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The Effect of Moringa leaves Extract on Coagulation Profile (PT, INR, and aPTT) Ex-vivo in High-Risk Individuals of Getting Blood Clot in Sudanese Living in Egypt

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Abstract

Background: Blood clotting is a critical process in hemostasis. Traditionally, the management of blood clotting has relied on pharmaceutical treatments. However, in recent years, herbal medicine has gained attention particularly in the field of coagulation. Moringa Oleifera, known for its rich nutritional profile and various health benefits, is being investigated for its potential anticoagulant properties. This study explores the ex-vivo anticoagulant effect of Moringa extract; aiming to prevent blood clot formation, especially in individuals with high-risk factors.
Method: This study was an in vitro experimental study, conducted at Nasr City, Cairo, Egypt from January to April 2025, using blood samples collected from individuals who had a high risk of developing blood clots, such as hypertension, diabetes, or obesity. The aqueous extract of Moringa Oleifera was prepared and applied to the collected samples. A coagulation profile (PT, INR and aPTT) was performed to assess the anticoagulant activity of the extract. To evaluate any change in clotting function compared to control samples.

Result: The results showed a significant prolongation in Prothrombin time and Partial Thromboplastin Time in plasma samples treated with Moringa Oleifera extract in different concentrations compared to the control ($P < 0.05$). The anticoagulant activity increased with higher extract concentrations.

Conclusion: The study concluded that Moringa Oleifera has concentration-dependent anticoagulant effects in vitro. These findings suggest that it may serve as a potential natural anticoagulant,

recommending further in vivo and clinical investigations

Key words: PT-PTT- Moringa Oleifera - Anticoagulant - Coagulation Profile

Introduction

Herbal medicine, also known as botanical medicine or phytomedicine, involves the use of plants and plant extract to treat various health conditions and promote overall well-being [1].

Moringa Oleifera is classified as an important herbal plant due to its immense medical and non-medical benefits. More than one hundred compounds from different parts of Moringa Oleifera have been characterized, including alkaloids, flavonoids, anthraquinones, vitamins, glycosides, and terpenes [2]. Moringa Oleifera is a highly nutritious plant containing a wide array of bio-active phytochemicals. The leaves are especially rich in vitamins (A, C and E), minerals (calcium, potassium, iron and magnesium) and essential amino acids [3].

Moringa contains numerous pharmacologically active compounds, including: Flavonoids (quercetin, kaempferol) known for their antioxidant and anti-inflammatory properties [4].

Phenolic acids contribute to free radical scavenging activity. Alkaloids and tannins are involved in antimicrobial and anti-parasitic activities. Saponins are linked to cholesterol-lowering effects, while Glucosinolates and isothiocyanates have been studied for their anticancer and detoxifying [5]

The coagulation is mediated by a complex enzymatic cascade known as coagulation cascade, which involves the sequential activation of specific plasma proteins called coagulation factors. These factors are usually present in an inactive form and are activated step-by-step to form a stable fibrin clot [6] .Prothrombin time is used to evaluate the extrinsic and common pathways of the coagulation cascade by measuring how long it takes blood to clot. It helps detect deficiencies in clotting factors such as I, II, V, VII, and X. The International Normalized Ratio (INR) is a standardized expression of the PT result, allowing comparison between laboratories. INR is essential for monitoring patients on oral anticoagulants like warfarin, ensuring effective and safe coagulation [7].APTT evaluates the intrinsic and common pathways of coagulation. It helps detect deficiencies or inhibitors of factors VIII, IX, XI, and XII. APTT is important in monitoring patients receiving heparin therapy and in diagnosing bleeding disorders like hemophilia [8].

Literature review

Several studies highlight moringa's anticoagulant properties. Adedapo et al.

(2016) reported that aqueous leaf extracts exhibited mild anticoagulant activity in rats [9]. Similarly,

Adisakwattana et al. (2012) found that flavonoids like quercetin in moringa inhibit platelet aggregation [10]. Bhattacharya et al. (2011) demonstrated fibrinogenolytic and fibrinolytic

effects in leaf and root extracts [11], while Gurib-Fakim et al. (2007) observed suppression of ADP-, collagen-, and epinephrine-induced platelet aggregation in vitro [12]. Additionally, Monera & Maponga (2010) and Olorunfemi et al. (2019) noted reduced fibrinogen levels in animal models [13, 14].Conversely, some studies suggest moringa may promote clotting under certain conditions. Arise et al. (2016) found that specific extracts could have procoagulant effects depending on solvent type and concentration [15]. The plant's high vitamin K content (Olayaki et al., 2015) may also enhance coagulation [16]. Mohammed et al. (2019) reported mixed outcomes, with increased platelet counts and prothrombin time but decreased partial thromboplastin time [17].

While preclinical studies indicate moringa's influence on coagulation, human trials remain limited. The variability in results underscores the need for further in vitro and clinical research to clarify its mechanisms and potential therapeutic and preventive application. Blood clots are major health concern due to their serious complications such as stroke, pulmonary embolism, and heart attacks. Despite the availability of pharmaceutical anticoagulants, their side effects and the need for close monitoring make them less suitable for long-term or preventive use in some cases. In recent years, there has been growing interest in herbal medicine as a safer and more natural alternative. *Moringa Oleifera,* known for its rich nutritional and medical properties, is widely used in traditional medicine. Some studies suggest that it may affect blood coagulation, but scientific evidence remains limited and sometimes contradictory.

This study aims to explore the effect of moringa on blood clotting in vitro, contributing to a better understanding of its potential role as a natural agent in managing coagulation related disorders. The research is also driven by personal interest in supporting the use of herbal medicine in Sudan, where it can provide an affordable and accessible health solution for many people

Objective

To assess the In Vitro effect of *Moringa Oleifera* extract on blood coagulation, aiming to understand how it prevents the individuals with risk factors from getting blood clot.

Methodology:

An in vitro experimental study. Nasr City, Cairo, Egypt. From January to April 2025. Samples were collected from individuals who have at least one or more of the risk factors for blood clot formation (hypertension, diabetes mellitus, obesity, family history or smoking) with age range from 25-65 years. Exclusion criteria include individuals on anticoagulant therapy or diagnosed with bleeding disorders. A total of 30 sample was collected; according to available time period. Sampling 1.8 ml of blood collected in sodium citrate container from individuals with inclusion criteria.

About 25g of clean, dried moringa leave extract were taken and grind into a fine powder using a grinder. This powder was mixed with distilled water in a 1:20 ratio. The mixer was soaked for 24 hours at room temperature. After that the mixer was filtered using Whatman No.1 filter paper to remove leave residues. Then centrifuged at 3000 rpm for 10 minutes to get a clearer superannuate. Different dilutions were prepared (15%- 25%- 50%) using distilled water

Collected blood samples were placed immediately into an ice bag to maintain low temperature and

preserve sample integrity. The samples were stored in the ice bag for approximately 4 hours until they were transported to the laboratory for further processing and coagulation testing .The collected samples were centrifuged at 3000 rpm for 10 minutes to separate platelet poor plasma (PPP). The plasma was then carefully transferred into clean, labeled plain centrifuge tubes (Eppendorf tubes) .
For testing the separated plasma was mixed with moringa extract of different concentrations at 1:1 ratio (150µ of plasma with 150µ of diluted extraction) and then used immediately for coagulation analysis. A control group was prepared by mixing 150µ of plasma with 150µ of distilled water instead of moringa extract.
The mixture was tested for clotting time using a coagulation analyzer. Both Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) were performed using appropriate reagents according to the manufacturer's instructions. Internal quality control procedures were followed throughout the testing process. The coagulation analyzer was calibrated according to the manufacturer's guidelines before use, and control samples were run alongside the test samples to ensure the accuracy and reliability of the results.
Data had been reported as mean ±SD, independent T test had been used to compare different concentration of extracts with control and also to calculate P value.
This study has been approved by the faculty of medical laboratory sciences. Al-Zaim Al-Azhari University. The purpose of this study had been classified and discussed with the participants. Informed consent was obtained from all participants before blood samples were collected

Results

The result of this study reflects the effect of various concentrations (15%, 25%, and 50%) of aqueous Moringa Oleifera extraction on prothrombin time, INR and PTT in 30 platelet-poor plasma of individuals with risk factors of getting blood clots, to detect its potential anticoagulant properties. Shapiro-wilk test was used to define normal data distribution. Only concentration 50% group follow the normal, but both concentration 15% and 25% group doesn't follow the normal distribution. So, Wilcoxon Signed Rank Test was used to compare between the medians of groups and calculated P-value.
The median of PT (50% aqueous extraction) showed highest prolongation (58.25 sec.) when compared with control group (15.95 sec.) and PT (extraction 15%, 25%), which
recorded (22.30 sec. and 52.50 sec.) respectively. That mean the prolongation of PT is increased when an aqueous extraction concentration is increased.
The median of INR (50% aqueous extraction) showed highest level (4.53) when compared with control group (1.24) and other INR (extraction 15%, 25%), which recorded 1.72 and 4.11 respectively. That indicate the increase of INR is also increased when an aqueous extraction concentration is increased.
The *P value* was < 0.01 for all comparisons between the median of control group and the different concentrations, so we conclude that there is significant difference between them.

able 4.1 shows the PT and INR significant difference between the control group and the extraction groups with P-value less than 0.05.

PT					**INR**				
(I)	(II)	Median (I)	Median (II)	P	(I)	(II)	Median (I)	Median (II)	P
Control	Conc15%	15.95	22.30	< 0.01	Control	Conc. 15%	1.24	1.72	< 0.01
	Conc 25%		52.20	< 0.01		Conc. 25%		4.11	< 0.01
	Conc50%		58.25	< 0.01		Conc. 50%		4.53	< 0.01

The **P value** was < 0.01 for all comparisons between the medians of the groups with different concentrations. That mean there is a **significant difference** in PT and INR

Table 4.2 shows the PT and INR median significant difference among different concentration of aqueous extraction groups

PT					**INR**				
(I)	(II)	Median (I)	Median (II)	P value	(I)	(II)	Median (I)	Median (II)	P
Conc 50%	Conc25%	22.30	85.50%	< 0.01	Conc. 50%	Conc 25%	4.53	4.11	< 0.01
Conc 50%	Conc15%	22.30	83.00%	< 0.01	Conc. 50%	Conc 15%	4.53	1.72	< 0.01
Conc 25%	Conc15%	85.50%	83.00%	< 0.01	Conc. 25%	Conc 15%	4.11	1.72	< 0.01

In PTT, all groups follow the normal distribution when test by using Shapiro-wilk test. So, The Paired Sample T-Test was used to compered between the means of groups. The mean of PTT (50% aqueous extraction) showed highest prolongation (82.05 sec.) when
compared with control group (41.24 sec.) and other PTT (extraction 15%, 25%), which recorded (51.35 sec. and 68.87sec.) respectively. That mean the

prolongation of PTT is increased when an aqueous extraction concentration is increased.

The *P value* was < 0.01 for all comparisons between the means of control group and the different concentrations, so we conclude that there is significant difference between them.

Table 4.3 shows the PTT significant difference between the control group and the extraction groups with P-value less than 0.05.

PTT				
(I)	(II)	Mean (I)	Mean (II)	P
Control	Conc. 15%	41.24	51.35	0.00
	Conc. 25%		68.87	0.00
	Conc. 50%		82.05	0.00

The P value was < 0.01 for all comparisons between the means of the groups with different concentrations. That mean there is a significant difference in PTT.

Table 4.4 shows the PTT means significant difference among different concentration of aqueous extraction groups

PTT				
(I)	(II)	Mean (I)	Mean (II)	P value
Conc. 50%	Conc. 25%	82.05	68.87	0.00
Conc. 50%	Conc. 15%	82.05	51.35	0.00
Conc. 25%	Conc. 15%	68.87	51.35	0.00

Discussion :

Moringa Oliefera, which has shown various pharmacological properties including antioxidant, anti-inflammatory, and possible anticoagulant effects.

This in vitro study was conducted on 30 blood samples obtained from Sudanese individual at high risk of getting blood clot, to assess the anticoagulant effect of Moringa Oliefera aqueous extract. The result demonstrated a significant increase in clotting time for PT, INR and PTT in plasma treated with different concentration of moringa extract (15%, 25%, 50%), compared to the control group (P value < 0.05). this indicates that Moringa Oliefera has components that may interfere with coagulation pathways, leading to delay clot formation.

The findings of this study, which demonstrated a prolongation in PT, INR, and PTT

following treatment with Moringa extract, is agreed with several previous studies that reports
anticoagulant properties of Moringa. Adedapo et. Al (2016) and Adisakwattana et. al (2012) mentioned the ability of Moringa aqueous extracts and its flavonoid components to inhibit platelet aggregation and delay clot formation [9,10]. Also, Bhattachrya et. al (2011) and Olorunfemi et. al (2019) reported fibrinolytic and anticoagulant effects in both leaf and roots extracts [11,14]. Conversely, it contradicts studies such as Arise et al. (2016) and Mohammed et al. (2019), which observed procoagulant or mixed effects of moringa, including shortness clotting time and increased platelet counts [15,17]. These differences may be due to extraction methods (alcoholic vs. aqueous), concentrations used, or type of biological models (animals vs. human plasma). Overall, these founding support the hypothesis that Moringa has anticoagulant properties under certain experimental conditions by measuring Prothrombin Time, INR, and Partial Thromboplastin Time, which were prolonged when compared with control and the increase of prolongation depend on increase of extraction concentration.

Conclusion

This study demonstrates that the aqueous extract of Moringa Oliefera has a potential anticoagulant effect tested in vitro on human plasm. A significant prolongation in both Prothrombin Time (PT), International standardized Ratio (INR), and Partial Thromboplastin Time (PTT) was observed across the tested concentrations.
The anticoagulant activity was found to be concentration-dependent, with higher concentrations producing longer clotting times.

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