signaling pathways, which play critical roles in coordinating cytoskeletal reorganization and secretion during thrombus formation [7]. Considering the close interplay between platelet activation and the coagulation cascade, modulation of platelet function remains critical in regulating coagulation.

Coumarins are a class of naturally occurring benzopyrone compounds widely distributed in plants, many of which exhibit diverse pharmacological activities, including anticoagulant, antimicrobial, and anticancer effects, and they have given rise to clinically useful oral anticoagulants such as warfarin, acenocoumarol, and phenprocoumon for preventing and managing thromboembolic disorders [8,9]. These agents act as anticoagulants by inhibiting vitamin K epoxide reductase complex subunit 1 (VKORC1), thereby limiting the regeneration of reduced vitamin K required for the γ-carboxylation of vitamin K-dependent coagulation factors [9]. Consequently, functionally inactive clotting factors are produced, leading to reduced fibrin formation [10]. However, their clinical use is associated with several adverse effects, including an increased risk of bleeding, particularly intracranial and gastrointestinal hemorrhaging, as well as rare complications such as skin necrosis and vascular calcification, necessitating careful and cautious use [11,12,13,14].

The genus *Ferula* comprises a group of medicinal plants known to be rich in coumarin derivatives [15]. *Ferula communis*, a species widely distributed in the Mediterranean region, exists in two distinct chemotypes: one enriched in daucane esters and the other containing prenylated coumarins. Among these, ferulenol was identified as a major bioactive prenylated coumarin [16,17]. Previous studies demonstrated that ferulenol exhibits notable antimicrobial activities, primarily through interfering with cellular energy metabolism [18]. Furthermore, ferulenol was shown to inhibit VKORC1, suggesting that it may share mechanistic features with coumarin-derived anticoagulants through modulating vitamin K-dependent coagulation pathways [17,19]. Given the functional link between the coagulation cascade and platelet activation, it is possible that ferulenol may exert inhibitory effects on platelet activation beyond its anticoagulant properties. However, its direct impact on platelet activation remains unclear. Therefore, the objective of the present study was to elucidate the antiplatelet effects of ferulenol and its possible mechanisms.

## 2. Methods

### *2.1 Chemicals and Reagents*

Ferulenol (purity >98%), Thromboxane $B_2$ ($TXB_2$) enzyme-linked immunosorbent assay (ELISA) kit, and warfarin were obtained from Cayman Chemical (Ann Arbor, MI, USA). Aprotinin, dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), arachidonic acid (AA), sodium pyrophosphate, leupeptin, sodium fluoride, phenylmethylsulfonyl fluoride, luciferin-luciferase, sodium orthovanadate, bovine serum albumin (BSA),

and thrombin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The protein assay dye reagent concentrate was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Primary antibodies used in this study included anti-phosphorylated (p)-c-Jun N-terminal kinase (JNK; Thr183/Tyr185; #9251), anti-p-p44/p42 extracellular signal-regulated kinase (ERK; Thr202/Tyr204; #9101), anti-p-phospholipase Cγ2 (PLCγ2; #50535), and anti-p-phosphoinositide 3-kinase (PI3K p85 Tyr458/p55 Tyr199; #4257) polyclonal antibodies, which were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-p-cytosolic phospholipase A2 (cPLA2; Ser505; AF3329) and anti-p-p38 mitogen-activated protein kinase (p38 MAPK; Thr180/Tyr182; AF4001) polyclonal antibodies were obtained from Affinity Biosciences (Cincinnati, OH, USA). The anti-p-Akt (Ser473; orb127667) polyclonal antibody was purchased from Biorbyt (Biorbyt Ltd., Cambridge, UK), whereas anti-p-glycogen synthase kinase 3α/β (GSK3α/β; sc-81496) and anti-α-tubulin (sc-32293) monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fura-2 acetoxymethyl ester (Fura-2 AM) was obtained from Molecular Probes (Eugene, OR, USA). The fluorescein isothiocyanate (FITC)-conjugated anti-human CD62P (P-selectin) monoclonal antibody was purchased from BioLegend (San Diego, CA, USA). Alexa Fluor® 488-labeled goat anti-rabbit immunoglobulin G (IgG) and Alexa Fluor® 647-labeled goat anti-mouse IgG were obtained from Abcam (Cambridge, UK). Hybond-polyvinylidene difluoride (PVDF) membranes, enhanced chemiluminescence (ECL) detection reagents, and horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG and sheep anti-mouse IgG secondary antibodies were obtained from Amersham (Buckinghamshire, UK).

### *2.2 Preparation of Human Platelets and Assessment of Platelet Aggregation and ATP Release*

The protocol for this study complied with the ethical standards of the Declaration of Helsinki and was approved by the Institutional Review Board of Taipei Medical University (Approval No. TMU-JIRB-N202412004). All participants provided written informed consent before blood sampling. Only healthy adult volunteers who had refrained from medications or dietary supplements with possible effects on platelet function for at least 14 days before donation were included. Blood was anticoagulated with acid–citrate–dextrose (ACD) solution at a blood-to-ACD ratio of 9:1 (v/v). After centrifugation, platelet-rich plasma (PRP) was collected and treated with EDTA (2 mM) and heparin (6.4 IU/mL) prior to the washing procedure. The washed platelets were resuspended in Tyrode's buffer containing 3.5 mg/mL BSA, and the final $Ca^{2+}$ concentration was adjusted to 1 mM. For aggregation assays, washed platelets were incubated with ferulenol (1–80 μM) for 3 min and subsequently stimulated with collagen (2 μg/mL), thrombin (0.02 U/mL), or AA (60 μM). DMSO (0.1%) served as the solvent control. Platelet aggregation was measured using a Lumi-Aggregometer (Payton, Scarborough, Ontario, Canada). ATP release, used as an indicator of dense-granule secretion, was also determined. In brief, 20 μL of luciferin-luciferase reagent was added to the platelet suspension 1 min prior to agonist stimulation, and luminescence signals were recorded with a fluorescence spectrophotometer (F-7000; Hitachi, Tokyo, Japan).

### *2.3 Assessment of P-Selectin Expression by Flow Cytometry*

To assess P-selectin expression, washed human platelets were adjusted to $3.6 \times 10^8$ cells/mL and pretreated for 3 min with either 0.1% DMSO as the solvent control or ferulenol at 20 or 40 μM. FITC-conjugated anti-P-selectin monoclonal antibody (2 μg/mL) was added during this pretreatment period. After 15 min of collagen stimulation (2 μg/mL), samples were subjected to FACScan flow cytometric analysis (Becton Dickinson, San Jose, CA, USA), with 50,000 events recorded per sample. Platelets were identified and gated according to their forward scatter (FSC) and side scatter (SSC) profiles to exclude debris and non-platelet particles. FITC fluorescence was subsequently analyzed within the gated platelet population.

### *2.4 Determination of Intracellular $Ca^{2+}$ Levels*

Intracellular $Ca^{2+}$ mobilization was examined using Fura-2 AM-loaded platelets. Briefly, PRP obtained from citrated whole blood was incubated with Fura-2 AM (5 μM) for 1 h. After labeling, platelets were resuspended in Tyrode's buffer supplemented with 1 mM $Ca^{2+}$ and adjusted to a final concentration of $3.6 \times 10^8$ cells/mL. Before collagen stimulation, platelet suspensions were incubated with either 0.1% DMSO as the solvent control or ferulenol at 20 or 40 μM for 3 min. Collagen (2 μg/mL) was then added, and changes in $[Ca^{2+}]_i$ were recorded using a fluorescence spectrophotometer (F-7000; Hitachi, Tokyo, Japan). According to a previously reported method, fluorescence was monitored using excitation wavelengths of 340 and 380 nm, with emission collected at 500 nm [20].

### *2.5 Quantification of $TXB_2$ Levels*

$TXB_2$ generation was assessed using platelet suspensions adjusted to $3.6 \times 10^8$ cells/mL. Platelets were first treated with either 0.1% DMSO as the solvent control or ferulenol (40 μM) for 3 min, followed by collagen stimulation (2 μg/mL) for 6 min. EDTA (2 mM) and indomethacin (50 μM) were added to terminate the reaction. The samples were centrifuged at 2000 ×g for 5 min, after which the supernatants were collected for $TXB_2$ analysis using a commercial ELISA kit in accordance with the manufacturer's instructions.

*2.6 Immunoblot Analysis of Platelet Signaling Pathways*

Immunoblotting was performed using washed platelets adjusted to $1.2 \times 10^9$ cells/mL. Platelet suspensions were treated with either 0.1% DMSO as the solvent control or ferulenol at 20 or 40 μM before collagen stimulation (2 μg/mL) for 5 min, while unstimulated platelets were used as resting controls. After platelet stimulation, 80 μL of lysis buffer was added to terminate the reactions. The resulting lysates were then centrifuged at 5000 ×g for 5 min to remove insoluble debris. The protein content of each clarified lysate was quantified with a Bradford protein assay kit (Bio-Rad, Hercules, CA, USA). Samples containing equal amounts of protein were separated on 10% SDS-polyacrylamide gels and then transferred onto membranes for probing with target-specific primary antibodies diluted at 1:1000. Band densities were measured using Bio-profil Biolight software (version V2000.01; Vilber Lourmat, Marne-la-Vallée, France), and α-tubulin was used as the internal control for normalization.

*2.7 Confocal Imaging Analysis*

Confocal fluorescence microscopy was performed using unstimulated and collagen-stimulated platelets attached to poly-L-lysine–coated coverslips. After activation with collagen (2 μg/mL), attached platelets were fixed in 4% paraformaldehyde for 1 h and subsequently treated with 0.1% Triton X-100 for permeabilization. Samples were incubated with 5% BSA in PBS for 1 h to minimize nonspecific binding, and were subsequently treated with primary antibodies against the target proteins for 24 h. The coverslips were extensively washed with PBS and then incubated with fluorescently labeled goat anti-rabbit and goat anti-mouse IgG secondary antibodies for 1 h. Confocal images were obtained using a Leica TCS SP5 microscope (Mannheim, Germany) equipped with a 100× oil-immersion objective.

*2.8 Real-Time Visualization of Vascular Thrombosis*

The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Taipei Medical University (LAC-2025-0261), and mice were maintained under a 12-h dark–light cycle with free access to food and water. The study was carried out in accordance with the ARRIVE guidelines. A total of 24 six-week-old male ICR mice (BioLASCO Taiwan Co., Ltd., Taipei, Taiwan) were treated intraperitoneally with 50 μL of 0.1% DMSO as the solvent control or ferulenol (15 mg/kg). No animals were excluded from the analysis. Following drug administration, the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; 10 mg/mL solution prepared in sterile normal saline), and sodium fluorescein (15 μg/kg) was subsequently injected through the lateral tail vein. For the thrombosis assay, mesenteric venules with a diameter range of 30–40 μm were selected. Thrombus formation was triggered by light irradiation at <520 nm, and the time required for complete vascular occlusion after irradiation began was defined as the occlusion time. After the experiment, mice were euthanized by $CO_2$ inhalation at 20–30% chamber volume/min, and death was confirmed by the absence of respiration and reflexes.

*2.9 Measurement of Tail Bleeding Time in Mice*

The mouse tail transection assay was used to evaluate bleeding time. The animals were intraperitoneally injected with 50 μL of 0.1% DMSO as the solvent control, ferulenol at 15 mg/kg, or warfarin at 15 mg/kg. Twenty-four hours later, the animals were anesthetized, and a 3 mm segment of the distal tail was cut using a scalpel. The tail was immediately immersed in 37 °C saline, and bleeding time was measured until blood flow had completely ceased. For animals in which bleeding lasted more than 10 min, a maximal value of 600 s was assigned. All outcome assessments were performed by investigators blinded to the treatment allocation.

*2.10 Statistical Analysis*

Results are shown as the mean ± standard error of the mean (SEM). Independent experiments using platelets from different donors were used to determine the n value. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), with the Student–Newman–Keuls test applied for post hoc analysis. Statistical significance was set at $p < 0.05$. SAS software (version 9.2; SAS Institute, Cary, NC, USA) was used to conduct the analyses.

## 3. Results

*3.1 Ferulenol Suppresses Platelet Aggregation*

The present study investigated the effects of ferulenol on human platelet activation. As shown in Fig. 1A,B, ferulenol (10–40 μM) suppressed collagen (2 μg/mL)-induced platelet aggregation, achieving almost complete inhibition at 40 μM. At a concentration of 80 μM, ferulenol significantly inhibited platelet aggregation induced by AA (60 μM). In contrast, ferulenol did not significantly affect thrombin (0.02 U/mL)-induced platelet aggregation, even at concentrations up to 80 μM. The $IC_{50}$ for collagen-induced platelet aggregation was estimated to be approximately 20 μM. The solvent control (0.1% DMSO) did not produce any detectable change in platelet aggregation (data not shown). The LDH assay further demonstrated that ferulenol treatment at concentrations ranging from 20 to 160 μM for 20 min did not promote LDH leakage from platelets, suggesting that its inhibitory effects were not attributable to platelet cytotoxicity (**Supplementary Fig. 1**).

*3.2 Ferulenol Attenuates P-Selectin Expression, ATP Secretion, and Intracellular $Ca^{2+}$ Mobilization*

ATP release and P-selectin expression were measured to assess platelet granule secretion. ATP release reflects

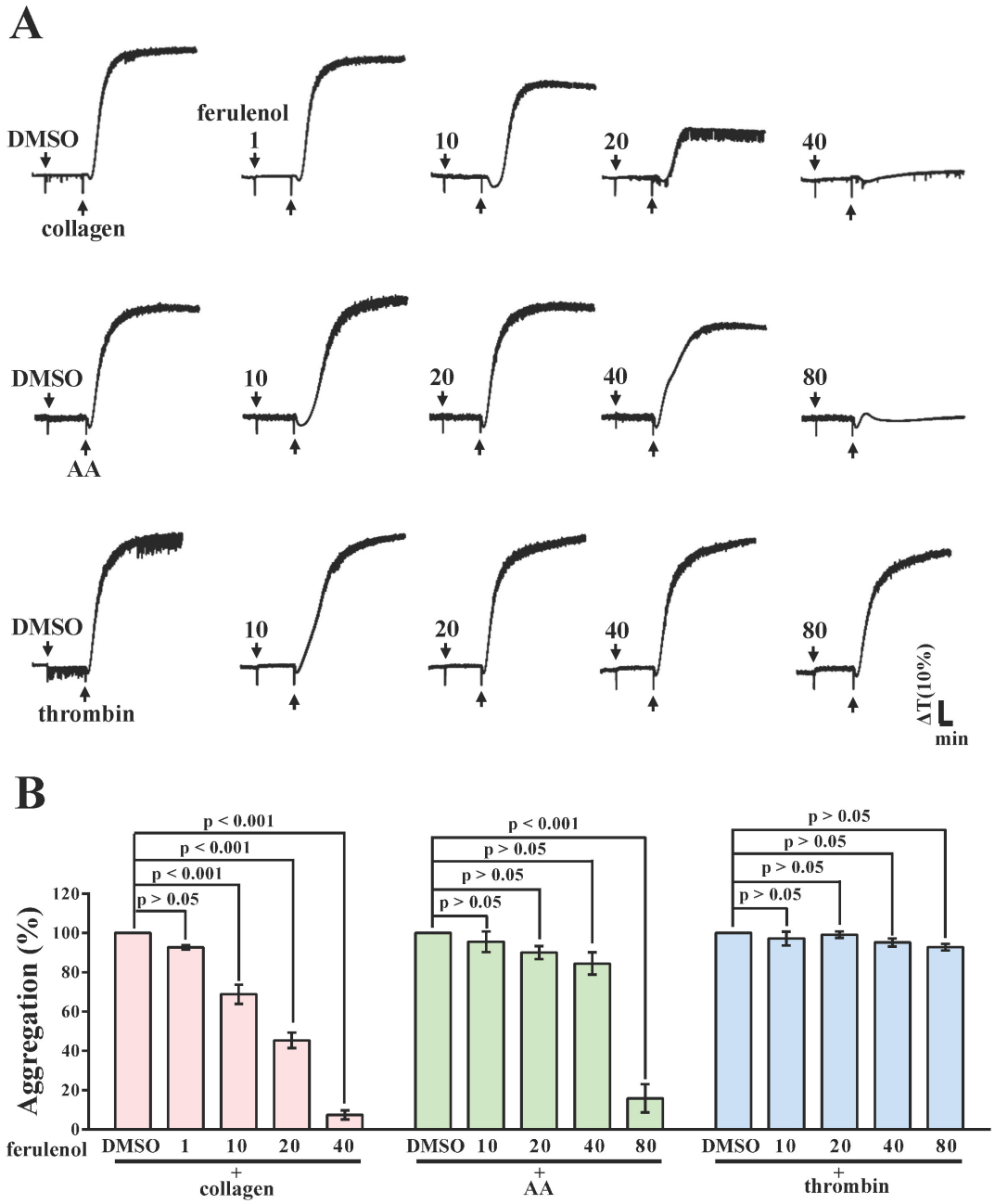


**Fig. 1. Ferulenol suppresses platelet aggregation induced by different agonists**. (A) Washed platelets were incubated with either 0.1% dimethyl sulfoxide (DMSO) as the solvent control or various concentrations of ferulenol (1–80 μM) before being activated by collagen (2 μg/mL), thrombin (0.02 U/mL), or arachidonic acid (AA, 60 μM). (B) Concentration–response histograms summarizing the inhibitory effects of ferulenol on agonist-induced platelet aggregation (%). Results are shown as the mean ± standard error of the mean (SEM) ($n = 4$). $p < 0.001$ compared with the 0.1% DMSO-treated group.

dense granule secretion, whereas P-selectin expression indicates α-granule release; both responses are closely involved in the amplification of platelet aggregation [21]. In collagen-stimulated platelets, ATP release was gradually reduced following treatment with increasing concentrations of ferulenol (Fig. 2A). Elevation of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) promotes platelet aggregation. Intracellular $Ca^{2+}$ mobilization was assessed using a fluorescence-based detection method. As illustrated in Fig. 2B, the rise in $[Ca^{2+}]_i$ was strongly attenuated by ferulenol treatment at both 20 and 40 μM, and the reduction was approximately 74% for each concentration. In resting platelets, P-selectin is retained within α-granules. Following platelet activation, granule secretion and membrane rearrangement promote the redistribution of P-selectin to the platelet surface [21]. Fig. 2C shows that P-selectin ex-

pression on the platelet surface was minimal under resting conditions without collagen stimulation (Tyrode's solution). Collagen markedly increased P-selectin expression, whereas this response was markedly reduced following treatment with ferulenol (20 and 40 μM). As illustrated in Fig. 2D, unstimulated platelets produced minimal $TXB_2$ (52.81 ± 19.46 ng/mL), while collagen markedly enhanced $TXB_2$ generation (811.8 ± 39.53 ng/mL). Ferulenol at 40 μM reduced this collagen-induced $TXB_2$ production, reducing the level to 621.0 ± 45.84 ng/mL.

### *3.3 Ferulenol Suppresses Collagen-Induced PLCγ2 Phosphorylation*

Activation of PLCγ2 represents a key step in collagen-induced platelet signaling [22]. Following platelet stimulation, PLCγ2 cleaves phosphatidylinositol 4,5-bisphosphate ($PIP_2$), resulting in the generation of two second messengers, diacylglycerol (DAG) and inositol trisphosphate ($IP_3$). Among these, $IP_3$ regulates intracellular $Ca^{2+}$ mobilization by promoting $Ca^{2+}$ release from the dense tubular system (DTS) together with $Ca^{2+}$ influx [23]. In the current study, ferulenol (20 and 40 μM) significantly decreased PLCγ2 phosphorylation by collagen-stimulated platelets (Fig. 3A). Furthermore, confocal fluorescence microscopy further verified the inhibitory effect of ferulenol on PLCγ2 activation. As shown in Fig. 3B, activated PLCγ2 was visualized by green fluorescence, whereas α-tubulin was detected as red fluorescence in both unstimulated and collagen-activated platelets. An increase in the p-PLCγ2 fluorescence intensity was observed following collagen stimulation compared to resting platelets, whereas this elevation was attenuated by treatment with ferulenol (40 μM) (Fig. 3B). In contrast, the fluorescence intensity of α-tubulin remained unchanged across the different groups (Fig. 3B). These findings indicated that the antiplatelet effects of ferulenol were associated with inhibition of PLCγ2 activation.

### *3.4 Ferulenol Attenuates the Phosphorylation of Collagen-Induced cPLA2 and MAPKs*

By promoting the liberation of AA, cPLA2 serves as a key mediator in platelet activation and helps amplify platelet aggregation [24]. In the context of our study, treatment with ferulenol at 20 and 40 μM markedly reduced cPLA2 phosphorylation in collagen-stimulated platelets (Fig. 4A). MAPK pathways, including ERK, JNK, and p38 MAPK, are involved in several biological processes, such as inflammation, platelet activation, apoptosis, and cell proliferation [25]. As shown in Fig. 4B–D, treatment with ferulenol at both 20 and 40 μM markedly decreased the phosphorylation of the examined MAPKs in collagen-stimulated platelets. Collectively, these observations suggest that alterations in cPLA2 and MAPK signaling may contribute to the antiplatelet activity of ferulenol.

### *3.5 Ferulenol Inhibits Collagen-Induced PI3K/Akt/GSK3β Signaling*

Under conditions of elevated shear stress, the PI3K/Akt/GSK3β pathway is closely involved in the regulation of thrombus formation [26]. Activation of Akt is dependent on PI3K, highlighting its critical role in regulating downstream signaling events [26]. Akt signaling is activated by a variety of platelet agonists and plays an important role in regulating platelet activation and hemostasis. In addition, GSK3β, a key downstream component, is controlled by PI3K/Akt in platelets [27]. Our results demonstrated that collagen stimulation markedly increased phosphorylation of PI3K, Akt, and GSK3β in platelets, which was significantly inhibited by ferulenol (20 and 40 μM) (Fig. 5A–C). Collectively, these results suggest that ferulenol may inhibit platelet activation, at least in part, by affecting the PI3K/Akt/GSK3β signaling pathway.

### *3.6 Ferulenol Prolongs Thrombotic Occlusion Time Without Increasing Bleeding Time in Mice*

To further assess the antithrombotic activity of ferulenol *in vivo*, thrombus formation was analyzed in mouse mesenteric venules using a fluorescein-induced thrombosis model and real-time intravital microscopy. As shown in Fig. 6A, thrombotic platelet plug formation occurred more slowly after ferulenol treatment (15 mg/kg) than in the 0.1% DMSO solvent control group. Quantitative analysis showed that ferulenol significantly increased the time required for vessel occlusion, from 183 ± 13 s in the 0.1% DMSO group to 253 ± 20 s in the ferulenol-treated group ($n = 8$; Fig. 6B). We also determined bleeding time after intraperitoneal administration of 0.1% DMSO (solvent control), ferulenol (15 mg/kg), or warfarin (15 mg/kg) (Fig. 6C). Bleeding time was not significantly prolonged by ferulenol treatment, increasing from 187 ± 18 s in the 0.1% DMSO-treated group ($n = 8$) to 233 ± 27 s in the ferulenol-treated group ($p > 0.05$). In contrast, warfarin treatment significantly prolonged bleeding time to 600 ± 0 s ($p < 0.001$). These results suggest that ferulenol at 15 mg/kg significantly delayed thrombotic vessel occlusion without significantly prolonging bleeding time under these experimental conditions.

## 4. Discussion

Ferulenol has been reported to inhibit VKORC1, with a Ki of approximately 0.08 μM, which is a principal molecular target of coumarin-derived anticoagulants [17]. Warfarin and other clinically used vitamin K antagonists produce anticoagulant effects largely by targeting VKORC1, which interferes with the activation of vitamin K-dependent clotting factors and limits thrombin formation. Previous studies have indicated that ferulenol shows stronger VKORC1-inhibitory activity than warfarin [17], suggesting a potentially stronger impact on vitamin K-dependent coagulation processes. Although warfarin effectively sup-

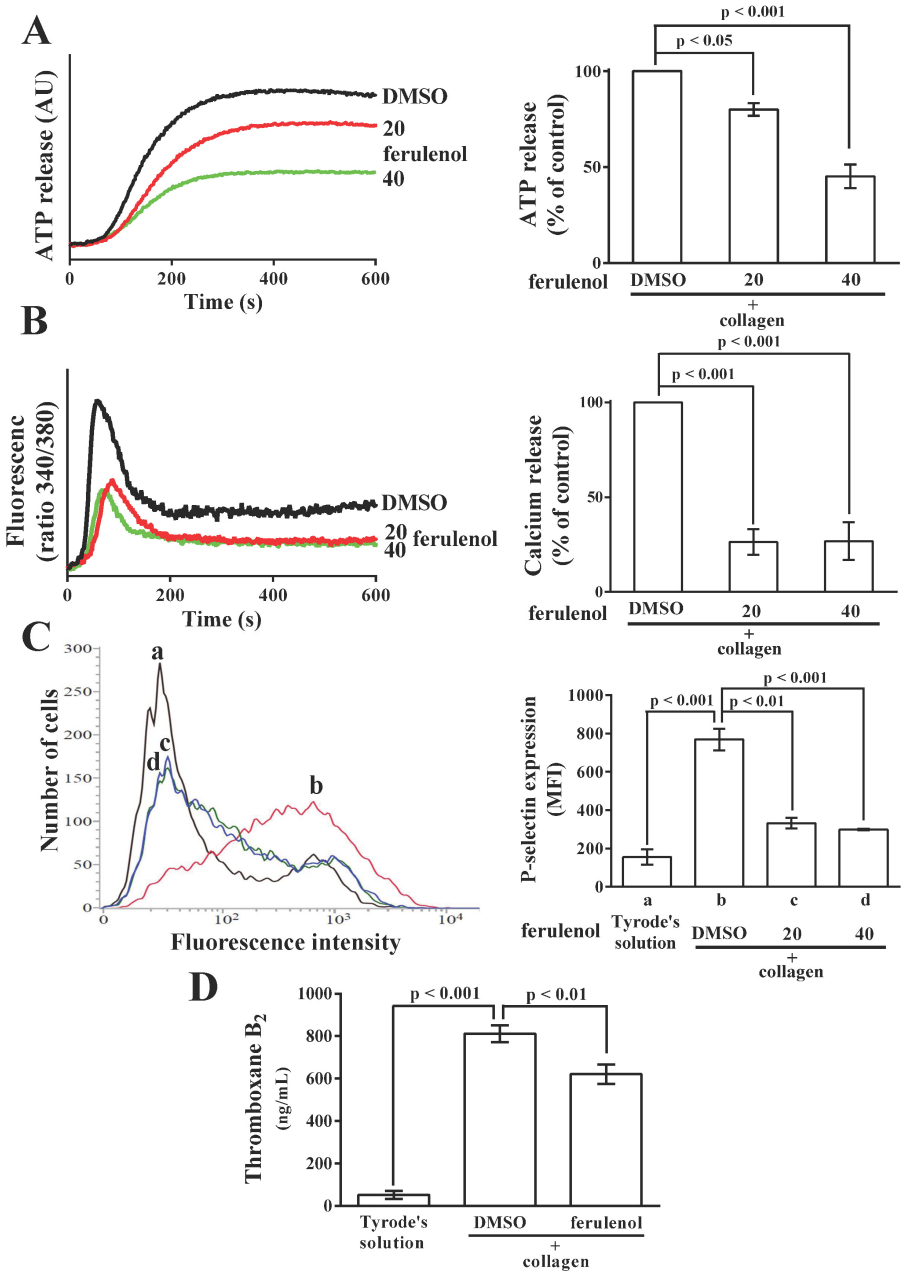


**Fig. 2. Ferulenol attenuates collagen-induced ATP release, $Ca^{2+}$ mobilization, P-selectin expression, and thromboxane $B_2$ formation**. Washed platelets at a concentration of $3.6 \times 10^8$ cells/mL were treated with either 0.1% DMSO as the solvent control or ferulenol at 20 and 40 μM before collagen stimulation (2 μg/mL). Ferulenol-mediated changes in platelet activation were analyzed by measuring (A) ATP secretion, expressed as arbitrary units (AU); (B) intracellular $Ca^{2+}$ mobilization ($[Ca^{2+}]_i$); and (C) surface expression of P-selectin, expressed as mean fluorescence intensity (MFI). The P-selectin groups were designated as follows: (a) Tyrode's solution, (b) 0.1% DMSO with collagen, (c) 20 μM ferulenol with collagen, and (d) 40 μM ferulenol with collagen. The experimental procedures are described in detail in the Methods section. (D) $TXB_2$ production was additionally evaluated. Quantitative analyses are shown in the corresponding bar graphs. Values are presented as the mean ± SEM ($n = 4$). For panels (A,B), $p < 0.05$ and $p < 0.001$ indicate significant differences compared with the 0.1% DMSO-treated group. In panels (C,D), $p < 0.001$ represents a significant difference from the resting control group (Tyrode's solution). Significant differences versus the 0.1% DMSO-treated group are denoted by $p < 0.01$ and $p < 0.001$.

presses coagulation, our results showed that it did not inhibit collagen-induced platelet activation, even at concentrations up to 80 μM (**Supplementary Fig. 2**), which is consistent with previous reports [28,29]. Furthermore, warfarin had no effect on collagen-induced ATP release or cPLA2 phosphorylation (**Supplementary Fig. 2**), indicating that warfarin does not affect platelet activation. Interestingly, in contrast to warfarin, we found that feru-

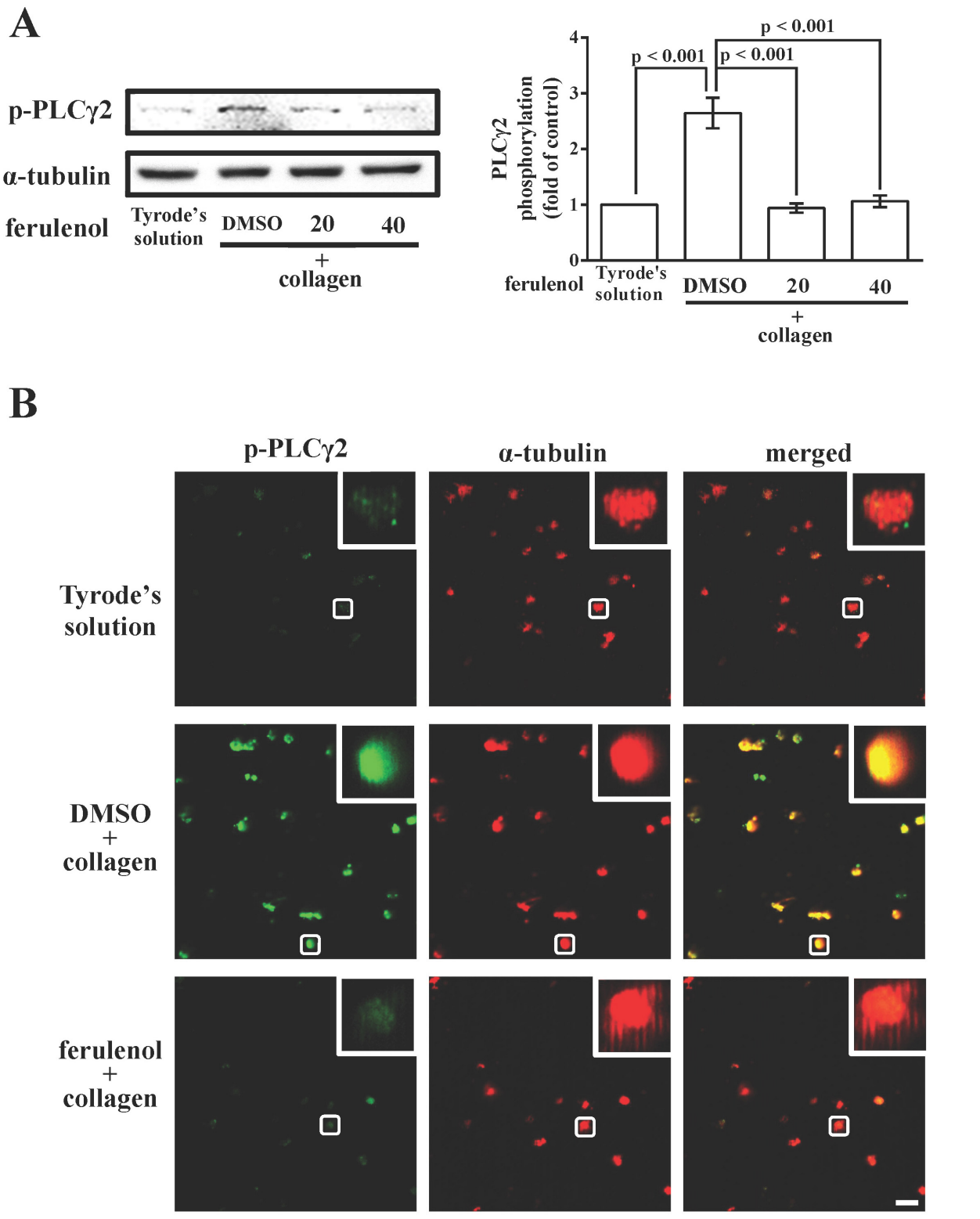


**Fig. 3. Ferulenol inhibits collagen-induced PLCγ2 phosphorylation in human platelets**. Washed platelets were exposed to 0.1% DMSO as the solvent control or ferulenol at 20 and 40 μM before activation with collagen (2 μg/mL). (A) The phosphorylation level of PLCγ2 was determined by immunoblot analysis. In the confocal microscopy experiment, 40 μM ferulenol was used for platelet treatment. (B) Green fluorescence represents phosphorylated PLCγ2 labeled with Alexa Fluor® 488-conjugated goat anti-rabbit IgG, whereas red fluorescence indicates α-tubulin labeled with Alexa Fluor® 647-conjugated goat anti-mouse IgG. Scale bar = 5 μm. Data are shown as the mean ± SEM ($n$ = 4). In panel (A), $p < 0.001$ indicates a significant difference versus the resting control group (Tyrode's solution), and $p < 0.001$ indicates a significant difference versus the collagen-stimulated 0.1% DMSO group.

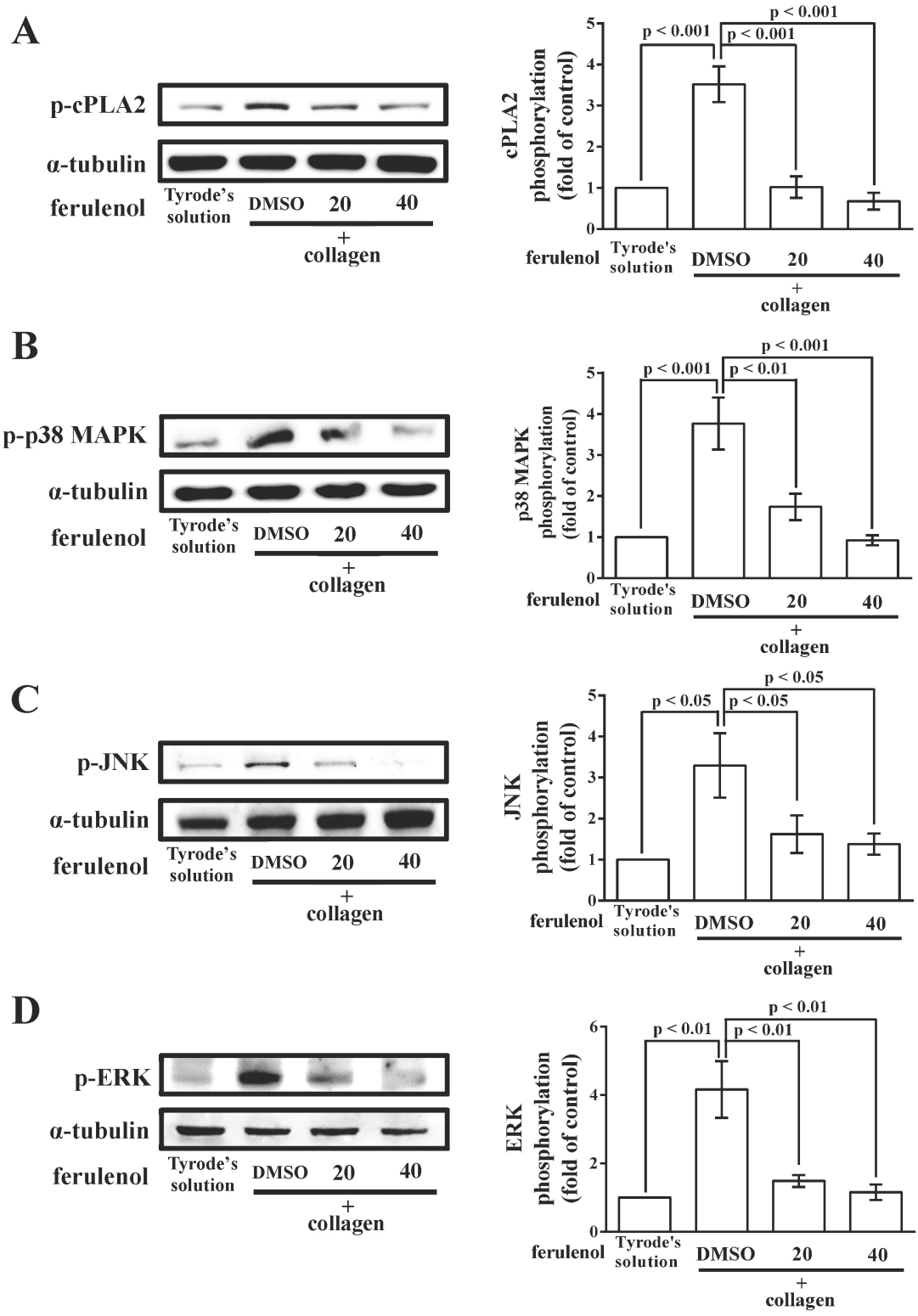


**Fig. 4. Effects of ferulenol on signaling by collagen-induced cytosolic phospholipase A2 (cPLA2) and mitogen-activated protein kinases (MAPKs) in human platelets**. Washed platelets were incubated with 0.1% DMSO as the solvent control or ferulenol (20 and 40 μM), followed by stimulation with collagen (2 μg/mL). Phosphorylation levels of (A) cytosolic phospholipase A2 (cPLA2), (B) p38 MAPK, (C) c-jun N-terminal kinase (JNK), and (D) extracellular signal-regulated kinase (ERK) were examined by immunoblot analysis. Tyrode's solution was used as the resting control. Data are shown as the mean ± SEM (n = 4). $p < 0.05$, $p < 0.01$, and $p < 0.001$ indicate significant differences compared with the resting control group (Tyrode's solution); $p < 0.05$, $p < 0.01$, and $p < 0.001$ indicate significant differences compared with the collagen-stimulated 0.1% DMSO group.

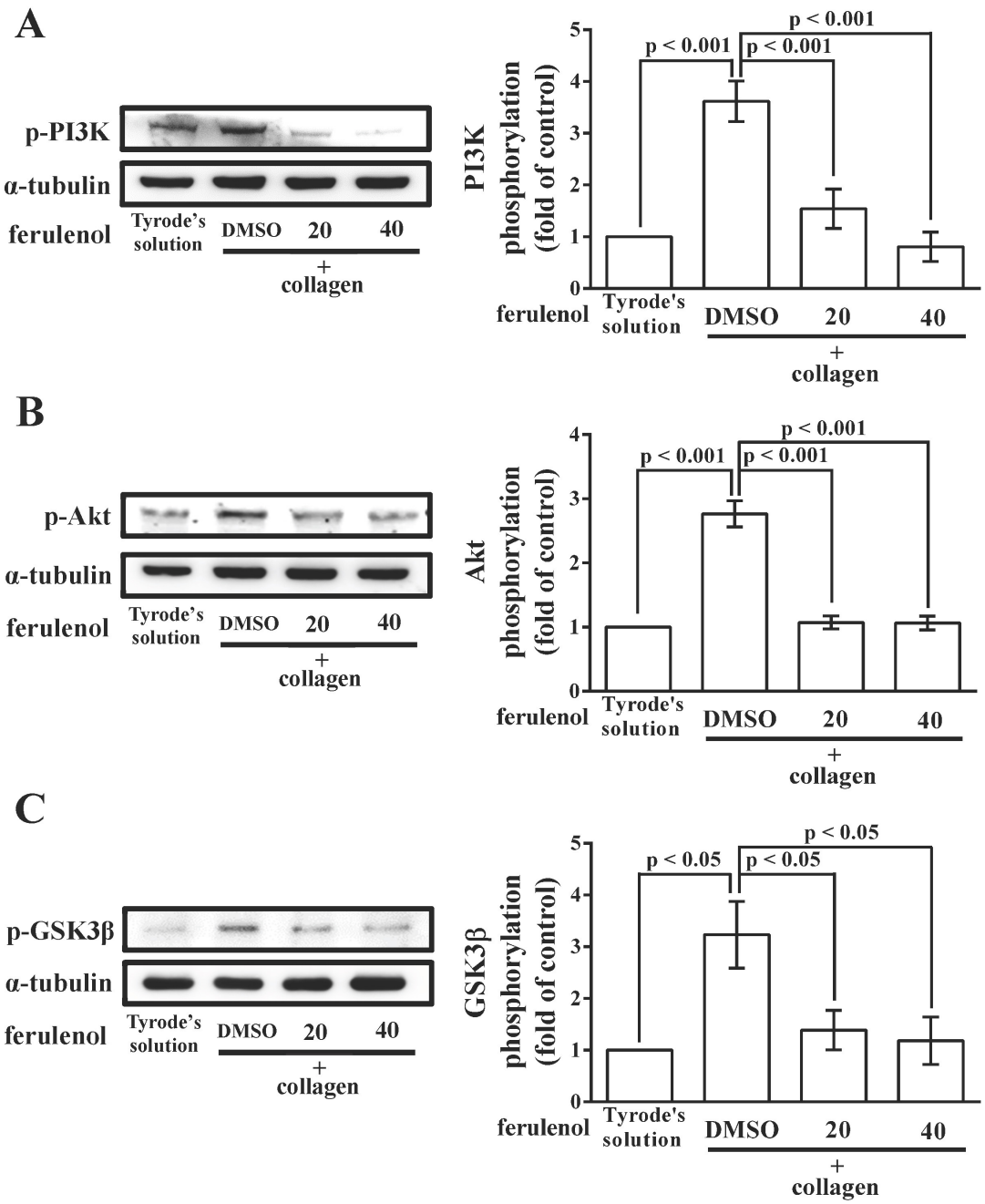


**Fig. 5. Ferulenol suppresses collagen-induced PI3K/Akt/GSK3β signaling in platelets**. Washed platelets were treated with either 0.1% DMSO as the solvent control or ferulenol at 20 and 40 μM before collagen stimulation (2 μg/mL). Immunoblot analysis was performed to determine the phosphorylation levels of (A) PI3K, (B) Akt, and (C) GSK3β. Tyrode's solution was included as the resting control. Results are presented as the mean ± SEM ($n$ = 4). $p < 0.05$ and $p < 0.001$ denote significant differences compared with the resting control group (Tyrode's solution); $p < 0.05$ and $p < 0.001$ denote significant differences compared with the collagen-stimulated 0.1% DMSO group.

lenol markedly inhibited collagen-induced platelet activation. These findings indicate that although ferulenol shares a similar mechanistic basis with classical coumarin anticoagulants in the coagulation pathway, it exhibits distinct activity on platelet activation.

Following platelet stimulation, tyrosine kinase-dependent signaling cascades are initiated, resulting in increased intracellular $Ca^{2+}$ levels and the secretion of granule contents, including ADP/ATP and P-selectin. α-Granules are the principal protein-containing granules in platelets and store a variety of molecules, such as

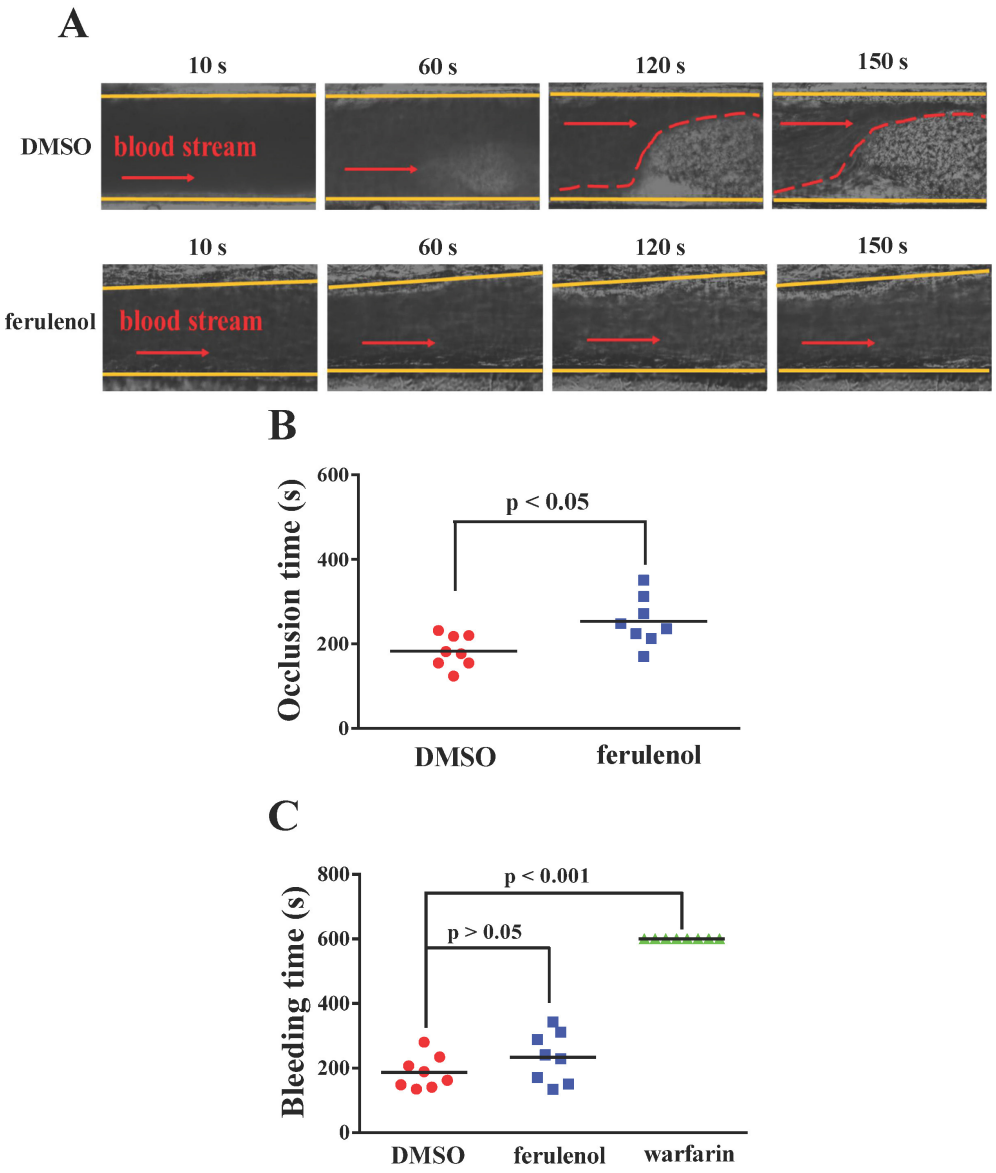


**Fig. 6. Ferulenol affects thrombotic vessel occlusion and tail bleeding time in mice**. (A) Mice were intraperitoneally injected with either 0.1% DMSO as the solvent control or ferulenol at 15 mg/kg. Thrombus formation in mesenteric venules was triggered by fluorescein irradiation. Representative time-lapse images were obtained from both the 0.1% DMSO and ferulenol groups over 10–150 s after irradiation. The red arrow denotes the direction of blood flow, while the gray-shaded region outlined by the red dashed lines indicates the site of platelet plug formation (200× magnification). (B) The time required for vessel occlusion was used as a parameter of thrombus formation. (C) At 24 h after intraperitoneal injection of 0.1% DMSO, ferulenol (15 mg/kg), or warfarin (15 mg/kg), tail bleeding time was assessed. Results are shown as the mean ± SEM ($n$ = 8). $p < 0.05$ and $p < 0.001$ denote significant differences versus the 0.1% DMSO solvent control group.

P-selectin, fibrinogen, and platelet-derived growth factor. Upon activation, α-granule secretion promotes the translocation of P-selectin to the platelet surface [21]. Accordingly, the detection of surface P-selectin expression provides a reliable approach for assessing platelet activation, as presented in Fig. 2C.

Activation of PLC represents a key step in platelet signal transduction initiated by various agonists. Human platelets express two major PLC classes, PLCβ and PLCγ, which participate in distinct signaling pathways depending on the agonist involved. The two PLCγ isoforms do not appear to participate equally in agonist-induced platelet signaling. Although both PLCγ1 and PLCγ2 are present in platelets, PLCγ2 is considered the major isoform mediating signaling responses induced by collagen and AA, whereas their relative involvement may vary depending on the platelet agonist [30,31]. Following vascular injury, collagen, a major structural element of the extracellular

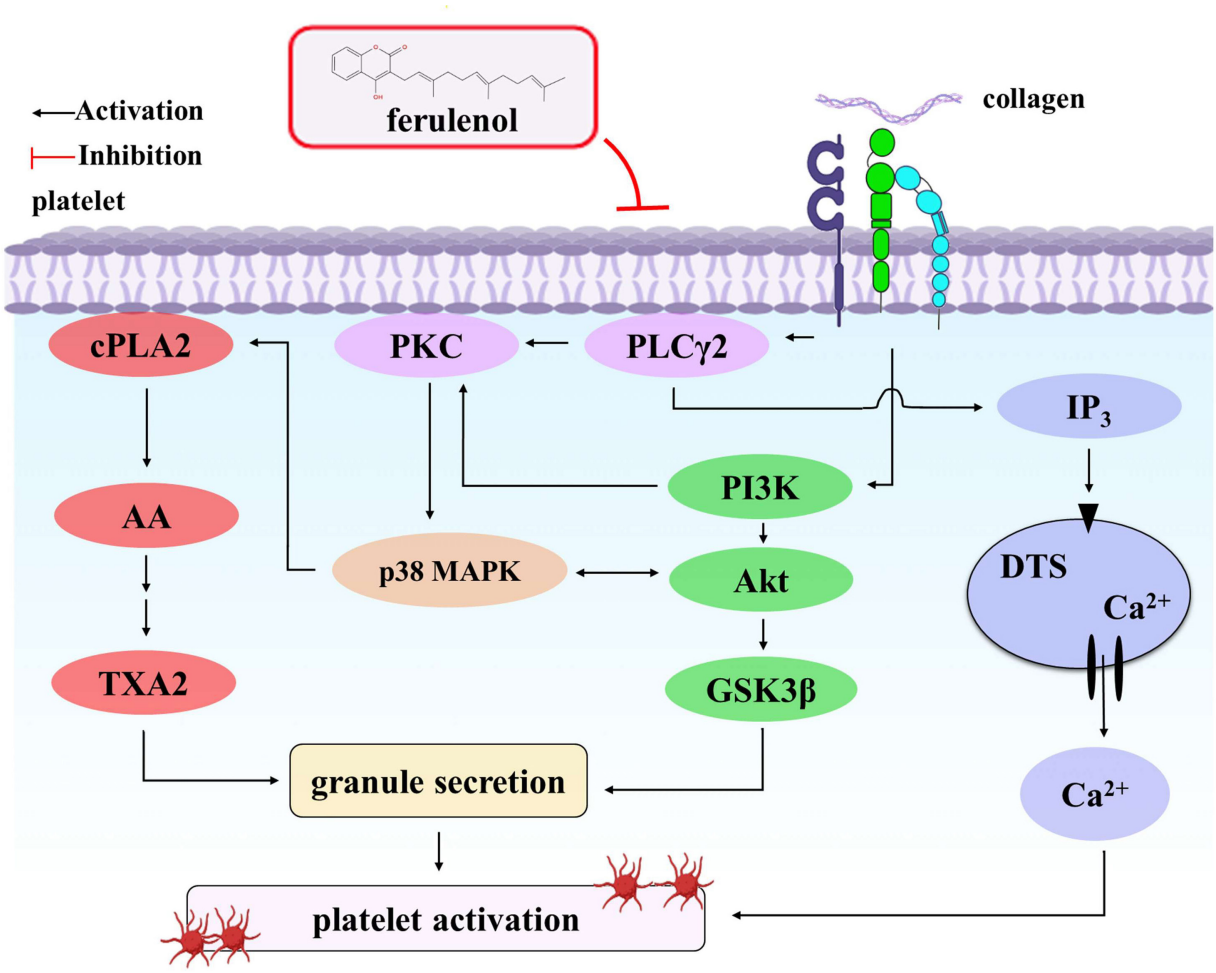


**Fig. 7. Proposed signaling mechanism by which ferulenol suppresses collagen-induced platelet activation**. Ferulenol suppresses platelet activation mainly through inhibition of PLCγ2 signaling, with subsequent modulation of downstream cPLA2, PI3K/Akt/GSK3β, and MAPK pathways. This coordinated regulation leads to reduced intracellular calcium ($[Ca^{2+}]_i$) levels, ultimately suppressing platelet aggregation.

matrix, becomes exposed and promotes platelet activation through its interaction with receptors such as glycoprotein VI (GPVI), leading to the initiation of PLCγ2-dependent signaling pathways [31]. Upon stimulation by different agonists, including collagen, phospholipase enzymes such as cPLA2 become activated. These enzymes hydrolyze membrane phospholipids to release AA, which is subsequently converted by cyclooxygenase into $TXA_2$, thereby enhancing platelet activation. Consistent with this pathway, our results showed that ferulenol markedly inhibited $TXB_2$ formation, a stable metabolite of $TXA_2$. In platelet activation, the PLCγ2–PKC pathway and the $cPLA_2$–AA–$TXA_2$ axis are functionally interconnected. PLCγ2 participates in the early phase of signaling by promoting second messenger production, while the AA–$TXA_2$ pathway further strengthens platelet activation at a downstream level (Fig. 7). In parallel, stimulation of Gαq-coupled GPCRs results in the separation of Gαq from the receptor complex and subsequent activation of PLCβ. This signaling step is required for platelet aggregation triggered by GPCR agonists such as thrombin, serotonin, and ADP [32]. The distinct signaling pathways activated by these agonists may help explain why ferulenol suppresses collagen- and AA-induced platelet aggregation, whereas thrombin-induced aggregation remains largely unaffected. Our findings showed that ferulenol strongly attenuated PLCγ2 activation in collagen-stimulated platelets. This result suggests that the inhibitory effect of ferulenol on platelet activation may be closely associated with suppression of downstream PLCγ2/cPLA2 signaling.

MAPK signaling pathways regulate various cellular processes, including cell growth, differentiation, and apoptosis. Evidence from pharmacological inhibitor studies and genetic knockout models has shown that p38 MAPK, JNK, and ERK contribute to platelet activation [25]. However, the specific contributions of JNK and ERK to platelet activation remain unclear. Although these kinases have been suggested to exert negative regulatory effects on integrin

$\alpha_{IIb}\beta_3$ activation, they are also considered important participants in collagen-induced platelet aggregation [25]. This regulatory network also involves cPLA2, a key enzyme responsible for AA release, the phosphorylation of which is driven by p38 MAPK activation, leading to the hydrolysis of membrane phospholipids and subsequent AA liberation, which in turn serves as a precursor for $TXA_2$ generation, thereby amplifying platelet activation (Fig. 7) [24,33]. Our findings indicated that ferulenol markedly suppresses activation of p38 MAPK, JNK, and ERK, as well as activation of cPLA2. This inhibitory effect may account for the enhanced ability of ferulenol to suppress platelet activation triggered by collagen or AA.

Platelet activation is regulated by complex signaling networks, among which PI3K plays a central role. PI3K functions downstream of several platelet receptors, including GPVI, and promotes PLC$\gamma$2 activation and intracellular $Ca^{2+}$ mobilization, thereby amplifying platelet signaling [26]. Akt as a major downstream mediator of PI3K, and its functional importance is highlighted by findings that Akt-deficient mice exhibit diminished platelet aggregation and defective stable adhesion under flow conditions [26]. Accordingly, activation of Akt downstream of PI3K represents a potential therapeutic target for antithrombotic drug development. In contrast, the role of Akt-mediated signaling to platelet activation is not yet fully clarified, although potential effectors such as GSK3, including its both isoforms, have been reported in platelets. Among these, GSK3$\beta$ represents the predominant isoform expressed in platelets [27]. Therefore, clarifying the downstream substrates of GSK3 in platelets may provide useful insight for identifying new antithrombotic targets. Moreover, previous studies have shown that PI3K/Akt and MAPK pathways, including p38 MAPK, can mutually regulate each other in human platelets, with PKC acting upstream to control MAPK signaling (Fig. 7) [34].

In the *in vivo* thrombosis model, fluorescein sodium administration followed by continuous light irradiation caused endothelial injury in mesenteric venules, leading to thrombotic platelet plug formation during the experimental period. The ferulenol dose administered to mice (15 mg/kg) was selected according to body surface area-based interspecies dose conversion principles and was used in the animal experiments [35]. Under these conditions, ferulenol at 15 mg/kg significantly prolonged the occlusion time, suggesting that its antithrombotic effect may be associated, at least in part, with inhibition of platelet activation. To further evaluate whether ferulenol affected bleeding tendency, a mouse tail transection assay was performed. Coumarin-based anticoagulants are widely used for the prevention and treatment of thromboembolic disorders; however, their clinical application is often limited by bleeding risk [36]. Therefore, warfarin was included as a representative coumarin anticoagulant for comparison. In our study, warfarin markedly prolonged bleeding time but did not substantially inhibit collagen-induced platelet activation, supporting the view that its major effect is related to anticoagulation rather than direct inhibition of platelet activation. By contrast, ferulenol-treated mice showed only a slight, non-significant increase in bleeding time compared with the DMSO group. Under the present experimental conditions, ferulenol showed antiplatelet and antithrombotic activity, while bleeding time was not significantly prolonged. Therefore, ferulenol may be a potential antiplatelet compound for further investigation in thromboembolic disease-related studies.

## 5. Limitations

This study has several limitations. Ferulenol primarily inhibited collagen-induced platelet activation via suppressing PLC$\gamma$2 activation and downstream cPLA2, MAPKs, and PI3K/Akt/GSK3$\beta$ signaling pathways; however, other potential mechanisms cannot be excluded. Although bleeding time was evaluated *in vivo*, future studies may further characterize the effects of ferulenol on bleeding time under different dosing conditions.

## 6. Conclusions

We demonstrated for the first time that ferulenol exerts antiplatelet effects, at least in part, by inhibiting PLC$\gamma$2 activation, leading to suppression of cPLA2, MAPKs, and PI3K/Akt/GSK3$\beta$ signaling pathways. These effects result in reductions in ATP release, intracellular $Ca^{2+}$ mobilization, and P-selectin expression, ultimately attenuating platelet aggregation. However, we could not rule out the possibility that other unidentified signaling pathways are involved in its mechanisms. These findings may provide insights into the antiplatelet activity of ferulenol in thromboembolic disorders.

## Availability of Data and Materials

The data generated in the present study may be requested from the corresponding author.

## Author Contributions

C-YC: Writing—review & editing, Writing—original draft, Supervision, Project administration, Funding acquisition, Conceptualization. W-CH: Writing—review & editing, Software, Methodology, Project administration. YC and Y-XL: Writing—review & editing, Methodology, Investigation, Conceptualization. C-YH, C-WH and TJ: Writing—review & editing, Investigation, Data curation. C-CC: Writing—review & editing, Writing—original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Funding acquisition. J-RS: Writing—review & editing, Writing—original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Funding acquisition. All authors read and approved the final manuscript.

All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The human study protocol was approved by the Institutional Review Board of Taipei Medical University (TMU-JIRB-N202412004) and complied with the Declaration of Helsinki. All participants provided written informed consent before being enrolled in the study. The conduct of all animal experiments and care protocols followed the guidelines set forth in the Guide for the Care and Use of Laboratory Animals (Approval No. LAC-2025-0261) and obtained approval from the Institutional Animal Care and Use Committee of Taipei Medical University.


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## Conflicts of Interest

The authors declare no conflicts of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/Pharmazie52496.

## References


[1] Björkegren JLM, Lusis AJ. Atherosclerosis: Recent developments. Cell. 2022; 185: 1630–1645. https://doi.org/10.1016/j.cell.2022.04.004

[2] Vilahur G, Fuster V. Interplay between platelets and coagulation: from protective haemostasis to pathological arterial thrombosis. European Heart Journal. 2025; 46: 413–423. https://doi.org/10.1093/eurheartj/ehae776

[3] Gierula M, Ahnström J. Anticoagulant protein S-New insights on interactions and functions. Journal of Thrombosis and Haemostasis : JTH. 2020; 18: 2801–2811. https://doi.org/10.1111/jth.15025

[4] Zou J, Sun S, De Simone I, Ten Cate H, de Groot PG, de Laat B, et al. Platelet Activation Pathways Controlling Reversible Integrin αIIbβ3 Activation. TH Open. 2024; 8: e232–e242. https://doi.org/10.1055/s-0044-1786987

[5] Reddy EC, Rand ML. Procoagulant Phosphatidylserine-Exposing Platelets *in vitro* and *in vivo*. Frontiers in Cardiovascular Medicine. 2020; 7: 15. https://doi.org/10.3389/fcvm.2020.00015

[6] Nardin M, Verdoia M, Cao D, Nardin S, Kedhi E, Galasso G, et al. Platelets and the Atherosclerotic Process: An Overview of New Markers of Platelet Activation and Reactivity, and Their Implications in Primary and Secondary Prevention. Journal of Clinical Medicine. 2023; 12: 6074. https://doi.org/10.3390/jcm12186074

[7] Tian Y, Zong Y, Pang Y, Zheng Z, Ma Y, Zhang C, et al. Platelets and diseases: signal transduction and advances in targeted therapy. Signal Transduction and Targeted Therapy. 2025; 10: 159. https://doi.org/10.1038/s41392-025-02198-8

[8] Gao L, Wang F, Chen Y, Li F, Han B, Liu D. The antithrombotic activity of natural and synthetic coumarins. Fitoterapia. 2021; 154: 104947. https://doi.org/10.1016/j.fitote.2021.104947

[9] Kasperkiewicz K, Ponczek MB, Owczarek J, Guga P, Budzisz E. Antagonists of Vitamin K-Popular Coumarin Drugs and New Synthetic and Natural Coumarin Derivatives. Molecules. 2020; 25: 1465. https://doi.org/10.3390/molecules25061465

[10] Kholmukhamedov A, Subbotin D, Gorin A, Ilyassov R. Anticoagulation Management: Current Landscape and Future Trends. Journal of Clinical Medicine. 2025; 14: 1647. https://doi.org/10.3390/jcm14051647

[11] Battistella M, Mamdami MM, Juurlink DN, Rabeneck L, Laupacis A. Risk of upper gastrointestinal hemorrhage in warfarin users treated with nonselective NSAIDs or COX-2 inhibitors. Archives of Internal Medicine. 2005; 165: 189–192. https://doi.org/10.1001/archinte.165.2.189

[12] Kakagia DD, Papanas N, Karadimas E, Polychronidis A. Warfarin-induced skin necrosis. Annals of Dermatology. 2014; 26: 96–98. https://doi.org/10.5021/ad.2014.26.1.96

[13] El-Helou N, Al-Hajje A, Ajrouche R, Awada S, Rachidi S, Zein S, et al. Adverse drug events associated with vitamin K antagonists: factors of therapeutic imbalance. Vascular Health and Risk Management. 2013; 9: 81–88. https://doi.org/10.2147/VHRM.S41144

[14] Linkins LA. Bleeding risks associated with vitamin K antagonists. Blood Reviews. 2013; 27: 111–118. https://doi.org/10.1016/j.blre.2013.02.004

[15] Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)-a review. Journal of Ethnopharmacology. 2011; 134: 1–10. https://doi.org/10.1016/j.jep.2010.11.067

[16] Salehi M, Naghavi MR, Bahmankar M. A review of Ferula species: biochemical characteristics, pharmaceutical and industrial applications, and suggestions for biotechnologists. Industrial Crops and Products. 2019; 139: 111511. https://doi.org/10.1016/j.indcrop.2019.111511

[17] Louvet MS, Gault G, Lefebvre S, Popowycz F, Boulven M, Besse S, et al. Comparative inhibitory effect of prenylated coumarins, ferulenol and ferprenin, contained in the 'poisonous chemotype' of Ferula communis on mammal liver microsomal VKORC1 activity. Phytochemistry. 2015; 118: 124–130. https://doi.org/10.1016/j.phytochem.2015.08.012

[18] Kabongo AT, Acharjee R, Sakura T, Bundutidi GM, Hartuti ED, Davies C, et al. Biochemical characterization and identification of ferulenol and embelin as potent inhibitors of malate:quinone oxidoreductase from *Campylobacter jejuni*. Frontiers in Molecular Biosciences. 2023; 10: 1095026. https://doi.org/10.3389/fmolb.2023.1095026

[19] Gebauer M. Synthesis and structure-activity relationships of novel warfarin derivatives. Bioorganic & Medicinal Chemistry. 2007; 15: 2414–2420. https://doi.org/10.1016/j.bmc.2007.01.014

[20] Huang WC, Shu LH, Kuo YJ, Lai KSL, Hsia CW, Yen TL, et al. Eugenol Suppresses Platelet Activation and Mitigates Pulmonary Thromboembolism in Humans and Murine Models. International Journal of Molecular Sciences. 2024; 25: 2098. https://doi.org/10.3390/ijms25042098

[21] Hou Y, Li H, Zhu L, Li Y, Zeng Y, Quan T, et al. A review of natural compounds to regulate platelet aggregation: molec-

ular mechanism and research advance. Frontiers in Pharmacology. 2025; 16: 1537776. https://doi.org/10.3389/fphar.2025.1537776

[22] Fuentes E. Modulation of Glycoprotein VI and Its Downstream Signaling Pathways as an Antiplatelet Target. International Journal of Molecular Sciences. 2022; 23: 9882. https://doi.org/10.3390/ijms23179882

[23] Vilahur G, Gutiérrez M, Arzanauskaite M, Mendieta G, Ben-Aicha S, Badimon L. Intracellular platelet signalling as a target for drug development. Vascular Pharmacology. 2018; 111: 22–25. https://doi.org/10.1016/j.vph.2018.08.007

[24] Wong DA, Kita Y, Uozumi N, Shimizu T. Discrete role for cytosolic phospholipase A(2)alpha in platelets: studies using single and double mutant mice of cytosolic and group IIA secretory phospholipase A(2). The Journal of Experimental Medicine. 2002; 196: 349–357. https://doi.org/10.1084/jem.20011443

[25] Patel P, Naik UP. Platelet MAPKs-a 20+ year history: What do we really know? Journal of Thrombosis and Haemostasis : JTH. 2020; 18: 2087–2102. https://doi.org/10.1111/jth.14967

[26] Moroi AJ, Watson SP. Impact of the PI3-kinase/Akt pathway on ITAM and hemITAM receptors: haemostasis, platelet activation and antithrombotic therapy. Biochemical Pharmacology. 2015; 94: 186–194. https://doi.org/10.1016/j.bcp.2015.02.004

[27] Li D, August S, Woulfe DS. GSK3beta is a negative regulator of platelet function and thrombosis. Blood. 2008; 111: 3522–3530. https://doi.org/10.1182/blood-2007-09-111518

[28] Chylova M, Motovska Z, Fialova A, Stetkarova I, Peisker T, Kalvach P. The effect of warfarin administration on platelet aggregation. Bratislavske Lekarske Listy. 2021; 122: 320–324. https://doi.org/10.4149/BLL_2021_054

[29] Nilsson B, Back V, Wei R, Plane F, Jurasz P, Bungard TJ. Potential Antimigraine Effects of Warfarin: An Exploration of Biological Mechanism with Survey of Patients. TH Open. 2019; 3: e180–e189. https://doi.org/10.1055/s-0039-1692989

[30] Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2010; 30: 2341–2349. https://doi.org/10.1161/ATVBAHA.110.207522

[31] Zheng Y, Adams T, Zhi H, Yu M, Wen R, Newman PJ, et al. Restoration of responsiveness of phospholipase Cγ2-deficient platelets by enforced expression of phospholipase Cγ1. PLoS ONE. 2015; 10: e0119739. https://doi.org/10.1371/journal.pone.0119739

[32] Zhang M, Chen T, Lu X, Lan X, Chen Z, Lu S. G protein-coupled receptors (GPCRs): advances in structures, mechanisms, and drug discovery. Signal Transduction and Targeted Therapy. 2024; 9: 88. https://doi.org/10.1038/s41392-024-01803-6

[33] Lin W, Wang S, Liu R, Zhang D, Zhang J, Qi X, et al. Research progress of cPLA2 in cardiovascular diseases (Review). Molecular Medicine Reports. 2025; 31: 103. https://doi.org/10.3892/mmr.2025.13468

[34] Jayakumar T, Chen WF, Lu WJ, Chou DS, Hsiao G, Hsu CY, et al. A novel antithrombotic effect of sulforaphane via activation of platelet adenylate cyclase: ex vivo and in vivo studies. The Journal of Nutritional Biochemistry. 2013; 24: 1086–1095. https://doi.org/10.1016/j.jnutbio.2012.08.007

[35] Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy. 2016; 7: 27–31. https://doi.org/10.4103/0976-0105.177703

[36] Bhatia K, Ladd LM, Carr KH, Di Napoli M, Saver JL, McCullough LD, et al. Contemporary Antiplatelet and Anticoagulant Therapies for Secondary Stroke Prevention: A Narrative Review of Current Literature and Guidelines. Current Neurology and Neuroscience Reports. 2023; 23: 235–262. https://doi.org/10.1007/s11910-023-01266-2