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Phytochemical Characterization and In-Vitro Anticoagulant Potential of *Cyamopsis Tetragonoloba* (L.) Taub.: Exploring a Traditional Medicinal Plant for Natural Antithrombotic Therapy

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ABSTRACT

Background: Thromboembolic disorders are among the leading causes of cardiovascular morbidity and mortality worldwide. Although synthetic anticoagulants are effective in preventing thrombus formation, their clinical use is often limited by bleeding complications, drug interactions, and the need for continuous therapeutic monitoring. Consequently, there is growing interest in identifying plant-derived anticoagulant agents with improved safety and therapeutic efficacy. *Cyamopsis tetragonoloba* (L.) Taub., commonly known as cluster bean or guar, is a medicinal plant rich in polyphenols, flavonoids, tannins, and galactomannan, which have been reported to possess diverse pharmacological properties.

Objective: The present study aimed to investigate the phytochemical constituents and evaluate the in vitro anticoagulant activity of the ethanolic seed extract of *Cyamopsis tetragonoloba* using human plasma by measuring prothrombin time (PT).

Materials and Methods: The seeds of *Cyamopsis tetragonoloba* were shade-dried, powdered, and extracted with ethanol using the Soxhlet extraction method. Preliminary phytochemical screening was performed using standard qualitative methods. Anticoagulant activity was assessed in vitro by incubating different concentrations of the ethanolic extract with plasma collected from healthy volunteers. Prothrombin time (PT) was measured to evaluate anticoagulant activity and compared with untreated control samples.

Results: Qualitative phytochemical analysis confirmed the presence of phenolic compounds, flavonoids, tannins, saponins, carbohydrates, and other bioactive constituents in the ethanolic extract. The extract exhibited concentration-dependent prolongation of prothrombin time, indicating significant anticoagulant activity. The observed pharmacological effect may be attributed to the synergistic action of polyphenolic compounds and galactomannan, which may interfere with the coagulation cascade and thrombin-mediated fibrin formation.

Conclusion: The findings demonstrate that the ethanolic extract of *Cyamopsis tetragonoloba* possesses promising in vitro anticoagulant activity and supports its traditional medicinal use as a potential natural anticoagulant. Further phytochemical characterization, mechanistic studies, toxicity evaluation, and clinical investigations are warranted to identify the active constituents and establish their therapeutic potential in the prevention and management of thromboembolic disorders.

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide, accounting for nearly one-third of all global deaths. Thromboembolic disorders, including myocardial infarction, ischemic stroke, pulmonary embolism, and deep vein thrombosis, represent major contributors to cardiovascular morbidity and mortality. These conditions arise from abnormal activation of the coagulation cascade, resulting in excessive fibrin clot formation and vascular obstruction. Despite significant advances in anticoagulant therapy, thrombotic disorders continue to impose a substantial healthcare burden, particularly in developing countries [1,2].

Current anticoagulant drugs, including unfractionated heparin, low-molecular-weight heparins, warfarin, and direct oral anticoagulants (DOACs), have significantly improved the prevention and treatment of thromboembolic diseases. However, their clinical use is associated with several limitations, including an increased risk of bleeding, narrow therapeutic index, drug-food and drug-drug interactions, inter-individual variability, and the requirement for regular laboratory monitoring. These drawbacks have encouraged researchers to explore naturally occurring bioactive compounds as safer and cost-effective alternatives for anticoagulant therapy [3,4].

Medicinal plants have served as an important source of therapeutic agents since ancient times and continue to play a vital role in modern drug discovery. According to the World Health Organization (WHO), nearly 80% of the global population depends on traditional herbal medicines for primary healthcare. Plant-derived phytochemicals such as flavonoids, phenolic acids, tannins, saponins, alkaloids, polysaccharides, and terpenoids have demonstrated diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, cardioprotective, and anticoagulant effects [5,6].

Cyamopsis tetragonoloba (L.) Taub., commonly known as cluster bean or guar, belongs to the family Fabaceae and is widely cultivated in India, Pakistan, and other semi-arid regions. The plant is economically important because of its high galactomannan gum content, which is extensively used in the food, pharmaceutical, cosmetic, textile, and petroleum industries. Besides its industrial applications, *C. tetragonoloba* has been traditionally used for the management of diabetes, hyperlipidemia, gastrointestinal disorders, inflammation, constipation, and wound healing. Phytochemical investigations have reported that the seeds contain galactomannan, flavonoids, phenolic compounds, tannins, saponins, steroids, proteins, dietary fiber, and essential minerals, which contribute to its diverse pharmacological activities [7–9].

Recent pharmacological studies have highlighted the therapeutic potential of *C. tetragonoloba* in the management of oxidative stress, diabetes mellitus, dyslipidemia, obesity, microbial infections, and inflammatory disorders. Sulfated derivatives of guar galactomannan have demonstrated anticoagulant activity by modulating thrombin generation and interfering with fibrin clot formation. Polyphenolic constituents have also been reported to inhibit platelet aggregation and reduce oxidative damage associated with vascular diseases. However, limited scientific evidence is available regarding the anticoagulant potential of crude ethanolic seed extracts evaluated using human plasma-based coagulation assays [10,11].

Prothrombin Time (PT) is one of the most widely accepted laboratory methods for evaluating the extrinsic and common pathways of blood coagulation. Measurement of PT provides a reliable assessment of anticoagulant activity by determining the time required for clot formation after activation of the coagulation cascade. Therefore, PT-based assays are commonly employed in the preliminary screening of natural products with potential anticoagulant properties [12].

The present study was undertaken to investigate the phytochemical constituents and evaluate the in vitro anticoagulant activity of the ethanolic seed extract of *Cyamopsis tetragonoloba* using human plasma by measuring Prothrombin Time (PT). The findings of this study may provide scientific evidence supporting the traditional medicinal use of *C. tetragonoloba* and contribute to the development of plant-derived anticoagulant

agents for the prevention and management of thromboembolic disorders. The experimental procedures, extraction process, phytochemical screening, and anticoagulant evaluation were conducted according to the methodology described in the uploaded study.

2. Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used in the present investigation were of analytical grade. Normal saline served as the negative control, while ethylenediaminetetraacetic acid (EDTA) solution was used as the positive anticoagulant control. Calcium chloride solution was employed to initiate coagulation by restoring calcium ions required for activation of the coagulation cascade. Dimethyl sulfoxide (DMSO) was used for dissolving the concentrated plant extract to prepare the required test concentrations.

2.2 Study Design

The present investigation was designed as an in vitro experimental study to assess the anticoagulant potential of the ethanolic seed extract of *Cyamopsis tetragonoloba* (L.) Taub. using the Prothrombin Time (PT) assay. The study also included qualitative phytochemical screening to identify the major classes of secondary metabolites present in the extract that could contribute to its anticoagulant activity. All experimental procedures were performed following standard pharmacognostic and laboratory protocols under controlled conditions [13].

2.3 Collection and Authentication of Plant Material

Mature seeds of *Cyamopsis tetragonoloba* (L.) Taub. belonging to the family Fabaceae were procured from a local agricultural market. The collected seeds were manually inspected to remove damaged seeds and foreign matter before being washed thoroughly with distilled water. The cleaned seeds were shade dried at ambient temperature to preserve heat-sensitive phytoconstituents. After complete drying, the plant material was authenticated using standard taxonomic descriptions and published botanical literature prior to experimental use [14].

2.4 Preparation of Plant Material

The dried seeds were pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was sieved to ensure uniform particle size, which improves solvent penetration during extraction. The processed powder was transferred into clean, airtight containers and stored in a cool, dry place until extraction was performed [15].

2.5 Preparation of Ethanolic Extract

Extraction of phytoconstituents was carried out using a Soxhlet extraction apparatus with ethanol as the extraction solvent. Approximately the required quantity of powdered seed material was packed into a cellulose extraction thimble and placed inside the Soxhlet chamber. Ethanol was heated to reflux, allowing repeated cycles of evaporation, condensation, and solvent percolation through the powdered material. The extraction process was continued until the siphoned solvent became colourless, indicating exhaustive extraction of soluble constituents. The collected extract was filtered, concentrated under reduced temperature to remove the solvent, and stored in airtight containers under refrigeration until further analysis [16].

2.6 Preliminary Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical analysis using established phytochemical screening procedures. The extract was examined for the presence of alkaloids, flavonoids, tannins, phenolic compounds, steroids, saponins, and carbohydrates. [17]. Dragendorff's reagent was employed for alkaloids, ferric chloride solution for tannins and phenolics, sodium hydroxide test for flavonoids, Salkowski reaction for steroids, foam test for saponins, and Molisch's, Benedict's, and Fehling's tests for carbohydrates. The appearance of characteristic colour changes or precipitates was considered indicative of the corresponding phytochemical constituents [18].

2.7 Preparation of Test Solutions

The concentrated ethanolic extract was dissolved in DMSO to prepare four different concentrations, namely 0.062 g/mL, 0.125 g/mL, 0.250 g/mL, and 0.500 g/mL. Fresh solutions were prepared immediately before each experiment to ensure stability and reproducibility of the anticoagulant assay.

2.8 Collection of Blood Samples and Plasma Preparation

Fresh venous blood samples were collected aseptically from healthy adult volunteers after obtaining informed consent. Blood was transferred immediately into tubes containing 3.8% trisodium citrate in the proportion of 1 part anticoagulant to 9 parts blood to prevent coagulation. The samples were centrifuged at 3000 rpm for 15 minutes to obtain platelet-rich plasma. The separated plasma was carefully transferred into sterile tubes and maintained under refrigerated conditions until used for the Prothrombin Time assay [19].

2.9 Evaluation of Anticoagulant Activity by Prothrombin Time Assay

The anticoagulant activity of the ethanolic extract was evaluated using the Prothrombin Time (PT) assay. The experiment consisted of six treatment groups, including one negative control, one positive control, and four extract-treated groups.

- **Group I:** 0.2 mL plasma + 0.1 mL normal saline + 0.3 mL calcium chloride (Negative control)
- **Group II:** 0.2 mL plasma + 0.1 mL EDTA (50 mg/mL) + 0.3 mL calcium chloride (Positive control)
- **Group III:** 0.2 mL plasma + 0.1 mL extract (0.062 g/mL) + 0.3 mL calcium chloride
- **Group IV:** 0.2 mL plasma + 0.1 mL extract (0.125 g/mL) + 0.3 mL calcium chloride
- **Group V:** 0.2 mL plasma + 0.1 mL extract (0.250 g/mL) + 0.3 mL calcium chloride
- **Group VI:** 0.2 mL plasma + 0.1 mL extract (0.500 g/mL) + 0.3 mL calcium chloride

Immediately after the addition of calcium chloride, each tube was maintained at an angle of approximately 45° and gently tilted at 30-second intervals until the formation of a visible fibrin clot. The clotting time was recorded using a digital stopwatch and expressed as the Prothrombin Time (PT). Each experimental condition was evaluated in triplicate, and the average clotting time was calculated for subsequent analysis [20].

2.10 Statistical Analysis

All experimental observations were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical evaluation was carried out using appropriate descriptive statistical methods to

compare the anticoagulant activity among different treatment groups. Statistical significance was considered at a p-value less than 0.05 [21].

3. Results

3.1 Preliminary Phytochemical Screening

The ethanolic extract of *Cyamopsis tetragonoloba* (L.) Taub. seeds were subjected to qualitative phytochemical analysis to identify the major classes of secondary metabolites that may contribute to its biological activity. The screening demonstrated that the extract contained flavonoids, tannins, phenolic compounds, steroids, and saponins, whereas alkaloids were absent. These phytoconstituents are widely recognized for their antioxidant, anti-inflammatory, and cardiovascular protective properties and have also been associated with modulation of platelet aggregation and the coagulation cascade.

Among the detected constituents, flavonoids and phenolic compounds are known to exhibit anticoagulant and antithrombotic activities through inhibition of platelet activation, suppression of oxidative stress, and regulation of thrombin generation. Similarly, tannins have been reported to interfere with platelet adhesion and fibrin formation, while saponins may influence membrane permeability and contribute to vascular protective effects. The detection of these bioactive constituents provides a phytochemical basis for the anticoagulant activity observed in the subsequent pharmacological evaluation.

Table 1. Qualitative phytochemical screening of the ethanolic extract of *Cyamopsis tetragonoloba* seeds

S.No.	Phytochemical constituent	Test performed	Observation
1	Alkaloids	Dragendorff's test	Absent (−)
2	Flavonoids	Sodium hydroxide test	Present (+)
3	Tannins	Ferric chloride test	Present (+)
4	Phenolic compounds	Ferric chloride test	Present (+)
5	Steroids	Salkowski test	Present (+)
6	Saponins	Foam test	Present (+)

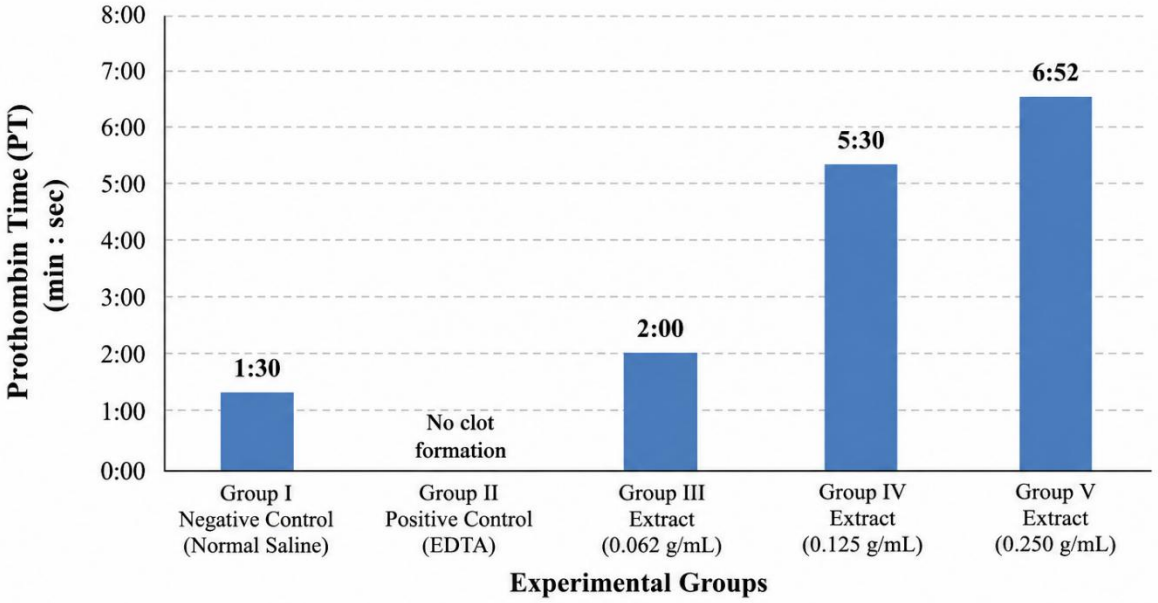
(+): Present; (−): Absent

3.2 Evaluation of Anticoagulant Activity by Prothrombin Time Assay

The anticoagulant potential of the ethanolic seed extract of *Cyamopsis tetragonoloba* was assessed by measuring the Prothrombin Time (PT) using platelet-rich plasma obtained from healthy volunteers. The PT assay evaluates the integrity of the extrinsic and common coagulation pathways by measuring the time required for fibrin clot formation following the addition of calcium chloride.

The negative control (normal saline) exhibited a normal clotting time of 1 min 30 s, representing the physiological coagulation response in untreated plasma. In contrast, the positive control (EDTA) completely prevented clot formation during the observation period because EDTA chelates calcium ions, which are essential for activation of multiple coagulation factors. These findings confirmed the validity of the experimental procedure.

Figure 1. Effect of Ethanolic Extract of *Cyamopsis tetragonoloba* Seeds on Prothrombin Time (PT)



PT: Prothrombin Time; EDTA: Ethylenediaminetetraacetic acid.
Group I: Negative control (normal saline).
Group II: Positive control (EDTA, 50 mg/mL).
Group III–V: Ethanolic extract of *Cyamopsis tetragonoloba* seeds at different concentrations.

Administration of the ethanolic extract resulted in a marked prolongation of Prothrombin Time compared with the negative control. At the lowest tested concentration (0.062 g/mL), the clotting time increased to 2 min 00 s, indicating an initial anticoagulant effect. Increasing the extract concentration to 0.125 g/mL produced a substantial increase in PT to 5 min 30 s, representing a pronounced delay in clot formation. The 0.250 g/mL concentration produced the greatest reported prolongation of PT (6 min 52 s), demonstrating enhanced anticoagulant activity with increasing extract concentration.

Overall, the results indicate a concentration-dependent increase in clotting time, suggesting that the ethanolic extract interferes with one or more steps of the coagulation cascade. The progressive prolongation of PT observed with increasing extract concentration supports the presence of bioactive phytochemicals capable of modulating coagulation mechanisms under in vitro conditions.

Table 2. Effect of the ethanolic extract of *Cyamopsis tetragonoloba* seeds on Prothrombin Time

Group	Treatment	Extract concentration	Prothrombin Time
I	Normal saline (Negative control)	—	1 min 30 s
II	EDTA (Positive control)	—	Absence of clot formation
III	Ethanolic extract	0.062 g/mL	2 min 00 s
IV	Ethanolic extract	0.125 g/mL	5 min 30 s
V	Ethanolic extract	0.250 g/mL	6 min 52 s

3.3 Comparative Analysis of Prothrombin Time

Comparison of the experimental groups revealed a clear difference in clotting time between untreated plasma and extract-treated samples. The normal saline control showed rapid clot formation, whereas EDTA completely inhibited coagulation, confirming its established anticoagulant activity. Treatment with the ethanolic extract progressively delayed clot formation as the extract concentration increased.

The increase in PT from 1 min 30 s in the negative control to 6 min 52 s at 0.250 g/mL demonstrates a substantial prolongation of clotting time. This trend indicates that the extract possesses measurable anticoagulant activity in vitro and that the effect increases with concentration over the tested range. The observed activity is consistent with the presence of flavonoids, tannins, and phenolic compounds identified during phytochemical screening, which have previously been associated with inhibition of platelet activation and modulation of coagulation pathways.

3.4 Overall Findings

The qualitative phytochemical investigation confirmed that the ethanolic seed extract of *Cyamopsis tetragonoloba* contains several bioactive secondary metabolites, including flavonoids, tannins, phenolic compounds, steroids, and saponins. These constituents provide a plausible phytochemical basis for the observed biological activity.

The Prothrombin Time assay demonstrated that the ethanolic extract prolonged clotting time in healthy human plasma compared with the negative control. A concentration-dependent increase in PT was observed across the tested extract concentrations, indicating that the extract exhibits in vitro anticoagulant activity. The strongest reported effect was obtained at 0.250 g/mL, which produced the longest clotting time among the concentrations documented in the thesis. These findings suggest that *Cyamopsis tetragonoloba* seeds may serve as a promising natural source of anticoagulant compounds and justify further studies aimed at isolating the active constituents and elucidating their mechanisms of action.

4. Discussion

The present study investigated the in vitro anticoagulant activity of the ethanolic seed extract of *Cyamopsis tetragonoloba* (L.) Taub. using the Prothrombin Time (PT) assay. The findings demonstrated that the extract significantly prolonged clotting time in healthy human plasma compared with the negative control, indicating its ability to interfere with the coagulation process. The anticoagulant effect increased progressively with increasing extract concentration, suggesting a concentration-dependent pharmacological response. These observations support the traditional medicinal use of *C. tetragonoloba* and indicate its potential as a natural source of anticoagulant compounds.

Qualitative phytochemical screening confirmed the presence of flavonoids, tannins, phenolic compounds, steroids, and saponins in the ethanolic seed extract, while alkaloids were absent. These phytochemicals have been extensively investigated for their beneficial effects on cardiovascular health. Phenolic compounds and flavonoids possess strong antioxidant activity that reduces oxidative stress and endothelial dysfunction, both of which contribute to thrombus formation. In addition, flavonoids have been reported to inhibit platelet activation and aggregation, thereby reducing thrombin generation and delaying fibrin clot formation. The phytochemical profile observed in the present study is therefore consistent with the anticoagulant activity demonstrated by the PT assay.

The Prothrombin Time assay specifically evaluates the extrinsic and common pathways of blood coagulation. Compared with the negative control, the ethanolic extract produced a progressive increase in PT from 2 min at 0.062 g/mL to 6 min 52 s at 0.250 g/mL, indicating inhibition of one or more coagulation factors involved in fibrin clot formation. The absence of clot formation in the EDTA-treated positive control validated the experimental procedure and confirmed the reliability of the assay system. These findings suggest that the extract may interfere with thrombin generation or fibrin polymerization, although the precise molecular mechanism requires further investigation.

The anticoagulant activity observed in the present investigation is in agreement with previous reports on *Cyamopsis tetragonoloba*. Singh and Devi described the plant as a rich source of pharmacologically active

phytochemicals possessing antioxidant, anti-inflammatory, antimicrobial, and anticoagulant properties. Similarly, Badugu reported abundant flavonoids and phenolic compounds in *C. tetragonoloba*, which contribute significantly to its biological activities. Other investigators have demonstrated that sulfated derivatives of guar galactomannan exhibit potent anticoagulant and antithrombotic activities in both in vitro and in vivo experimental models, supporting the hypothesis that polysaccharides and phenolic constituents collectively contribute to the anticoagulant potential of this medicinal plant.

The observed anticoagulant activity may be explained through multiple complementary mechanisms. Flavonoids are capable of suppressing platelet activation and reducing thromboxane-mediated aggregation, whereas phenolic compounds protect vascular endothelial cells by scavenging reactive oxygen species. Tannins may inhibit platelet adhesion and fibrin network formation, while saponins have been reported to influence membrane-associated signalling pathways involved in coagulation. The combined action of these phytoconstituents could account for the prolonged Prothrombin Time recorded in the present study, suggesting that the extract acts through multiple targets rather than a single coagulation factor.

Overall, the findings indicate that the ethanolic seed extract of *Cyamopsis tetragonoloba* possesses promising anticoagulant activity, which is likely attributable to the synergistic action of flavonoids, phenolic compounds, tannins, and other bioactive phytochemicals. These results provide preliminary scientific evidence supporting the traditional medicinal use of the plant and justify further studies aimed at isolating active compounds, elucidating molecular mechanisms, evaluating safety, and validating therapeutic efficacy through preclinical and clinical investigations.

5. Conclusion

The present study demonstrated that the ethanolic seed extract of *Cyamopsis tetragonoloba* (L.) Taub. possesses promising in vitro anticoagulant activity, as evidenced by the concentration-dependent prolongation of Prothrombin Time in healthy human plasma. Preliminary phytochemical screening confirmed the presence of flavonoids, tannins, phenolic compounds, steroids, and saponins, which are likely to contribute to the observed anticoagulant effect through their synergistic biological actions.

The results indicate that the ethanolic extract effectively delayed blood clot formation when compared with the negative control, suggesting its potential to modulate the extrinsic pathway of the coagulation cascade. The progressive increase in clotting time with increasing extract concentration further supports a concentration-dependent anticoagulant response under in vitro conditions. These findings provide preliminary scientific evidence supporting the traditional medicinal use of *C. tetragonoloba* and highlight its potential as a natural source of bioactive compounds for the development of plant-derived anticoagulant agents.

Although the present investigation provides encouraging evidence of anticoagulant activity, it was limited to an in vitro Prothrombin Time assay using crude ethanolic extract. Further investigations are required to isolate and characterize the active phytoconstituents responsible for the observed activity and to elucidate their molecular mechanisms of action. Future studies should include additional coagulation assays such as activated partial thromboplastin time (aPTT), thrombin time (TT), platelet aggregation studies, toxicity evaluation, animal experiments, pharmacokinetic analysis, and well-designed clinical investigations to establish the safety, efficacy, and therapeutic potential of *Cyamopsis tetragonoloba* as a novel herbal anticoagulant.

The findings of this study contribute to the growing body of evidence supporting the pharmacological importance of *Cyamopsis tetragonoloba* and provide a scientific basis for its further development as a complementary or alternative therapeutic agent for the prevention and management of thromboembolic disorders.

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The authors declare that no external funding was received for this study.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical Approval

Blood samples were collected from healthy adult volunteers after obtaining informed consent, and all experimental procedures were conducted in accordance with accepted ethical principles for biomedical research involving human participants.

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