Sudan University of Science and Technology

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Effect of Fenugreek Aqueous Extract on Prothrombin Time in the Blood of Healthy Adult Volunteers

تاثير المستخلص المائي للحلبة على زمن البروثرومبين في دم متطوعين بالغين أصحاء

Dissertation submitted in a partial fulfillment of the requirements for the award of the degree of M.Sc. in Hematology and Immunohematology

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بسم الله الرحمن الرحيم

الآيـــــــة

قَالَ تَعَالَىٰ:

﴿ قُلْ إِنَّ صَلَاتِي وَنُسُكِي وَمَحْيَايَ وَمَمَاتِي لِلَّهِ رَبِّ الْعَالَمِينَ ﴿١٦٢﴾ ﴾

صدق الله العظيم

سورة الأنعام: الآية (162)

Dedication

To…my lovely mother and father

To…my dears sisters and brothers

To …my soulmate Waad Ismail

To…my best friend and partner Samah

To…all friends and people who help and support me

To….my self Maymona

I dedicate this work…

Acknowledgment

First of all I thank **Allah** for giving me the strength and
Thanking you is not just enough to express the gratitude that
should be bestowed upon you.
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thanks for **anybody** help or try to help ,support and stand with
me thanks from the bottom of my heart

My thank also to my **friends** and my **teacher**
extended to **all people** whom the blood samples has been collected from.

Abstract

Haemostasis is the process of forming clots in the walls of damaged blood vessels to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. Fenugreek is largely universal staple herb, popular throughout history and it has been consumed for treatment of different disorders.

This study aimed to examine the effect of Fenugreek aqueous extract on Prothrombin Time among Adult Healthy Volunteers.

Total Fifty (50) healthy adult volunteers in this study; 22 of them were females and 28 were males; age ranged between 21 and 30 years. In vitro anticoagulant effects of Fenugreek aqueous extract (5%) in different volumes (25, 50 and 75 µL) were examined in the blood samples of normal individuals by measuring prothrombin time (PT).

The result show that mean of Prothrombin time level was found significantly higher in the samples collected after addition of fenugreek aqueous extract (25,50 and 75%) than that of samples collected before addition of extract (Mean±SD:18.04 (±1.26), 24.34(±2.54),45.67 (±6.61) and 13.60 (±1.12) respectively, *P.value* 0.000).

No statistically significant difference was found in the mean of Prothrombin level between females and males in both pre- and post-fenugreek aqueous extract (50and75%) samples (*p.value*= 0.769, 0.345 and 0.107 respectively) except on 25% of fenugreek extract is show significant different between male and female with (p.value= 0.024)

No significant difference when we compared mean of prothrombine time level between aged groups (21-25, 26-30) in both pre-and post fenugreek extracts(25,50.75%) (p.value=0.919, 0.863, 0.153 and 0.371.

This study was concluded that the prothrombin time affected when adding fenugreek aqueous extract among healthy volunteers samples.

مستخلص البحث

التخثر هو عملية تكوين جلطات في جدران الأوعية الدموية التالفة لمنع النزيف غير الطبيعي وللحفاظ على الدم داخل الأوعية الدموية في حالة سيولة.

الحلبة إلى حد كبير نبات عالمي ، و عرفت عبر التاريخ كعلاج لأمراض مختلفة.

تهدف هذه الدراسة إلى معرفة تأثير الحلبة على زمن البروثرومبين بين المتطوعين البالغين الأصحاء.

تم جمع 50 متطوعًا بالغًا سليمًا في هذه الدراسة ؛ 22 منهم من الإناث و 28 من الذكور. تراوحت الأعمار ما بين 21 و 30 سنة. تم فحص التأثيرات المضادة للتخثر في المختبر لمستخلصات الحلبة المائية (5٪) بتراكيز مختلفة (25 ، 50 و 75ميكرولتر) في عينات دم الأفراد الطبيعيين عن طريق قياس زمن البروثرومبين .

وجد أن متوسط مستوى زمن البروثرومبين اعلى بكثير في العينات التي تم جمعها بعد اضافة مستخلص الحلبة(25,50,75) حيث ان المتوسط (18.04 ،24.34، 45.67) على التوالي، من العينات التي تم جمعها قبل اضافة المستخلص حيث ان المتوسط (13.60) القيمة الاحصائية(0.000).

خلصت النتائج على وجود فروق ذات دلالة احصائية في متوسط مستوى البروثرومبين بين الاناث والذكور في عينات ما قبل وبعد اضافة الحلبة (50,75) القيمة الاحصائية (0.769، 0.345، 0.107) على التوالي باستثناء (25) مايكروليتر من مستخلص الحلبة تظهر فروق ذات دلالة احصائية بين الذكور والاناث مع قيمة احصائية تساوي (0.024).

لم يتم العثور على فرق احصائي عند مقارنة متوسط مستوى وقت البروثرومبين بين الفئات العمرية في المستخلصات ما قبل وبعد الحلبة (25,50.75) القيمة الاحصائية لها (0.919، 0.863، 0.153، 0.371) .

خلصت هذه الدراسة إلى أن زمن البروثرومبين يتأثر عند إضافة مستخلص الحلبة المائي إلى عينات المتطوعين الأصحاء.

List Of Abbreviations:

Abbreviation	Full Name
ADP	Adinin Diposphat
Ag	Antigen
APTT	Activated Partial Thromboplastin Time
BK	Brady Kinin
Ca2++	Ionized Calcium
CAMP	Cyclic Adenosine Monophosphate
CGMP	Cyclic Guanosine Monophosphate
CR	Clot Retraction
CT	Clotting Time
DDAVP	1-deamieno-8-D-arginine Vasopressin
ECs	Endothelial Cells
EDRF	Endothelium Derived Relaxing Factor
GP	Glycoproteins
HMWK	High Molecular Weight Kininogen
INR	International Normalize Ratio
ISI	International Sensitivity Index
MW	Molecular Weight
PC	Platelet Count
PT	Prothrombin Time
PPP	Platelet Poor Plasma
SPSS	Statistical Package for Social Sciences
TF	Tissue Factor
TFG	Fenugreek Trigonella foenum-graecum
TFPI	Tissue Factor Pathway Inhibitor
tPA	Tissue-Plasminogen Activator
TXA2	Thromboxane A2
uPA	Urokinase- Plasminogen Activator
'VWF	Von Willebnllld Factor

List of Contents

List of Table:

List of figure

Chapter I

Introduction

Chapter I

Introduction

1.1.Introduction :

Homeostasis is an interaction process between coagulation and anticoagulants that involves a complex interrelated systemic mechanism that maintains blood within the injured vascular system (Sirridge and Shannon, 1993).

Normal hemostasis is influenced by local factors in various organs and depends on the interactive hemostatic response to vascular damage between the blood vessel wall, circulating platelets, coagulation factors, coagulation inhibitors and fibrolytic agents.

To prevent the loss of blood from damaged blood vessels which is essential for life (Gelton and Hall, 2006). The relative importance of the hemostatic cascade depends on the type of vessel (arterial, venous or capillary) that has been injured (Rang et al., 2007).

The prothrombin time test (PT test) is a useful screening procedure for external coagulation mechanisms, including the common pathway. It detects deficiencies in factors II, V, VII and X. Prothrombin time tests are often used to follow oral anticoagulant therapy that inhibit factors II, VII, IX and X. Thromboplastin activates the extrinsic coagulation system in plasma in the presence of calcium. Ions. The time of subsequent clotting depends on the concentration offectors II, V, VII and X. Thus prolongation indicates a decrease in one or more of these factors. (Hoffbrand and Moss, 2008)

Normal PT is 11– 15 seconds and normal amounts of clotting factors VII and X whereas prolonged increases in prothrombin time are considered abnormal (Saxena et al., 2007).

Clotting factor II, or prothrombin, is a vitamin K-dependent proenzyme that acts in the blood coagulation cascade. Factor II deficiency is a rare, inherited or bleeding disorder. many

Specific outbreak mutations of the prothrombin gene have been documented (Akhavan et al., 2000).

Many drugs are used in hemostatic disorders including anticoagulants, fibrinolytics (thrombolytics), and antiplatelet. Warfarin and heparin are the most commonly used anticoagulant agents and bleeding is their major side effect. Also non-steroidal anti-inflammatory drugs (NSAIDs), especially aspirin, have the potential for anti-platelet activity (Hoffbrand et al., 2006).

Fenugreek is available as a dried, ripe seed and its extract is used as an artificial flavoring for maple syrup. The seeds contain 0.1 to 0.9% diosgenin and several coupemarin compounds have been noted in the seeds as well as several alkaloids such as trichonelin, gentianin, and carapine. Seeds also contain oil containing about 8% mildew. Fenugreek has been noted to reduce plasma cholesterol in animals when they contain 50% fenugreek seeds in their diet,

which can be attributed to its high fiber content, although it may be due to the steroid saponin . A hypoglycemic effect of fenugreek has also been reported.

When fenugreek is applied as an odor of maple syrup through urine and perspiration, fenugreek has limited toxicity, but generally the unpleasant effects of fenugreek have not been reported. It is generally considered and listed as a safe herb in the United States (Lambert and Cormier 2001). This study is an initial attempt to evaluate the in vitro anticoagulant effect of aqueous extracts of fenugreek in blood samples of normal individuals by measuring PT.

1.2.Rationale:

Prothrombin time a test that is done to gauge the integrity of part of the blood clotting process. Is commonly used to screen for bleeding disorders as well as to monitor the anticoagulant such are warfarin.

Although fenugreek is more frequently known to have beneficial effects on the human health, it is rarely used to make food and there are no documented data to our knowledge on beneficial effects on haemostasis at the national level except one thesis in sudan . We, therefore, conducted this cross sectional study into the effect of fenugreek on Prothrombin Time.

1.3.Objectives:

1.3.1.General objective:

To study the effect of fenugreek on blood coagulation (prthrombin time level) among healthy Sudanese volunteers.

1.3.2.Specific objectives:

1. To measure prothrombin time pre and post adding (25%,50%,75%) of fenugreek extract.
2. To evaluate prothrombin time (pre and post) according to Gender.
3. To evaluate prothrombin time (pre and post) according to Age.

Chapter II

Literature Review

Chapter II

Literature review

2.Literature review:

2.1.Hemostasis :

Normal hemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors An efficient and rapid mechanism for stopping bleeding from sites of blood The vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The hemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels (Hoff brand*et al*., 2006)

2.1.1.primary haemostasis:

2.1.1.1.Blood vessels :

2.1.1.1.1.Structure and function

The intimal surface is covered with endothelial cells (ECs) which rest on basement membrane of sub endothelial microfibrils, these being almost the only constituents of the capillaries. Have thin wall to facilitate both active and passive exchange of nutrients and waste products. with progressively larger vessels, particularly arteries, increasing amounts of elastin, innervated smooth muscle cells and collagen are found. The smooth muscle influence blood flow. Fibrillal collagen is necessary to support platelet adhesion via von willebrand factor and to activation of coagulation factor. Type 111collagen promotes platelet adhesion best and is also critical for the mechanical integrity of blood vessel.

Endothelial cells play key role in body defense response, they posses surface receptors for a variety of physiological substances, such as thrombin angiotensin11. Endothelial cell activities affecting platelet vessel wall interaction:-

ProstaglandinI2 and nitric oxide also known as endothelium derived relaxing factor (EDRF) have powerful vasodilatory activity ,acting on smooth muscle cells in the vessel wall and helping to modulate blood flow,

both substances inhibit aggregation of platelets and leukocytes by raising intra platelet levels of cyclic

adenosine monophosphate(CAMP)and cyclic guanosine monophosphate (CGMP).

ProstaglandinI2 is major prostaglandin synthesized by endothelial cells ,small amount produced by fibroblast and smooth muscle cells The precursor of prostaglandinI2 is arachidonic acid which is liberated

from phospholipids of the endothelial cell membrane by phospholipases Arachidonic acid is first converted to prostaglandin G2 and PGH2 PGG2 and PGH2 with thrombin generated at the site of injury, stimulate the synthesis of PGI2 by adjacent ECs, which counteracts the platelet aggregatingactivity of protease and helps to localized platelet plug formation In addition to nitric oxide and PGI2 the ECs also contain ectoenzyme which degrade adenosine diphosphate (ADP), which is vasoconstrictor and induce platelet aggregation VWF is large glycoprotein synthesized by ECs and megakaryocytes ,help in platelet vessel wall interaction (Hoffbrand*et al* .,2005)

2.1.1.1.2.Sub endothelium:

Consist of connective tissue composed of collagen,elastic fiber ,proteoglycan and non collagenous glycoproteins,including VWF and fibronectin After blood vessel wall damage has occurred these components are exposed andthen responsible for platelet adherence This appears to be mediated by VWF binding to collagen but also to microfibrilis which have greater affinity to VWF under some condition (Hoffbrand*et al* .,2005)

2.1.1.2. Platelets:

Platelets are made in the bone marrow. Huge cells known as megakaryocytes (derived from hematopoietic

stem cells) are the precursor to platelets, one megakaryocyte can produce 2,000 platelets Platelets bud off the edges of the megakaryocyte which eventually perishes by evaporating. It circulates in the blood for 7-10 days. Its either circulate freely or

sequestered in the spleen.at any given time one third of platelets are located in the spleen (Deloughery, 2004) Platelets are extremely small and discoid, 3.0 x 0.5 micrometer in diameter, with a mean volume 7-11 fL.Thrombopoietin is the major regulator of platelet production and is

constitutively produced by the liver and kidneys (Hoffbrand*et al*.,2006)

2.1.1.2.1.Role of platelets in hemostasis:

In a healthy blood vessel, and under normal blood flow, platelets do not adhere to surfaces or aggregate with each other. However, in the event of injury platelets are exposed to sub endothelial matrix, and adhesion and activation of platelets begins (Jackson, 2007)

2.1.1.2.2.PlateletsAdhesion:

platelets adhereto the sub endothelial collagen fibers, spread pseudopods along the surface, and clump together (aggregate) when vascular injury exposes the endothelial surface and underlying collagen ,its adhesion to sub endothelial connective tissues, especially collagen, occurs within 1 to 2 minutes after a break in the endothelium. Epinephrine and serotonin promote vasoconstriction. ADP increases the adhesiveness of platelets adhesion and aggregation of platelets are mediated by the binding of large

adhesion and aggregation of platelets are mediated by the binding of large soluble macromolecules to distinct glycoprotein receptors anchored in the platelet membrane, this increase the adhesiveness and cause circulating platelet to adhere to those already attached to the collagen resulting in cohesive platelets mass that rapidly increase in size to form platelet plug (Turgeon, 2001)

2.1.1.2.3.Platelets aggregation:

It is the process in which adherent platelets becomeactivated and release the contents of storage granules, recruiting nearby platelets in circulation to form an aggregate , the formation of the platelet aggregate or thrombus occurs via activation of GPIIb-IIIa and binding of multivalent adhesive ligands, fibrinogen, or von Willebrand factor (vWF), which crosslink the adjacent activated platelets (White andJennings,1999).

2.1.1.2.4.Platelets activation and release reaction:

Platelets undergo aggregation and release the content of their dense granule and *alpha* granule when exposed to agonist such as ADP, epinephrine, thrombin or collagen (Baklaja*et al.*,2008). ADP and serotoninreleased from the dense granules further enhance the platelet activation processes, for example, ADP released from the granules interacts with receptors on platelets to enhance the activation process (Schmaier and Lazarus, 2012)

2.1.1.3.Von Will brand factor' VWF:

Is involved in platelet adhesion to the vessel wall and to other platelets (aggregation). It also carries factor VIII and used to be referred to as factor VIII related antigen (VIII-Rag). It is a large cysteine-rich glycoprotein, with multimers made up on average of 2-50 subunits, with a molecular weight (MW) of 0.8-20 x106 VWF is encoded by a gene on chromosome 12 and is synthesized both in endothelial cells and megakaryocytes, and stored in Weiberl-Palade bodies and platelet agranule respectively Plasma VWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously secreted and a minority is stored in Weibel-Palade bodies. The stored VWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of decompressing (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from Seibel-Palade bodies is in the form of large and ultra large multiverse, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to monomeric VWF and smaller multiverse by the specific plasma metalloprotease, ADAMTS-13 (Hoff brand*et al.*,2006)

2.1.2.Secondary haemostasis:

Secondary haemostasis involves a series of blood protein reactions through a cascade-like process that

concludes with the formation of an insoluble fibrin clot This system involves multiple enzymes and several

cofactors as well as inhibitors to keep the system in balance. Coagulation factors are produced in the liver, except for factor VIII, which is believed to be produced in the endothelial cells. When the factors are in a precursor form, the enzyme or zymogen

is converted to an active enzyme or a protease The initiation of clotting begins with the activation the initiation of clotting begins with the activation of two enzymatic pathways that will ultimately lead to fibrin formation: the intrinsic and extrinsic pathways. Both pathways are necessary for fibrin formation, but their activating factors are different. Intrinsic activation occurs by trauma within the vascular system, such as exposed endothelium. This system is slower and yet more important versus the extrinsic pathway, which is initiated by an external trauma, such as a clot and occurs quickly (Pimenta and Perils, 2009)

2.1.3.Classification of Coagulation Factors:

Coagulation factors may be categorized into substrates, cofactors, and enzymes. Substrates are the substance upon which enzymes act. Fibrinogen is the main substrate. Cofactors accelerate the activities of the enzymes that are involved in the cascade. Cofactors include tissue factor, factor V, factor VIII, and Fitzgerald factor. All of the enzymes are serine proteases except factor XIII which is atransaminase

There are three groups in which coagulation factors can be

The fibrinogen group consists of factors I, V, VIII, and XIII. They are consumed during coagulation. Factors V and VIII are labile and will increase during pregnancy and inflammation

The Prothrombin group: Factors II, VII, IX, and X all are dependent on vitamin K during their synthesis. This group is stable and remains preserved in stored plasma

The contact group: Factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) are involved in the intrinsic pathway, moderatelystable, and not consumed during coagulation (Barbara*et al.*, 2007)

factor I, Fibrinogen:

Substrate for thrombin and precursor of fibrin, it is a large globulin protein Its function is to be converted into an insoluble protein and then back to soluble components. When exposed to thrombin, two peptides split from the fibrinogen molecule, leaving a fibrin monomer to form a polymerized clot

Factor II, Prothrombin:

Precursor to thrombin, in the presence of Ca2++, it is converted to thrombin (IIa), which in turn stimulates platelet aggregation and activates cofactors protein C and factor XIII. This is a vitamin K–dependent factor

Factor III, Thromboplastin:

Tissue factor activates factor VII when blood is exposed to tissue fluids

Factor IV, Ionized Calcium:

This active form of calcium is needed for the activation of thromboplastin and forconversion of Prothrombin to thrombin

Factor V, Proaccelerin or Labile Factor:

This is consumed during clotting and accelerates the transformation of Prothrombinto thrombin. A K dependent factor, 20% of factor V is found on platelets

Factor VI, Nonexistent

Factor VII, Proconvertin or Stable Factor:

This is activated by tissue thromboplastin, which in turn activates factor X. It is avitamin K–dependent factor

Factor VIII, Ant hemophilic:

This cofactor is used for the cleavage of factor X-Xa by IXa. Factor VIII is described as VIII/vWF:VIII:C active portion, measured by clotting, VIII: Ag is the antigenic portion, vWF Ag measures antigen that binds to endothelium for plateletfunction; it is deficient in hemophilia A

Factor IX, Plasma Thromboplastin Component:

A component of the thromboplastin generating system, it influences amount as opposed to rate. It is deficient in hemophilia B, also known as Christmas disease. It is sex linked and vitamin K–dependent

Factor X, Stuart-Prowers:

Final common pathway merges to form conversion of Prothrombin to thrombin, activity also related to factors VII and IX. It is vitamin K–dependent and can be independently activated by Russell's viper venom

Factor XI, Plasma Thromboplastin Antecedent:

Essential to intrinsic t hromboplastin generating of the cascade, it has increased frequency in the Jewish population. Bleeding tendencies vary, but there is the risk of postoperative hemorrhage.

Factor XII, Hageman factor:

This surface contact factor is activated by collagen. Patients do not bleed but have a tendency to thrombosis

Factor XIII, Fibrin Stabilizing Factor:

In the presence of calcium, this transaminase stabilizes polymerized fibrin monomers in the initial clot. This is the only factor that is not found in circulating plasma

High-Molecular-Weight Kininogen:

This surface contact factor is activated by kallikrein

Prekallikrein, Fletcher Factor:

This is a surface contact activator, in which 75% is bound to HMWK (Barbara *et al.*,2007)

2.1.4.Physiological Coagulation (In Vivo)

The original theory of coagulation used a cascade or waterfall theory. This description depicted the generation of thrombin by the soluble coagulation factors and the initiation of coagulation. This theory identified two starting points for the generation of thrombin: the initiation of the intrinsic pathway with factor XII and surface contact, and the extrinsic pathway with factor VIIa and tissue factor. These two pathways meet at the common pathway, where they both generate factor Xa from X, leading to a common pathway of thrombin from Prothrombin and the conversion of fibrinogen to fibrin. This process holds true under laboratory conditions the discovery of a naturally occurring inhibitor of hemostasis, tissue factor pathway inhibitor (TFPI), is able to block the activity of the tissue factor VIIa complex, soon after it becomes active (Barbara*et al.*,2007)

2.1.5.Extrinsic Coagulation Pathway:

The extrinsic pathway is initiated by the entry of tissue thromboplastin into the circulating blood. Tissue thromboplastin is derived from phospholipoproteins and organelle membranes from disrupted tissue cells. These membrane lipoproteins, termed tissue factors, are normally extrinsic to the circulation. Platelet phospholipids are not necessary for activation of the extrinsic pathway because tissue factor supplies its own phospholipids. Factor VII binds to these phospholipids in the tissue cell membranes and is activated to factor VIIa, a potent enzyme capable of activating factor X to Xa in the presence of ionized calcium. The activity of the tissue factor–

factor VII complex seems to be largely dependent on the concentration of tissue thromboplastin. The proteolytic cleavage of factor VIIa by factor Xa results in inactivation of factor VIIa. Factor VII participates onlyin the extrinsic pathway. Membranes that enter the circulation also provide a surface for the attachment and activation of factors II and V. The final step is the conversion of fibrinogen to fibrin by thrombin (Turgeon., 2001)

2.1.6.Intrinsic Coagulation Pathway:

The intrinsic system assumes that exposure of contactfactors (factor XII,highmolecular- of factor XI, which in turn activates factor IX, activated factor IX, in the presence of its weight kininogens, prekallikrein) to an abnormalinjured vascular surface leads to activation cofactor factor VIII, then activates factor X to factor Xa in the presence of phospholipid. In turn, factor Xa with its cofactor factor V together form the prothrombinase complex, which converts prothrombin to thrombin,thrombin then converts fibrinogen to fibrin (Shinton, 2008).

2.1.7.Common Pathways :

The common pathway is the point at which the intrinsic and extrinsic pathways come together and factors I, II, V, and X are measured. It is important to note that the PT and the APTT will not detect qualitative or
quantitative platelet disorders, or a factor XIII deficiency. Factor XIII is fibrin stabilizing factor and is responsible for stabilizing a soluble fibrin monomer into an insoluble fibrin clot. If a patient is factor XIII deficient, the patient will form a clot but will not be able to stabilize the clot and bleeding will occur later. Factor XIII is measured by a 5 mol/L urea test that looks not only the formation of the clot but also if the clot lazes after 24 hours (Barbara*et al.*,2007)

2.1.8.Formation of Thrombin :

When plasma fibrinogen is activated by thrombin, this conversion results in a stable fibrin clot. This clot is a visible result that the action of the protease enzyme thrombin has achieved fibrin formation. Thrombin is also involved in the XIII-XIIIa activation due to the reaction of thrombin cleaving a peptide bond from each of two alpha chains. Inactive XIII along with Ca2_ ions enables XIII to dissociate to XIIIa. If thrombin were allowed to circulate in its active form (Ia), uncontrollable clotting

would occur. As a result thrombin circulates in its inactive form Prothrombin (II).Thrombin, a protease enzyme, cleaves fibrinogen (factor I) which results in a fibrin monomer and fibrinogen peptides A and B. These initial monomers polymerize end to end due to hydrogen bonding

Formation of fibrin occurs in three phases :

-Proteolysis: Protease enzyme thrombin cleaves fibrinogen resulting in a fibrin - monomer, A and B fibrin peptide

- Polymerization: This occurs spontaneously due to fibrin monomer that line up end-to-end due to hydrogen bonding

- Stabilization: This occurs when the fibrin monomers are linked covalently by XIIIa into fibrin polymers forming an insoluble fibrin clot (Barbara*et al.*,2007).

2.1.9.Feedback Inhibition :

Some activated factors have the ability to destroy other factors in the cascade. Thrombin has the ability to temporarily activate V and VIII, but as thrombin increases it destroys V and VIII by proteolysis. Likewise, factor Xa enhances factor VII, but through a reaction with tissue factor pathway inhibitor (TFPI), it will prevent further activation of X by VIIa and tissue factor. Therefore, these enzymes limit their own ability to activate the coagulation cascade at different intervals.

Thrombin feedback activation of factor IX can possibly explain how intrinsic coagulation might occur in the absence of contact factors. Tissue factor is expressed following an injury forming a complex with VIIa, then activating X and IX. TFPI prevents further activation of X. Thrombin formation is further amplified by factors V, VIII, and XI, which leads to activation of the intrinsic pathway. This feedback theory helps to enforce why patients with contact factor abnormalities factors XI and XII) do not bleed

(Hoff brand*et al.*,2006)

2.1.10..Fibrinolytic system :

The role of the fibrinolytic system is to dissolve blood clots during the process of wound healing and to prevent blood clots in healthy blood vessels. The fibrinolytic system is composed primarily of three serine proteases that are present as zymogens (i.e., proenzymes) in the blood. Plasmin cleaves and breaks down fibrin. Plasmin is generated from the zymogen plasminogen by the proteases tissue-type plasminogen

activator (tPA) and urokinase-type plasminogen activator (uPA). TPA and plasminogen come together on the surface of a fibrin clot, to which they both bind. TPA then activates plasminogen, which ,subsequently cleaves fibrin. UPA activates plasminogen in the presence of the uPA receptor which is found on various cell types (Lijnen *et al.*, 2000). All three of these serine proteases are down-regulated by serpins that are present in blood. Alpha-2-antiplasmin inhibits plasmin, and plasminogen activator inhibitors 1 and 2 inhibit tPA and uPA (Rau *et al.*, 2007)

2.1.11.Coagulation inhibitor :

There are three separate mechanisms to this aspect,and they all are to do with control of the productionand function of thrombin which are Circulating antithrombin, The protein C/thrombomodulin mechanism and Tissue factor pathway inhibitor activation.Anti-thrombincomplexes with thrombin, thereby inactivating it, but in addition has other anti-coagulant actions by inactivating XIIa, XIa, IXa, and Xa Thrombomodulin on the surface of intact endothelial surfaces, binds both thrombin and protein C (and the binding to protein C is stronglyenhanced by the protein C receptor on the endothelium). Within this bound complex, thrombin loses its procoagulant properties and becomes an anti-coagulant,by the process of activating protein C. Activatedprotein C, on the surface of activated platelets (wherethe coagulation process is going on), degrades Va and VIIIa, thus inhibiting further local coagulation proteins C and S also require vitamin K-dependent post-translational carboxylation for effect this is important when considering coagulation disorders in liver disease and in instituting anti-coagulant therapy (Beck.,2009)

Tissue factor path way inhibitor (TFPI)bind to factor Xa and in this combination, binds to and inhibits tissue factor/factor VII complex and activated factor X (Xa), TFPI synthesize primarily by endothelium, other part found as blood porn and tiny portion is found in platelet (AbdelGader, 2009)

1.2.12.Role of Vitamin K in hemostasis:

Factors II, VII, IX, X, protein C and protein S have vitamin K dependent Glutamic Acid domains in amino terminusof the protein. These domains contains 9- 11 Glutamic acids modified to form gamma-carboxyglutamic acid. This modification allows calcium to bind to proteins. The binding of calcium changes

the conformations of the proteins and serves to bind them in turn to phospholipid surfaces. The hepatic Glutamic acid redox reaction is dependent on vitamin K. without this vitamin, dysfunctional coagulation proteins are produced which function poorly in coagulation reactions (Deloughery, 2004)

2.1.13.Screening tests of blood coagulation:

Screening tests provide an assessment of the 'extrinsic' and 'intrinsic' systems of blood coagulation and also the central conversion of fibrinogen to fibrin.The prothrombin time (PT) measures factors VII, X, V, prothrombin and fibrinogen. Tissue thromboplastin (a brain extract) and calcium are added to citrated plasma (Hoffbrand*et al.*, 2006)

The International Sensitivity Index is a method of standardizing prothrombin times obtained from different laboratories. The INR is derived by dividing the patients prothrombin times by the control and raising this to the International Sensitivity Index (ISI). The ISI is known for each prothrombin laboratory reagent and it adjusts the prothrombin time for the differing sensitives of reagents. Using the INR instead of prothrombin time has resulted in more accurate monitoring of warfarin dosage. Many laboratories now only report the INR and not prothrombin time (Deloughery,2004)

The activated partial thromboplastin time (APTT) measures factors VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen Three substances-phospholipid, a surface activator (e.g. kaolin) and calcium-are added to citrated plasma (Hoffbrand*et al.*, 2006)

2,1.14.Kinin System :

Another plasma protein system in coagulation is the kinin system. This system is capable of vascular dilatation leading to hypotension, shock, and end-organ damage by its capability to increase vascular permeability The kinins are peptides of 9 to 11 amino acids. The kinin system is activated by factor XII.

Hageman factor XIIa converts prekallikrein (Fletcher factor) into kallikrein, and kallikrein converts

kininogens into kinins. The most important is Brady kinin (BK). This is an important factor in vascular permeability as well as a chemical mediator of pain. BK is capable of reproducing many characteristics of an inflammatory state such as changes in

blood pressure, edema, and pain, resulting in vasodilation and increased microvessel permeability (Barbara *et al.*, 2007)

2.2. Fenugreek :

Fenugreek is a herb of the soy family, originated from India and the North Africa.(Bae *et al.*,2015). It's used as spices in food preparations to improve or impart flavor and are good sources of protein, fat, minerals, and dietary fiber. (Younesy *et al.*,2014)

Medical use of fenugreek :

-Fenugreek aqueous extract in different concentrations ,inhibits clot formation and increases PT. It also shows that increasing concentrations of fenugreek extract strongly inhibits the coagulation process and increases PT, and that aqueous extract of fenugreek
have anticoagulant properties through the prevention of clot formation.(Imadeldin et al.,2013)

- Fenugreek has been noticed to increase the anticoagulant effect of warfarin. It was also mentioned that there is one case of gastrointestinal bleeding in a premature infant (30 weeks) following introduction of Fenugreek to the mother (Manicam et al.,2010)

-Fenugreek promotes metabolic resiliency via significant and selected effects on glucose regulation, hyperlipidemia, and adipose pathology; but may not be as effective as behavioral modifications at preventing the adverse metabolic consequences of a high fat diet.(Knott *et al.*,2017).

-Fenugreek Trigonella foenum-graecum is used in many parts of the world for the treatment of diabetes. TFG seeds are used as an active ingredient in weight loss and anticholesterol ayurvedic formulation Ayurslim (The Himalaya Drug Com-pany, Bangalore, India). TFG seeds have been shown to possess hypoglycemic, hypolipidemic, and antioxidant effects (Kumar *et al.*,2014)

- dietary rich fenugreek (*Trigonella foenum-graecum*) seed extract, Furosap , enriched in 20% protodioscin extract is beneficial in significantly enhancing free testosterone level, sperm count, sperm motility, mental alertness, mood, reflex erection and overall performance in human volunteers (Maheshwari *et al.*,2017)

-The alkaloid and flavonoid content of fenugreek seeds can be responsible for antinociception and anti-inflammatory effects of the plant respectively (Mandegary *et al.*, 2012)

-Fenugreek reduced the severity of primary dysmenorrhea. Given that adverse effects were not reported for fenugreek, the herb can be administered safely for the management of this condition (Younesy *et al.*, 2014)

2.3. Previous study:

Fenugreek is largely universal staple herb, popular throughout history and it has been consumed for treatment of different disorders. But effect of fenugreek on blood coagulation don't documented data except study conducted by Imadeldin M Taj Eldin, Majed M Abdalmutalab, and Haydar E Bikir , Sudan J Paediatr. 2013 which reported that fenugreek extract has large effect on prothrombin time and can use beside anticoagulant therapy with highly significant different p.value (0.001).

Chapter III

Materials and Methods

Chapter III

Materials and Methods

3.Materials and Methods:

3.1 Study design:

This study was a cross sectional study (before –after).

3.2 Study area and duration:

The study was conducted at Khartoum state in the period from October to December 2019.

3.3 Study population and sample size:

This study was conducted on 50 apparently healthy volunteers to study the effect of fenugreek on prothrombin time ,prothrombin time was estimated for each participant before and after added seqeance concentration of fenugreek extract on plasma.

3.4 Study variables:

Prothrombin time level as dependent variable and the following are independent variables (gender and age).

3.5 Inclusion Criteria:

included only apparently healthy individual

3.6 Exclusion Criteria:

All individual that show any bleeding or thrombosis abnormality, pregnant women's , those who are under any treatment and patients with history of hypertension, diabetic patient

3.7 Sample collection:

Venous Blood samples were collected from all subjects in 3.2% tri- sodium citrate anticoagulant and prothrombin time was measured for each sample.

3.8 Principle and procedour:

3.8.1 Preparation of Platelet Poor Plasma (P.P.P):

It was prepared PPP by centrifugation for 15 minutes (approximately 4000 rev / min in a standard bench).

3.8.2 Preparation of Fenugreek extract:

Fresh, dried, ripe and recently cropped Fenugreek seeds were purchased from the local market in Khartoum city.

Fifty grams of the seeds were grinded into a fine powder, and five grams of the powder were weighed using sensitive

balance and then suspended in 100 ml of distilled water in a conical flask with continuous shaking for three hours. The supernatant of Fenugreek extract was filtrated using sucking pump. The final clear solution of Fenugreek aqueous extract was used for in vitro testing of anticoagulant activity in blood samples of normal individuals using the principles of PT test. (Imadeldin *et al*.,2013)

3.8.3 Principle of PT:

The PT was performed by manual testing measure the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) with calcium chloride (CaCl2) which indicates over all the efficiency of the extrinsic clotting system.

3.8.4 Reagents and materials:

Phospholipid **+** ca2+ **+** tissue factor.

Cotton, automatic piped, water path, alcohol, stop watch.

3.8.5 Method :

For determination of the prothrombin time, the plasma sample of each individual was divided into

four groups each of 50 µL. Group 1 (n=50) was tested first to determine the normal PT (positive control

group) using the stable, liquid, combined calcium/ thromboplastin rabbit brain (DiaMed LTD, UK) as a

gold standard. Three volumes of Fenugreek aqueous extract (25, 50 and 75 µL) were added separately to the remaining three groups of plasma samples in a water bath with gentle shaking. Then thromboplastin reagent (200µL) was added separately to the mixture of each plasma sample using pipetador volume adjustment. Stop watch was used for measuring the time of the clot formation . Thromboplastin reagent was added to the plasma in order to counteract the sodium citrate and allow clotting to proceed.

3.8.6 Normal Values:

10-20seconds (according to manufacturer

3.9 Data collection:

Patient's data collected using structural interview questionnaire.

3.10 Data Analysis:

The data were analyzed using SPSS software version 20; the paired t-test was applied to analyze the changes in the prothrombin time level. A P-value less than 0.05 was considered as significant.

3.11 Ethical consideration:

consent was taken from all subjects before sample collection.

Chapter IV

Results

Chapter IV

Results

4.Results:

This study was done in Khartoum state at Sudan university of since and technology in the period from August 2019 to March 2021 to evaluate the effect of fenugreek on prothrombin time test. 50 healthy Sudanese volunteers aged between 20-30 years were enrolled to participate in this study (22) of them were females with 44% and (28) of them were males with 56%.

In this study the effects of the fenugreek extract as an anticoagulant agent had been investigated, using the principles of pro thrombin time test in fifty normal individuals. The prothrombin time for all of them was found to be normal (13.6+1.12 Se). When fenugreek extract was added in different volumes (25,50 and 75 ul) to plasma samples of normal individuals, the results revealed highly significant differences (P <0.000) in clot formation comparing with control(pre prothrombin time) when added different volume of fenugreek extract also there werehighly significant differentces (P <0.000) between the groups.

Figure (4_1): Gender Distribution for the majority of the sample of the study is (56%) were(Male) and (44%) were (Female).

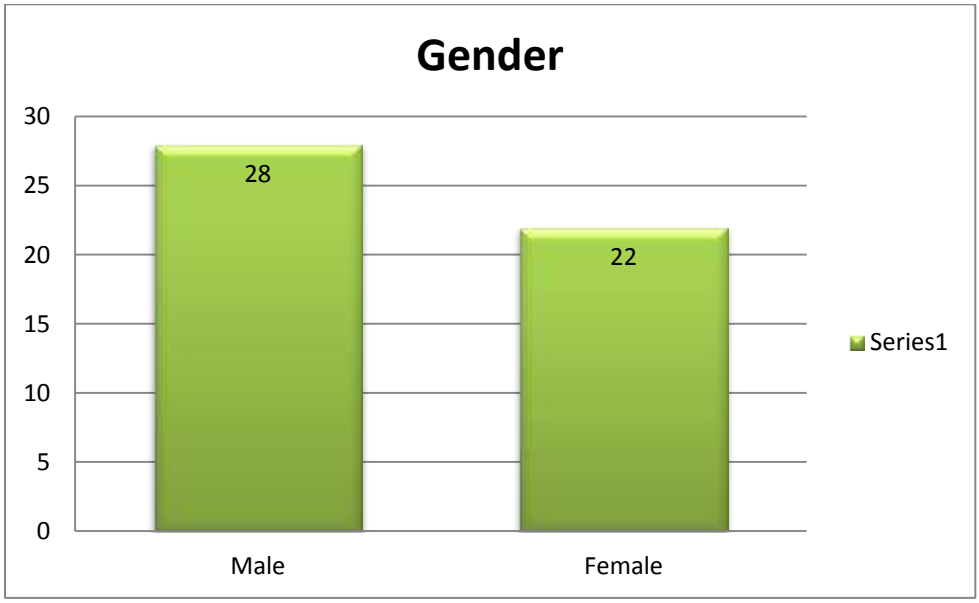

Table (4-1) Summary of mean of PT before and after fenugreek extract (25% , 50%, and 75%) addition

Test	**Mean (±SD)**	N	**p.value**
Pre fenugreek extract	13.60(±1.12)	50	0.000
Post 25% of fenugreek extract	18.04(±1.26)	50	
Pre fenugreek extract	13.60(±1.12)	50	0.000
Post 50% of fenugreek extract	24,34(±2.54)	50	
Pre fenugreek extract	13.60(±1.12)	50	0.000
Post 75% of fenugreek extract	45.67(±6.61)	50	

The statistical analysis of the results showed that there is a significant difference (P.value=0.000) among participants PT between before 13.60 (±1.12) and after 25% 18.04 (±1.26),50% 24.34(±2.54)and 75% 45.67 (±6.61) fenugreek extract addation samples (Table 3.3)

Table (4-2) PT according to the Gender :

parameter	Gender	**Mean (±SD)**	P-value
Pre fenugreek extract addition	Male	13.64 (±1.2)	0.764
	Female	13.54 (±1.14)	
Post 25% fenugreek extract addition	Male	18.39 (±1.10)	0.024
	Female	17.59 (±1.33)	
Post 50% fenugreek extract addition	Male	24.03 (±1.79)	0.345
	Female	24.72 (±3.26)	
Post 75% fenugreek extract addition	Male	47.00 (±7.60)	0.107
	Female	43.95 (±4.72)	

• Significant at ‹ 0.05

When we compared the PT in both males and females the mean of pre fenugreek extract addition

samples among females was 13.54(±1.14) and among males was 13.64(±1.12) , whereas the mean of PT post fenugreek extract (25%,50% and 75%) addation respectively samples collected from females was 17.59(±1.33) , 24.72(±3.26) and 43.95(±4.72) while it was 18.39(±1.10) , 24.03(±1.79) and 47.00(±7.60)in samples collected from males. The results showed that there is no significant difference between males and

females in the Pre and Post 50% and 75% of fenugreek extract addation samples (p.value = 0.764, 0.345 and 0.107 respectively) but there is significant difference between males and females in Post 25% fenugreek extract addation samples (p.value 0.024) .

Table (4.3) PT level according to age group :

parameter	Age group	**Mean (±SD)**	P-value
Pre fenugreek extract addition	20-25	13.61 (±1.7)	0.919
	26-30	13.57 (±1.07)	
Post 25% fenugreek extract addition	20-25	18.06 (±1.18)	0.863
	26-30	18.00 (±1.33)	
Post 50% fenugreek extract addition	20-25	23.93 (±2.75)	0.153
	26-30	25.00 (±2.05)	
Post 75% fenugreek extract addition	20-25	46.32 (±6.72)	0.371
	26-30	44.57 (±6.45)	

Significant at < 0.05

When we compared the pro thrombin time level in the different age group , the mean PT

for Pre- fenugreek extract samples among group one (20 – 25 years) was 13.61 (±1.7) and

among group two (26 – 30 years) was 13.57(±1.07) , while the mean of PT of post-fenugreek extract (25%,50% and 75%) addation respectively samples among group one 18.06(±1.18), 23.93(±2.75)and 46.32(±6.72) respectively and it was 18.00(±1.33), 25.00(±2.05) and 44.57(±6.45)respectively in samples collected from group two. The results showed that there is no significant difference between the two age groups in the pre and post 25%, 50% and 75% samples . (p.value 0.919, 0.863 0.153 and 0.371

respectively)

Chapter V

Discussion & Conclusion

Recommendation

Chapter V

5. Discussion & Conclusion, Recommendation

5.1. Discussion :

The prevalence of atherosclerosis and coronary artery diseases has focused attention on the influence of diet on the cardiovascular system. Natural anticoagulant agents that influence platelet function and inhibit coagulation process are of potential interest for primary prevention of cardiovascular diseases.

Pro thrombin time test is important test that used to evaluate the extrinsic and common pathway of coagulation and it is used to the monitoring of patients on oral anticoagulation therapy.

This study was carried out to evaluate the effect of fenugreek extract on pro thrombin time level in apparently 50 healthy sudanese volunteers aged between 21 – 30 years .

In this study we found that the mean of Prothrombin Time in the post samples were significantly higher than the mean of pre-samples, our results agrees with findings of study done by Imadeldin M Taj Eldin , Majed M Abdalmutalab and Haydar E Bikir in Wad Medani, Sudan (2013) reported that after addition aqueous extract of fenugreek(5%) in different volumes (25,50 and 75ML) showed increase on Prothrombin time level.

No significant difference when we compared the PT in both male and female the mean of pre-fenugreek extract add among female was 13.54(±1.14) and among male was 13.64(±1.2) ,where the mean of PT in post fenugreek extract 50 and 75% add to samples collected from female was 24.72(±3.26) and 43.95(±4.72) while it was 24.03(±1.79) and 47.00(±7.60) in samples collected from male.

But there is significant difference between male and female in post 25% fenugreek extract add sample , mean of PT among samples collected from female was 17.59(±1.33) while it was 18.39(±1.10) in samples collected from male (p.value <0.024).

No significant difference when we compared mean of prothrombine time level between

aged groups (21-25, 26-30) .mean of prothrombin time before adding of fenugreek extract was

13.61 (±1.7),13.57(±1.07) respectively .while the mean of PT after adding 25% of fenugreek extract was 18.06(±1.18), 18.00(±1.33) respectively ,mean of PT after adding 50% of fenugreek extract was 23.93(±2.75), 25.00(±2.05) respectively and mean of PT after adding 75% of fenugreek extract was 46.32(±6.72), 44.57(±6.45) respectively

5.2. Conclusion :

The study concludes that:

-Fenugreek aqueous extract in different concentrations (25, 50, 75 %) increases Prothrombin time.

-No signifigant different on the PT according to Gender in Pre and Post (50,75%) of Fenugreek aqueous extract , but there is difference between them in Post 25% of adding extract.

-No variation on PT according to age before and after adding (25, 50,75%) of Fenugreek aqueous extract.

5.3. Recommendations :

-Another study should be done with increase sample size, different concentration of fenugreek extract for more obvious finding.

-Perform intensive in-vivo study on this topic in different population.

-Further large studies are recommended to evaluate this effect and to determine the mode of action.

-Pharmaceutical companies do intensive studies to using fenugreek as supplementary anticoagulant agent to prevent cardiovascular diseases.

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Appendices

Appendices

Appendix I: Questionnaire

Sudan University of science and Technology

College of Graduate studies

Department of Hematology and Immunohematology

Questionnaire

A: General Information:

-Name : _____

-Age : _____

-Gender : Male ( ) , Female ( ) .

B: Personal History :

1-Underling disease: Yes ( ) , No ( ) .

If yes mention_____

2-Are you smoker ?

Yes ( ) , No ( ) .

3-Do you consume any type of drug ?

Yes ( ) , No ( ) .

If yes mention_____

4-Do you follow special diet ?

Yes ( ) , No ( ) .

5-Are you suffering of any coagulation disorder ?

Yes ( ) , No ( ) .

If yes mention_____

C: Family History :

1-Dose any one of your parents have one of the underling disease?

Yes ( ) , No ( ) .

2-Dose anyone in the family have coagulation problems?

Yes ( ) , No ( ) .

If yes mention_____

3-Is their history of sudden unexplained deaths in the family?

Yes ( ) , No ( ) .

D: investigations:

1-PT (Pre):_____

2-PT (Post):_____

Appendix II: Water Bath

Appendix III: Sucher Pump