Sudan University of Science and Technology

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Effect of Garlic Oil on Prothrombin Time in the Blood of Healthy Adults

تأثير زيت الثوم على زمن البروثرومبين في دم البالغين الاصحاء

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

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الايـة

بسم الله الرحمن الرحيم

قال تعالى :

قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا

صدق الله العظيم

سورة الكهف الآية (109)

Dedication

Thanks first and finally to Allah, Thanks to the queen of my heart my mother, to my
backbone my father , to my dear brother, to my special friends, teachers and colleages
who support me, finally great thanks to my best friend Maymona

Acknowledgment

Great thanks firstly and finally to Allah who gave me heath and
ability to complete this work.

Firstly I would like to thank my teacher Dr.Munsoor
for his advice and encouragement to conduct this study.

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Finally thanks extended to all people whom the blood samples has been collected from.

Abstract

This is crossectional study, conducted in Khartoum state to determine the effect of garlic oil on the prothrombin time during the period from November 2019 to March 2021.

Garlic Oil derived from the crushed bulbs of garlic, the bulbs contain 0,06% to 0,1% of volatile oil whose active constituents are propyl/ -disulphide, alliin and allicin.

Fifty (50) blood samples of healthy adult volunteers in this study; 22 of them were female and 28 were males, age ranged (21-30) years. In vitro anticoagulant effects of Garlic oil in different volumes (20% and 30%) were examined in blood samples of normal individuals by measuring prothrombin time (PT).

The data were analyzed using statistical package for social sciences (SPSS), version 20.paired sample t-test was used to compare the mean of PT before and after adding garlic oil in different volumes. A P-value less than 0.05 was considered significant.

The result showed that mean of PT before adding garlic oil was 13.6 seconds, and after adding garlic oil in different volumes(20% and 30%) were 23.9 and41.08 seconds respectively. was reflect that there is clear significant difference in PT (p. value 0.000).

The results showed that mean of PT in both sexes before adding garlic oil were 13.6 for male, 13.5 for female and after adding garlic oil in different volumes (20% and 30%) were24.1for male, 23.7for female in post 20% and 44.0for male, 37.3for female in post 30% . was reflect that there is no significant difference between males and females in PT before adding garlic oil and after adding 20% garlic oil (P. value 0.765 , 0.671) respectively, but there is significant difference between males and females in PT after adding 30 % garlic oil to samples(p.value0.002).

The results showed that No statistically significant difference was found in the two aged Group before and after adding Garlic oil in different volumes (20% and 30%), p.value (= 0.919 , 0.692 and 0.703) respectively.

This study was concluded that the prothrombin time was affected when adding garlic oil among healthy volunteers samples.

مستخلص البحث

هذه دراسة مقطعية أجريت في ولاية الخرطوم لتحديد تأثير زيت الثوم على زمن البروثرومبين خلال الفترة من نوفمبر 2019 الي مارس2021.
زيت الثوم يستخرج من سحق فصوص الثوم , تحتوي فصوص الثوم علي 0,06% الي 0,1% مادة زيتية متطايرة ذات مكونات نشطة هي بروبيل –ثنائي الكبريت , الين و الاليسين.
تم اخذ 50 عينة من متطوعين بالغين اصحاء في هذة الدراسة, 22منهم من الاناث و 28 من الذكور, تتراوح اعمارهم مابين (21-30) سنة. تم فحص تاثير زيت الثوم باحجام مختلفة(20%و30%) كمضاد للتحثر داخل المختبر في عينات الدم للافرادالطبيعين عن طريق قياس زمن البروثرومبين.

تم تحليل البيانات باستخدام الحزمة الاحصائية للعلوم الاجتماعية الاصدار 20. باستخدام العينة المزدوجة للمقارنة بين متوسط زمن البروثرومبين قبل و بعد اضافة زيت الثوم باحجام مختلفة, القيمة الاحصائية اقل من 0,05 حيث تعتبر ذات دلالة احصائية.

أظهرت النتائج أن متوسط البروثرومبين قبل اضافة زيت الثوم كان 13.6 ثانية , وبعد اضافة زيت الثوم بأحجام مختلفة (20% و 30%) كان 23.9 و 41.08 ثانية على التوالي, القيمةالاحصائية (0.000) .

كما خلصت النتائج أن متوسط البروثرومبين في كلا الجنسين قبل اضافة زيت الثوم كان 13.6للذكور و 13.5 للاناث و بعد اضافة زيت الثوم بكميات مختلفة (20% و30%) .كانت 24.1 و44.0 علي التوالي للذكور و23.7 و 37,3 علي التوالي للاناث , وقد ظهر عدم وجود فرق بين الذكور والاناث في البروثرومبين قبل اضافة زيت الثوم وبعد اضافة 20 % من زيت الثوم القيمة الاحصائية (0.765 ,0.671) علي التوالي. ولكن هناك فرق ذو دلاله احصائية بين الذكور و الاناث في زمن البروثرومبين بعد اضافة 30 % من زيت الثوم للعينات القيمة الاحصائية (0.002).

لم يتم العثور على فرق ذو دلالة احصائياً في المجموعتين العمريتين قبل و بعد اضافة زيت الثوم باحجام مختلفة (20% و 30%) .القيم الاحصائية (0.919 و 0.692 و 0.703) على التوالي.

خلصت هذه الدراسة الي أن زمن البروثرومبين يتأثر عند اضافة زيت الثوم الي 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
DMSO	Dimethyl sulfoxid
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
PPP	Platelet Poor Plasma
PT	Prothrombin Time
SPSS	Statistical Package for Social Sciences
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
tPA	Tissue-Plasminogen Activator
TXA2	Thromboxane A2
uPA	Urokinase- Plasminogen Activator
'VWF	Von Willebnllld Factor

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Chapter I
Introduction

CHAPTER I

1.1.Introduction

1.1.1. Coagulation:

Coagulation is a complex network of interactions involving blood vessels, platelets, and coagulation factors. The ability to form and to remove a clot is truly a system dependent on many synergistic forces. Hemostasis depends on a system of checks and balances between thrombosis and hemorrhage that includes both procoagulants and anticoagulants**,** this scale needs to be kept in balance (Ciesla, 2007).

The arresting of bleeding,depends on several components. The four major components are the vascular system, platelets (thrombocytes), blood coagulation factors, and fibrinolysis(Turgeon, 2005).

There are two main pathways initially described for triggering the blood clotting cascade:

The contact activation pathway (also known as the intrinsic pathway), and the tissue factor (TF) pathway (the extrinsic pathway) (Monica,2000) .

The prothrombin time (PT) is a screening test for the extrinsic clotting system(Monica,2000).

measures factors VII, X, V, prothrombin and fibrinogen. Tissue thromboplastin (a brain extract) and calcium are added to citrated plasma. The normal time for clotting is 10-14 s (Hoff brand and Moss,2011).

The activated partial thromboplastin time (APTT) is a screening test of the intrinsic clotting system(Monica,2000).

measures factors VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen. Three substances-phospholipid, a surfaceactivator (e.g. kaolin) and calcium-are added to citrated plasma. The normal time for clotting is approximately 30-40s(Hoff brand and Moss,2011).

The deposition of fibrin and it is removal are regulated by the fibrinolytic system. Although this is a complex multicomponent system with many activators and inhibitors, it centers around the fibrinogen- and fibrin-cleaving enzyme plasmin.

Plasmin circulates in it is inactive precursor form, plasminogen, which is activated by proteolytic cleavage (Bain *et al.*,2012).

1.1.2.Prothrombin Time:

The PT test measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system(Bain *et al.,* 2012).

1.1.3.Garlic Oil (Allium Sativum):

Garlic (Allium sativum) is a popular vegetable with a variety of medicinal properties. Garlic bulbs are edible, inexpensive and are readily available (Pakdel and Ghasemi, 2017). These are used as traditional dietary and medicinal purposes like anti-infective agents. The Garlic Porridge is a kind of herbal diet which lowering blood pressure and blood lipid, soften blood vessel. The tonic diet is given for nourishing and moistening the lung, nourish blood soothing the liver and lower the blood pressure. Garlic also found to be used as antiprotozoal activity against Entameoba histolytica, candidiasis. Cloves are known to possess antimicrobial, anticancer, antioxidant, antidiabetic, antiemetic, antihypertensive, hypoglycemic, hypolipidemic, and immune modulatory (Katey *et al.,* 2005). Garlic consumption is an alternative thrombolysis medicine, which has been used for many years in different cultures. Allicin, one of the garlic components, could have therapeutic effects, including antimicrobial effect, immunostimulating properties, improve fibrinolytic activity, inhibit platelet aggregation and adhesion and also reduce blood pressure (García Gómez and Sánchez-Muniz, 2000).

1.2.Rationale :

Prothrombin Time test measures the clotting time of recalcified plasma and indicates the overall efficiency of the extrinsic clotting system (Bain *et al.,* 2012).

Before the advent of modern medicins our ancestors relied on natures wonders to stay healthy. One of the most popular traditional medicines is garlic. Garlic is renowned for its remarkable ability to fight numerous diseases. Many studies had been carried out to examined the effects of Garlic oil on prothrombin Time and other coagulation parameters. Previous studies found change (prolongation) on prothrombin time and some of other parameters. So this study may highlight on the effect of garlic oil on blood coagulation parameters (prothrombin time test), and the ability to replace chemical drug with it to reduce side effect of chemical drug.

1.3. Objectives:

1.3.1. General objective:

-To study the effect of garlic oil on prothrombin time level among healthy sudanese volunteers.

1.3.2. specific objectives:

-To determine adose of garlic oil that has aminimum effect on prothrombin time level.

-To assess the effect of two dose 20% and 30% garlic oil on prothrombin time level.

-To determine the garlic oil effect on prothrombin time level according to gender,and age group.

Chapter II
Literature Review

CHAPTER II

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 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 :

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 aggregating activity 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).

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 and then 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).

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).

Platelets Adhesion:

platelets adhere to 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).

Platelets aggregation:

It is the process in which adherent platelets become activated 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).

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 serotonin released 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 multimters made up on average of 2-50 subunits, with a molecular weight (MW) of 0.8 - 20 x 106.

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 a transaminase.

There are three groups in which coagulation factors can be classified:

-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 For conversion of Prothrombin to thrombin.

Factor V, Proaccelerin or Labile Factor:

This is consumed during clotting and accelerates the transformation of Prothrombin to

thrombin. A vitamin 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 platelet function; 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 thromboplastin 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 abnormal injured 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 Pathway:

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 at not only the formation of the clot but also if the clot lazes after24 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 production and function of thrombin which are Circulating antithrombin, The protein C/thrombomodulin mechanism and Tissue factor pathway inhibitor activation. Anti-thrombin complexes with thrombin, there by 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 (the binding to protein C is strongly enhanced 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. Activated protein C, on the surface of activated platelets (where the 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 (Abdel Gader, 2009).

2.1.12. 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 terminus of 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 (Hoff brand *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. Garlic Oil :

Garlic (Allium sativum L) originated in central Asia. Its plant has been used as a flavoring agent and traditional medicine since time immemorial (Dharmatti ,2019). Garlic oil is derived from the crushed bulbs of garlic. They have powerful and obnoxious odour due to the presence of disulphides. The bulbs contain 0.06% to 0.1% of volatile oil whose active constituents are propyl / -disulphide, alliin and allicin. The yellow oil of garlic is a valuable flavouring agent in perfumery and it is called broad-spectrum antibiotics in medicine.

Garlic oil used in medicin in treatment of such symptoms as heart palpitations caused by high cholesterol, chest tightness, myocardial infarction, headache, limb numbness, dizziness, cerebral embolism, Antibacterial, disinfection, killing viruses, such as foot, ringworm, herpes, parasites, food poisoning and so on, inhibition of tumor growth, inhabitation or termination of cancer cell growth, Effective in promoting blood circulation, releasing fatigue, improving and strengthening the body, increasing immune function and beautifying, anti-aging and improving impotence function, lower blood glucose, and improve glucose tolerance functions (AL-saadi ,2013).

Garlic (Allium sativum) has the potential to modify the risk of developing atherosclerosis by reducing blood pressure, thrombus formation, and serum lipid and cholesterol levels (Stevinson *et al*, 2000**).** These effects are primarily attributed to the sulphur containing compounds, particularly allicin and its transformation products. Commercial garlic preparations may be standardized to a fixed alliin and allicin content (Rybak *et al.*, 2004).

Garlic inhibits platelet aggregation in vivo in a dose-dependent fashion . The effect of one of its constituents, ajoene, appears to be irreversible and may potentiate the effect of other platelet inhibitors such as prostacyclin, forskolin, indomethacin, and dipyridamole (Rahman, 2007). Although these effects have not been consistently demonstrated in clinical trials (Scharbert *et al.,* 2007), there are several cases in the literature on excessive dietary garlic intake or use of garlic as a medicine associated with coagulation alterations(Borrelli *et al.,*2007). In addition to bleeding concerns, garlic has the potential to decrease systemic and pulmonary vascular resistance in laboratory animals, an effect that was observed in clinical studies as well (Reinhart *et al.,*2008). in one clinical study garlic oil selectively inhibited CYP2E1 activity

(Gurley *et al* .,2002) , it is still difficult to predict drug interactions with garlic (Shi and Klotz , 2012).

2.3. Previous study:

Garlic was reported by many studies as anti hypertension and reducing thrombus formation and serum lipid and cholesterol levels (Stevinson *et al.*,2000).

beside this study done by Narjis Hadi Mansoor Al-saadi (2013) reported that garlic oil showed anticoagulant activity and there were highly significant different ($P<0.0001$) at(25, 50 and 75 ul) volume (AL-saadi ,2013).

other study that conducted by Omran M.O Alhamami, Jabbar Y.Al- Mayah, Najah R.Al-Mousawi, Alaa G.H.Al-Aoboodi in 2006, which reported that prolongation of APTT and PT in hyperlipidemic rats after 4 weeks of treatment with garlic (200 mg/kg) is highly significant ($p<0.001$) (Omran *et al.*,2006).

study conducted by Yeganeh M , Khojir Yeganeh Rad R in 2007, which reported that garlic has no significant change in values obtained for CT, PT, PC and CR($P>0.05$) (Yeganeh and Khojir ,2007).

Study conducted by Kung-Chi Chan, Mei-Chin Yin, Wan-Ju Chao in 2007, which reported that Garlic Oil supplement at 5 or 50 mg garlic oil\kg bodyweight significantly prolonged bleeding time, thrombin time, and enhanced anticoagulation factor activity, such as antithrombin III and protein C ($P<0.05$) (Kung-Chi *et al.*, 2007).

Study conducted by Noha Elrayah Eltieb Alaraky in 2018, Which reported that the prolongation OF Prothrombin time and INR in health population after eating garlic is highly significant ($P<0.001$) (Alaraky, 2018).

Chapter III
Materials and Methods

CHAPTER III

3.Materials and Methods:

3.1.Study design:

This was crossectional and interventional (before- after) study.

3.2. Study area and duration:

The study was conducted at Khartoum state in the period from November 2019 to March 2021 .

3.3. Study population :

Apparently healthy volunteers to study the effect of garlic oil on prothrombin time (PT). PT time was estimated for each participant before and after adding of garlic oil for plasma samples.

3.4. Inclusion criteria:

-Health people don't suffer from disease or taking any type of medication.

-Adult male and female from 20 to 30 years.

3.5. Exclusion Criteria:

-Smokers.

-children and elderly.

3.6. Sample Size:

This study included 50 healthy individuals .

3.7. Sample collection:

1.8ml of venous blood to 0.2 ml of 3.2% tri sodium citrate was collected from each participant using disposable sterile syringe after dis infecting collection site with 70% alcohol, platelet poor plasma was prepared by centrifugation at 4000 rpm for 15 minute. then PT test done within two hour of sample collection .

3.8. Data collection:

Data collected directly from participants using a design questioner by interviewed.

3.9. Reagent and Instruments:

-Calcified thromboplastin (fortress) .

-Dimethylsulfoxide (DMSO) solvent .

-Garlic oil (commercial from the local market).

-Water bath .

-Centerifuge .

-Automatic piped.

-Stop watch.

3.10. Principles and Procedures:

3.10.1. Principle of Prothrombin Time Test:

The PT test measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system(Bain *et al.*, 2012).

3.10.2.Assay Procedure:

-How I chose this (20% and 30%) volumes ?

Through multiple trials to define the affected volume of garlic oil on prothrombin time (PT) .

-Preparation of garlic mixsure (garlic oil + dimethylsulfoxide) 100 ul garlic oil to 200 ul DMSO in small glass tube and incubate for 5 minute at least in 37c° water bath.

-Plasma sample of each individual was divided into three group. Group 1 was tested first to determine the normal prothrombin time (pre sample positive control group), take 100 ul of plasma (ppp) into a glass tube place in a waterbath at 37c° wait for 1-3 min, then add 200 ul of thromboplastin reagent and start the time observe clot formation and stop watch at the appearance of the first fibrin web .

-other two groups in one small glass tube put 20 ul of garlic mixture + 80 ul of plasma (ppp) with gentle shaking and place in a waterbath at 37c° wait for 3-5 min, then add 200 ul of thromboplastin reagent and start the time observe clot formation and stop watch at the appearance of the first fibrin web . in other glass tube put 30 ul of garlic mixture + 70 ul of plasma (ppp) and then do the same procedure.

3.10.3. Normal Values:

10 – 20 seconds (depend on PT reagent).

3.11. Ethical consideration:

Participants were informed verbally in their simple language about the research, its

benefits and method of sample collection, then their approval taken.

3.12. Data analysis:

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

Chapter IV

Results

CHAPTER IV

4.Results:

This study was done in Khartoum state in the period from November 2019 to March 2021 to evaluate in-vitro the effect of Garlic oil on prothrombin time test .50 healthy Sudanese volunteers aged between 21-30 years were enrolled to participate in this study twenty two of them were females and twenty eight of them were males.

In this study the effects of the garlic oil as an anticoagulant agent (antithrombatic) 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). When garlic oil was added in different volumes (20% and 30%) to plasma samples of normal individuals, the results revealed highly significant differences (P <0.000) in clot formation comparing with control(pre prothrombin time) .

Table (4-1): Mean of Prothrombin Time before and after adding (20%,30%) garlic oil:

Statistical Analysis: paired sample T-test

Test	Mean (± SD)	N	P.value
Pre garlic oil	13.6 (±1.12)	50	0.000
Post 20% garlic oil	23.96 (±2.7)	50	
Pre garlic oil	13.6 (±1.12)	50	0.000
Post 30% garlic oil	41.08 (±7.8)	50	

*****Significant at <0.05

The statistical analysis of the results showed that there is a significant difference (P.value = 0.000) among participants PT between before 13.6 (±1.12) and after 20% garlic oil 23.96 (±2.7), 30% garlic oil 41.08(±7.8) samples (Table 4.1).

Table (4-2): Mean of Prothrombin Time in both sexes before and after adding (20%, 30%) garlic oil:

Statistical Analysis : Independent sample T-test.

Sex	Pre garlic oil	Post 20% garlic oil	Post 30% garlic oil
Male	13.64 (±1.12)	24.1 (±3.21)	44.00 (±6.63)
Female	13.54 (±1.14)	23.7 (±1.99)	37.36 (±7.9)
P value	0.765	0.671	0.002

***Significant at <0.05**

The mean of prothrobin time in male before was 13.64 (±1.12) and in female before was 13.54 (±1.14), whereas the mean of PT in post garlic oil (20%, 30%) adding respectively samples collected from females was 23.7(±1.99), 37.36(±7.9) while it was 24.1(±3.21), 44.00(±6.63) in samples collected from males. The results showed there is no significant difference between males and females in the Pre and Post 20% of garlic oil adding samples (p.value = 0.765 and 0.671) respectively, but there is significant difference between males and females in Post 30% garlic oil adding samples(p.value 0.002).

Table (4-3): Mean of Prothrombin Time before and after adding (20%, 30%) garlic oil according to age:

Statistical Analysis : Independent sample T-test.

Age group	Pre garlic oil	Post 20% garlic oil	Post 30% garlic oil
21 – 25	13.6 (±1.1)	23.8 (±2.7)	40.7 (±7.0)
26 – 30	13.5 (±1.0)	24.1 (±2.8)	41.6 (±9.2)
P value	0.919	0.692	0.703

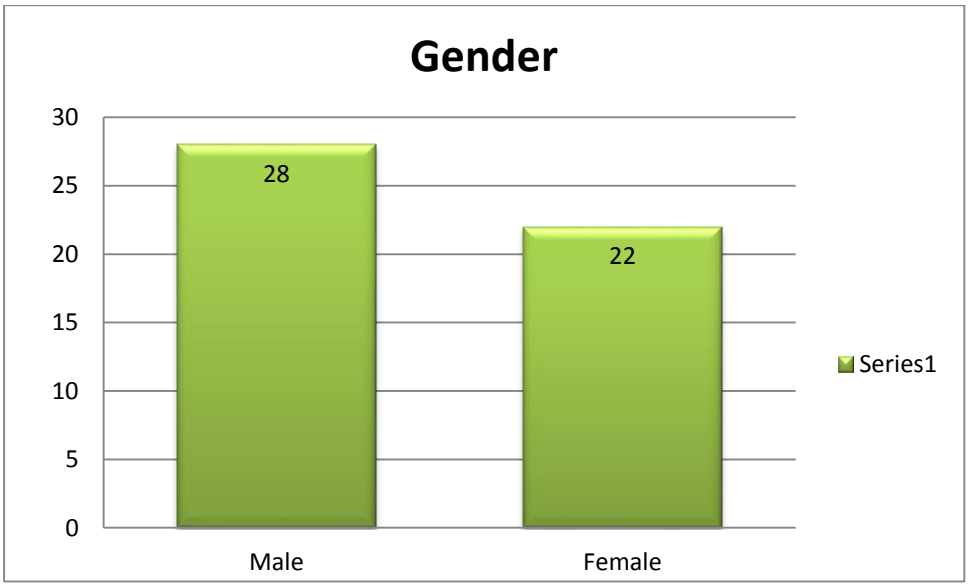
*Significant at <0.05

When we compared the pro thrombin time level in the different age group, the mean PT for Pre- garlic oil adding samples among group one (21 – 25 years) was 13.6 (±1.1) and among group two (26 – 30 years) was 13.5(±1.0) , while the mean of PT of post- garlic oil 20%, 30% adding respectively samples among group one 23.8(±2.7), 40.7(±7.0) respectively and it was 24.1(±2.8), 41.6(±9.2) 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 20%, 30% samples. (p.value 0.919, 0.692 and 0.703) respectively.

Table (4-4): Frequency of study group according to age groups:

Age group	Frequency	Percent %
21 - 25	31	62%
26 - 30	19	38%
Total	50	100%

Figure (4.1) distribution of study group according to gender:



Chapter V

Discussion, Conclusion, and Recommendation

CHAPTER V

5. Discussion , Conclusion, and Recommendation

5.1. Discussion :

In recent years, naturally occurring chemical substances derived from plants have attracted interest as possible treatmeants for coagulation disorders and as template molecules for the development of new drugs. Medical research has focused on garlic's potential value in treating cardiovascular disorders and as an anti-cancer agent.

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 garlic oil 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 pro thrombin time level in the post 20%, 30% garlic oil samples were significant higher than the mean of pre-samples, our results agrees with findings of study done by Narjis Hadi Mansoor Al-saadi (2013) reported that garlic oil showed anticoagulant activity and there were highly significant different ($P<0.0001$) at volume (25, 50 and 75 ul).

Another study that conducted by Omran M.O Alhamami, Jabbar Y .Al-Mayah, Najah R.AlMousawi,Alaa G.H.AI-Aoboodi in 2006 in Kufa Medical College animal house, which agreed with our study and reported that-prolongation of APTT and PT in hyperlipidemic rats after 4 weeks of treatment with garlic(200 mg/kg) is highly significant ($p<0.001$). But our result disagree with a study conducted by M.A Yeganeh , R Khojir Yeganeh Rad in 2007, which reported that garlic has no significant effect on pro thrombin time .

No significant difference when we compared the PT in both male and female the mean of pre-garlic oil add among female was 13.54(±1.14) and among male was 13.64(±1.12), where the mean of PT in post 20% garlic oil add samples collected from female was23.7(±1.99) while it was 24.1(±3.21) in samples collected from male.

But there is significant difference between male and female in post 30% garlic oil add samples , mean of PT among samples collected from female was 37.36(±7.9) while it was 44.00(±6.63) in samples collected from male (p.value <0.002).

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 garlic oil was 13.6(±1.1) , 13.5(±1.0) respectively .while the mean of PT after adding 20% of garlic oil was 23.8(±2.7) , 24.1(±2.8) respectively ,mean of PT after adding 30% of garlic oil was 40.7(±7.0) , 41.6(±9.2) respectively .

5.2. Conclusion :

The study concludes that:

-Garlic oil can affect pro thrombin time level.

-No variation on the PT according to sex in Pre and Post 20% of garlic oil, but there is difference between them in Post 30% of garlic oil adding.

-No variation on PT according to age before and after adding 20%, 30% of garlic oil.

-Garlic oil can be usful as antithrombotic agent ; the prolongation of PT suggests inhibition of the extrinsic coagulation pathway (Lee *et al.*, 2012).

5.3. Recommendation :

-Perform intensive in-vivo study on this topic in different population with increase sample size.

-And other study to evaluate effect of garlic oil on other coagulation parameter such as (APTT, CT, TT, BT).

- Perform intensive effort to using garlic as antithrombotic agent in acceptable form.

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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 : Dimethyl Sulfoxide (DMSO)