Research Article

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# The Impact of Extract from Astragalus *membranaceus* on Blood Coagulation in Individuals with Diabetes Mellitus

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**ABSTRACT**

Diabetes-related vascular problems might result from persistent hyperglycemia, and individuals with diabetes have an increased risk of blood clot formation compared to healthy controls. Anticoagulant drugs are medications used to dissolve blood clots; various agents have been employed to improve platelet function and minimize thrombosis in these patients. Medicinal plants can be used as alternatives with potentially fewer side effects. Interestingly, *Astragalus membranaceus* (also known as Huangqi) extract was obtained for *in vitro* investigation. This study aimed to assess the anticoagulant impact of Huangqi on human plasma. The research focused on prothrombin time (PT) and activated partial thromboplastin time (aPTT) among diabetic patients. *In vitro* anticoagulant effects were evaluated using plasma treated with the extract, while DMSO served as a negative control. The results showed that the baseline PT in untreated plasma from the healthy group was 11.79 seconds, compared to 11.69 seconds in the diabetic group. A significant difference ($P<0.05$) was observed between healthy donors and diabetic patients; the mean aPTT in diabetic patients was 26.03 seconds, while in controls, it was 28.52 seconds. The present study highlighted the potent antithrombin effect of Huangqi extract at a dosage of 50 mg/ml. The PT and aPTT assays were significantly prolonged by Huangqi ($P<0.001$), demonstrating effective anticoagulant activity. This suggests that Huangqi may benefit patients who are at high risk of thrombosis.

**KEYWORDS:** Astragalus, Coagulation, Diabetes mellitus, Hemostasis

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## 1. INTRODUCTION

The most prevalent type of diabetes in Thailand is non-insulin-dependent diabetes (type 2 diabetes), which is found worldwide and continues to increase. This is due to lifestyle changes that contribute to factors leading to the disease, such as being overweight or obese, which prevent the body from effectively using glucose. There has been ongoing research for herbs that can help the body restore glucose homeostasis. In individuals with diabetes, disturbances in glucose homeostasis can lead to platelet activation and coagulation disorders. Vascular damage caused by hyperglycemia can increase the risk of thrombosis. Although the use of antiplatelet drugs (e.g., aspirin) and anticoagulant drugs (e.g., anti-Xa agents) is well established in diabetic patients, the optimal treatment strategies remain controversial. (Li et al., 2021). Research shows the benefits of Thai and Chinese herbs in treating diabetes. Key examples include the effect of *Moringa oleifera* leaves on insulin secretion in the blood and the use of Huangqi (*Astragalus membranaceus*) extract to enhance insulin sensitivity (Thippayacharoentam et al., 2022; Liu et al., 2010). Long-term consequences of type 2 diabetes encompass thrombosis, platelet hyperactivity, and systemic inflammation (Kaur et al., 2018). Traditional Chinese medicine has been used in conjunction with Western medicine to treat diabetic nephropathy in recent years. Additionally, studies have revealed that Huangqi provided therapeutic benefits for renal failure associated with diabetes (Liao et al., 2017). The roots of Astragalus contain flavonoids, saponins, polysaccharides, amino acids, and trace elements, which vary among different species (Ma et al., 2002). *Astragalus membranace*us (Huangqi) is well-known for its major benefits, which include nourishing the body, lowering excessive perspiration, promoting blood formation, reducing inflammation, and aiding in wound healing. Clinical studies have demonstrated its use in treating a variety of illnesses, including hypothyroidism, diabetic control, and coronavirus-related respiratory infections (Law et al., 2020). In addition, it has been revealed that Astragaloside IV which is a principal active component of Huangqi prevents thrombosis via binding to prothrombin, according to target analysis using a computer-docking program. Research in cultured HUVEC also demonstrates increased fibrinolysis by activating t-PA and decreasing PAI-1 (Tang et al., 2021). Although Huangqi has shown promise in decreasing blood pressure, cholesterol, blood sugar levels, proteinuria, and inducing thrombolysis (Liao et al., 2017; Sabeena et al., 2020), there is still a scarcity of thorough evidence on its side effects in human trials. The exact processes by which Huangqi improves clinical outcomes in diabetes mellitus and has immunomodulatory effects in lung disease are still not well understood. (Guo et al., 2025). Furthermore, while this herb was marketed as safe, more research is required to fully understand the long-term effects and potential interactions of the main ingredient (*Astragalus polysaccharide*), particularly in susceptible people.

Prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count are all required to assess patients with suspected abnormal bleeding. Although Boshabor (2022) discovered that diabetes mellitus had no effect on platelets, Abdulrahaman et al. (2012) reported that abnormal PT and aPTT could be found in untreated diabetic individuals. The purpose of this study is to evaluate blood coagulation levels between healthy individuals and those with diabetes, with a focus on understanding differences in clotting mechanisms. Additionally, this research investigates the effects of

**Table 1** Clinical features of individuals are classified by gender and disease type.

| Characteristics | Diabetic patients | Healthy donors |
|---|---|---|
| | Frequency (Percentage) | Frequency (Percentage) |
| **Male** | 7 (38.9%) | 9 (50.0%) |
| **Female** | 11 (61.1%) | 9 (50.0%) |
| **Age range** | 41-74 years old | 20-24 years old |
| **Frequency of exercise Everyday** | | |
| **Sometimes** | | |
| **Raely** | 2 (11.1%) | 4(22.2%) |
| | 12 (66.7%) | 9 (50.0%) |
| | 4 (22.2%) | 5 (27.8%) |
| **DM alone** | 6 (33.3%) | - |
| **DM and lipidemia** | 3 (16.7%) | - |
| **DM and kidney disease** | 2 (11.1%) | - |
| **DM and hypertension** | 4 (22.2%) | - |
| **DM, lipidemia and hypertension** | 2 (11.1%) | - |
| **DM, kidney disease andhypertension** | 1 (5.6%) | - |
| **PT (mean±S.D.)** | 11.69±1.69 sec. | 11.79±0.84 sec. |
| **aPTT (mean±S.D.)** | 26.03±2.17 sec. | 28.52±3.47 sec. |

Huangqi extract on thrombus formation in both groups.

## 2. MATERIALS AND METHODS

### *2.1 Preparation of plant extracts*

The finely ground drug, Astragalus plant material was purchased from Jew Ngek Tueng pharmacy company limited in Bangkok, Thailand. The plant sample (20 g) was weighed into a Soxhlet extractor thimble and placed in the extraction. Ethanol was used as the solvent, with a herb-to-solvent ratio of 1:15. A heating mantle was used to reflux the mixture at 78 °C for 2 hours. The extract solution was allowed to cool to room temperature, filtered using Whatman No. 1 filter paper, and concentrated to dryness using a rotary evaporator under the following conditions: vacuum in 175 mbar and water bath 40 °C for 3 hours. Once the concentrated crude extract was obtained, it was weighed and stored in an amber bottle. Finally, the vial was chilled, and a stock solution (500 mg/ml) was prepared in 40% DMSO for later use.

### *2.2 The subjects studied*

The study was conducted in accordance with approval by the Human Research Ethics Committee, Huachiew Chalermprakiet University (Approval number HCU-EC1497/2567). The donors were recruited with the following criteria.

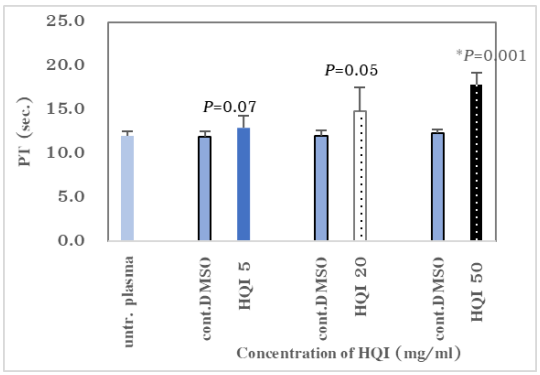
(a) Healthy subjects were people aged 19-22 years old who were studying at Huachiew Chalermprakiet University. The inclusion criteria included being free from disease, not suffering from any noncommunicable diseases (NCDs), having no history of coagulation disorders, and no recent use of medication.

(b) Diabetic patients (DM) aged 40-75 years old who were receiving treatment from doctors at Samutsakhon Hospital in Thailand were recruited for this study.

Exclusion criteria included donors taking medications that affect the blood system, such as warfarin, aspirin, and oral contraceptives, as well as those who smoke or consume alcohol regularly.

The sample size of 36 participants, comprising 18 healthy individuals and 18 diabetic patients, was

1A




1B

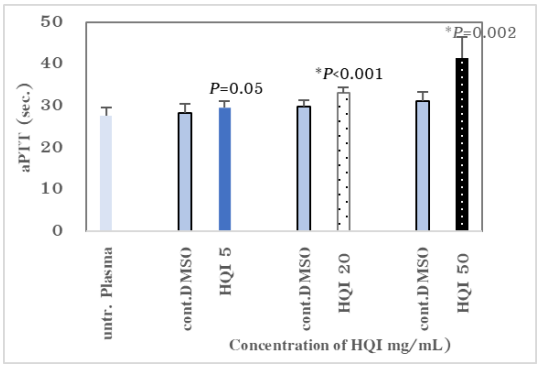



**Figure 1** In-vitro study of anticoagulant effects (PT: 1A, aPTT 1B) of Huangqi extracts at various concentration (N=5; single experiment). In each condition, the untreated PT plasma was not significantly different from the control DMSO. Notably, when compared to untreated plasma, DMSO in plasma had a minor effect on clotting time but no statistically significant difference (P>0.05). Both PT and aPTT indicated prolongation when treated with Huangqi 20 and 50

determined based on a priori power analysis using G*Power software (version 3.1.9.7).

### *2.3 Coagulation assay and control test*

To evaluate the antithrombogenicity of this extract, PT and aPTT were measured by a semi-automated blood coagulation analyzer CA-50 (Sysmex Corp., Kobe, Japan) as follows (Xiang, 2014). First, trisodium citrate blood was centrifuged at 3,500 rpm for 15 minutes to obtain platelet-poor plasma (PPP). Next, a sample of 50 ⌈L plasma was placed in a cuvette and incubated at 37 ℃ for 3 minutes. After that, 100 ⌈L tissue thromboplastin (Thromborel S, Siemens Healthcare Diagnostics GmbH, Germany) was added, and finally the PT was determined.

The 50 ⌈L aPTT reagent (Actin FS, Siemens Healthcare Diagnostics GmbH, Germany) was added into 50 ⌈L plasma and incubated for an additional 3 minutes. $CaCl_2$ (0.025 M) was then added to initiate the coagulation cascade, and the aPTT was measured as the time required to form a fibrin clot.

To ensure that the semi-automated blood coagulation analyzer was functioning properly, Ci-Trol control plasma (Siemens Healthcare Diagnostics GmbH, Germany) was tested as part of the experimental design. Our laboratory has normal ranges for PT and aPTT, as 11.95±1.25 seconds and 26.62±2.19 seconds respectively. To serve as a positive control, commercial heparin was acquired from Troikaa (India) and diluted in distilled water before being added to plasma (final concentration 4.5 ⌈g/ml). The test tubes were prepared with the following conditions: 50 μL of untreated plasma, 40% DMSO (5 μL of DMSO plus 45 μL of plasma), and 50 mg/ml Huangqi (5 μL of Huangqi stock plus 45 μL of plasma).

### *2.4 Statistical analysis*

All values are expressed as mean ± standard deviation for all experiments. Statistical comparisons were made using the IBM SPSS statistics 30.0 (Student version).

The analysis was conducted under the assumption of a two-tailed paired t-test with an alpha level of 0.05 and a desired statistical power of 0.90. This high level of power was selected to ensure a strong potential for detecting true effects, thereby minimizing the risk of Type II errors. Based on these parameters and an expected medium effect size (Cohen's *dz* = 0.6), the required sample size was calculated to be 32 participants. The actual sample size used in this study (n = 36) exceeded this requirement, further enhancing the reliability and robustness of the statistical conclusions.

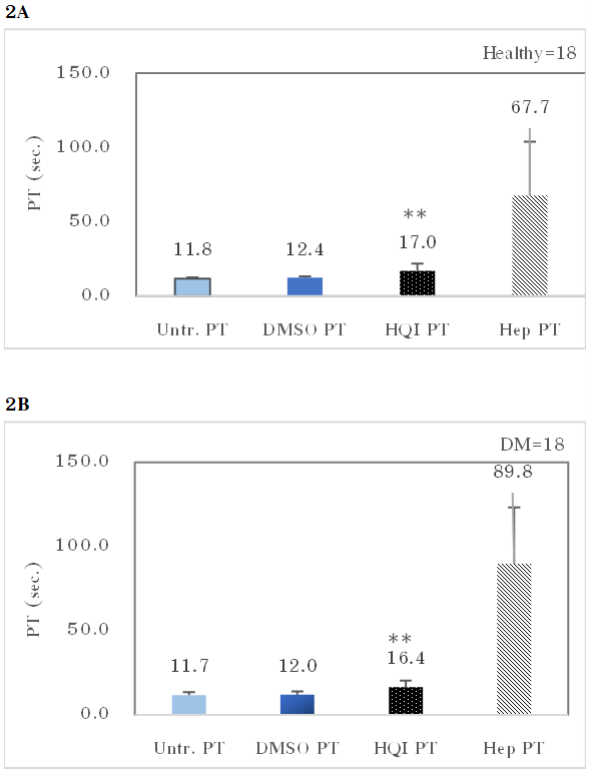



**Figure 2** Effect of Huangqi extracts (50 mg/ml) on prothrombin time in human plasma. (2A): Plasma conditions of healthy donors, (2B): Plasma conditions of diabetics subjects. A paired t-test revealed a significant difference (**P<0.001) between Huangqi and the DMSO tube. As calculated, the proportion of increment prolongation was 27.1% in HQI-treated healthy plasma and 26.8% in HQI-treated DM plasma.

## 3. RESULTS AND DISCUSSION

The results of the history-taking in a sample group of healthy individuals, from a total of 18 respondents, as shown in Table 1, revealed the following: there were 9 females and 9 males. The average age was 21.22±0.81 years. The diabetic group also consisted of 18 participants, including 11 females and 7 males, with an average age of 55.39 ± 9.68 years. This study included diabetic patients who were receiving modern medical treatment. Furthermore, some patients had comorbidities such as hypertension, hyperlipidemia, and kidney disease.

### *3.1 Comparison of PT and aPTT between patients and healthy controls*

An independent t-test was used to assess the mean difference in PT and aPTT values between diabetic and healthy groups. Baseline PT and aPTT levels in samples are shown in table 1. For PT, the difference between diabetic group and healthy donors was not significant ($P>0.05$). However, our study showed that diabetic patients had significantly lower aPTT scores than healthy individuals ($P < 0.05$). Notably, shortened aPTT levels may be associated with coagulopathy in diabetes (mean aPTT in healthy individuals =28.52 seconds; DM=26.03 seconds).

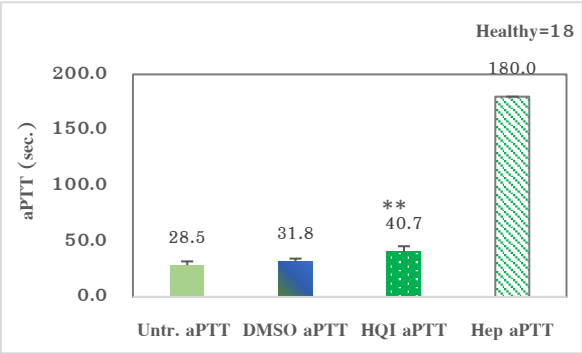
### *3.2 Optimal dosage of Huangqi extract for coagulation tests*

Preliminary experiments were conducted in five healthy donors to determine the optimal Huangqi concentration for use in our study. The baseline mean for PT value (12.0±0.5 seconds) and aPTT value (27.7±2.0 seconds) of five donors were indicated as untreated plasma (Figure 1). Huangqi extract showed prolonged PT in a dose dependent manner (concentration 5-50 mg/ml). As a negative control, plasma treatment with DMSO resulted in PT values of 11.9, 12.0, and 12.3 seconds. Huangqi treated plasma at various doses increased PT from 12.9 to 14.8 and 17.8 seconds, respectively (Figure 1A). The extract also showed concentration dependent anticoagulant properties as assayed by aPTT. The aPTT in extract-treated plasma at doses ranging from 5 mg to 50 mg/ml increased to 29.6, 33.2, and 41.5 seconds, whereas in the DMSO control it was approximately 28.4, 29.8, and 31.2 seconds (Figure 1B). Bars marked with an asterisk (HQI) showed significant difference compared to 40% DMSO (cont.DMSO) Here, we selected a dose of 50 mg/ml Huangqi extract as a test and used heparin 4.5 ⌈g/ml as a standard drug for comparison.

### *3.3 Effect of Huangqi extract on coagulation test*

In a single experiment, the coagulation test was performed on each sample. The baseline values of

3A




3B

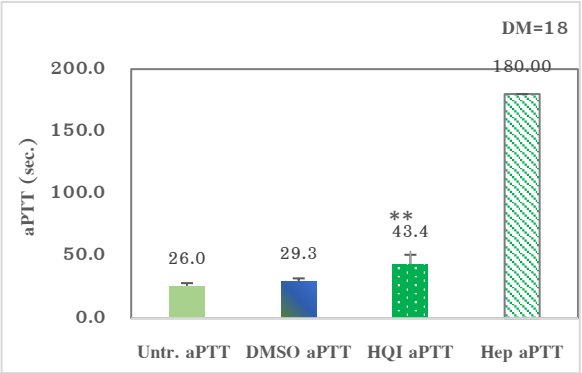



**Figure 3** Effect of Huangqi extract (50 mg/ml) on activated partial thromboplastin time in human plasma. (3A): Plasma conditions of healthy donors, (3B): Plasma conditions of diabetics subjects. Here, all condition tubes were tested in one replicate. A paired t-test was used to compare the tube containing Huangqi with the DMSO treated plasma (**$P$<0.0001). The calculated percentage of increment prolongation in HQI-treated healthy plasma was 21.9%, while in HQI-treated DM plasma,

PT and aPTT were measured as untreated plasma (plasma alone). DMSO-treated plasma was used as a negative control. In addition, plasma was treated with heparin as a positive control.

**3.3.1 Anticoagulant effect of Huangqi extract on partial thromboplastin time**

At a dose of 50 mg/mL, significant increase in PT was observed both in normal and patients (Figure 2). Huangqi extract markedly increased the PT compared to the control DMSO. (2A: **P<0.001, 2B: **P<0.001).

**3.3.2 Anticoagulant effect of Huangqi extract on activated partial thromboplastin time**

Anticoagulation potential of 50 mg/ml Huangqi was observed *in vitro* using the aPTT test(Figure 3). Huangqi significantly increased compared to the DMSO control (Figure 3A: **$P$<0.001, 3B: **$P$<0.001).

As illustrated by figures 2 and 3, in the healthy group, plasma treatment with Huangqi (50 mg/ml) raised PT and aPTT from baseline values by 44.1% and 42.8%, respectively. Overall, the extract prolonged PT and aPTT in the patient group by 40.0% and 66.9%, respectively.

Prolongation of PT and aPTT revealed that Huangqi inhibits the extrinsic pathway (coagulation factor VIIa) and the intrinsic pathway (coagulation factors XIIa, XIa, IXa, and VIIa), respectively. This plant extract may also suppress other coagulation cascades in the common pathway, comprising factor Xa, thrombin, and fibrin (Tripatara and Chumpia, 2004).

## 4. DISCUSSION

Our work clearly demonstrated the prolongation of clot formation via both the intrinsic and extrinsic pathways by Huangqi extract. Figure 2 shows that the percentage increase in PT prolongation was 26.8% in HQI-treated DM plasma and 27.1% in HQI-treated healthy plasma. Accordingly, as shown in Figure 3, percentage increase in aPTT prolongation in HQI-treated healthy plasma was 21.9%, whereas it was 32.5% in HQI-treated DM plasma. This recent study supports the findings of Liu et al. (2025), which indicated that Huangqi, in combination with Honghua (Carthamus tinctorius), may have anti-thrombotic properties by promoting the removal of blood stasis in ischemic stroke rats. In the same way, a previous study by Mazhar et al., (2020) revealed that extract from Astragalus genus (*A. sarcocolla*) exhibited in vitro anticoagulant activity, potentially paving the way which could combat cardiovascular diseases.

The main components of the Astragalus genus include flavonoids and saponins (Li et al., 2014).

*Astragalus sarcocolla* was noted for its antiplatelet and anticoagulant properties (Mazhar et al., 2022). Our research has validated the *in vitro* antithrombotic potential of the Astragalus plant. Therefore, many Astragalus species have been widely used in traditional medicine for their antiviral, antioxidant, and cardioprotective properties, as well as in preventing typical consequences from diabetes, such as damage to the kidneys, blood vessels, and nerves. (Danthaiwattana et al., 2021; Law et al., 2020). Astragaloside IV (AS-IV), one of the major components of the aqueous extract of Astragalus membranaceus, has been reviewed for its pharmacological effects, including neuroprotection, liver protection, anti-cancer, and anti-diabetic properties (Zhang et al., 2020). Hence, Cai et al. (2025) identified AS-IV as a potent inhibitor of von Willebrand factor-binding protein, which is involved in prothrombin activation and disrupts the coagulation factor cascade. Therefore, AS-IV effectively prolongs coagulation time and reduces the virulence of Staphylococcus aureus, a virulent bacterium that evades host immune responses. These findings are relevant to our study, which confirms the effect of Huangqi extract on the prolongation of PT and aPTT.

Primary hemostasis (platelet plug formation) and secondary hemostasis (fibrin clot formation) are two essential steps in the blood clotting process. Subsequently, thrombotic blockages are dissolved through fibrinolysis (Tripatara and Chumpia, 2004). The fibrinolytic activity of Huangqi was also tested using the euglobulin lysis time and clot lysis assays in healthy blood (data not shown). As anticipated, Huangqi-treated samples showed increased fibrin clot lysis and mild hemolysis in clotted blood, which aligned with the findings of Kim (2012) and Sabeena et al. (2022), respectively. Nevertheless, the methods described above are rarely used for routine analysis in hematology laboratories.

Due to the limited volume of blood available for testing, this study was unable to perform fibrinolysis tests in diabetic individuals. This limitation reduces the scope of the findings, as including diabetic subjects could have provided more information about the thrombolytic efficacy of Huangqi extract in a group at risk of coagulation problems. Future studies with larger sample sizes and duplicate experiments are recommended to allow a more thorough investigation across various patient groups. Here, we suggest that testing blood clotting time and performing a clot lysis test using a modified method, as previously described by Janwitayanuchit I. *et al*. (2024), could serve as an alternative approach.

Aspirin is a cornerstone of antiplatelet therapy for cardiovascular thrombotic disorders. As a result, many patients on long-term aspirin therapy are required to co-administer gastric-protective agents or switch to alternative anti-platelet drugs (Chen et al., 2025) Our findings suggest that Huangqi extract could serve as a promising therapeutic option for alleviating the side effects of patients with long-term aspirin use. However, further investigation is needed to determine the optimal dose that balances antiplatelet and antithrombotic effects. Maschirow et al. (2015) discovered a link between inflammation and coagulation during the asymptomatic stage of diabetes. To the best of our knowledge, this study demonstrates the potential of Huangqi for long-term disease prevention, particularly in preventing endothelial impairment associated with diabetes, which can lead to prothrombotic events. Importantly, Huangqi should be used in conjunction with conventional medical treatments under the guidance of healthcare

professionals to minimize potential risks associated with herbal use.

## 5. CONCLUSION

This investigation showed significant effects on coagulation *in vitro*, with Huangqi extract (at a concentration 50 mg/ml) able to prolong PT and aPTT. By considering the above characteristics, the study discovered Huangqi's potential therapeutic effects on blood coagulation, which could lead to new approaches for managing thrombosis in high-risk groups. However, further studies on the toxicity of the crude ethanol extract of the herb should be conducted to ensure that the active compounds derived from the Huangqi herb can safely reduce blood clotting in diabetic patients with vascular complications.

Differences in growing conditions, harvesting schedules, and processing techniques could result in variations in the Huangqi extract. These differences may impact the concentration of active ingredients such as saponins and polysaccharides, which may impact the herb's efficacy. Overall, Huangqi appears to have few negative effects when taken appropriately; nonetheless, excessive doses may interfere with immunosuppressive medications.

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