بسم الله الرحمن الرحيم

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Effect of Allium Sativum (Garlic) Intake on Prothrombin Time and International Normalize Ratio

A thesis Submitted for partial fulfillment of the Msc Degree in Medical Laboratory Sciences in (Haematology)

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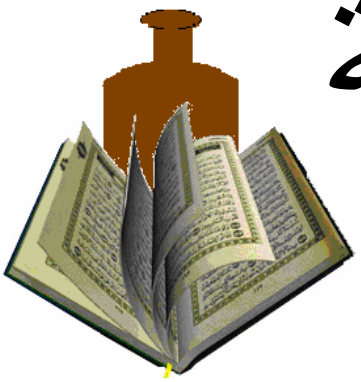
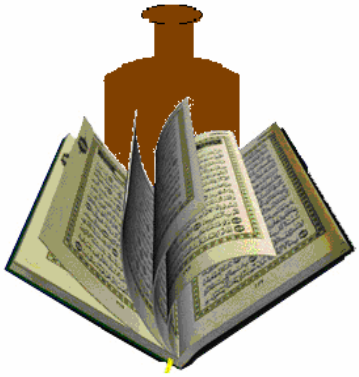


الآيـــــة

بسم الله الرحمن الرحيم

قال تعالي :

﴿ لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ ﴾



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Dedication

To the one who was there to love and care for me when the skies were grey and when I was down, she was always there to comfort me ,no one else could be what she has been to me

to the queen of my heart,

To my mother .

To my father.

It's insipid without you to anyone who one day drew a smile on my face, the person who perfume my life with happiness

My husband,,,

To the driving forces in my life,

Sisters and brothers

To my favorite friends,

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, but my love and respect which is there for you ever since u accepted me as your student is the least I can give for my whole life. Very grateful to you
Dr: Omkalthoum Osman Hamad
thanks my all friends who always like my post and support and help me and stand with me thanks from the bottom of my heart to my all true helping and lovely friends
special thank to my friend **Omila Fath Alaleem**
My thank also extend to my college and my teacher
lastly, I offer my regards to all of those who supported me in any aspect during the completion of this research.

List of abbreviation

Abbreviation	Term
GP	Glycoproteins
(MW)	Molecular Weight
VWF	Von Willebnllld Factol'
ADP	Adinin Diposphat
HMWK	High Molecular-Weight Kininogen
Ag	Antigen
PT	Prothrombin Time
APTT	Activated Partial Thromboplastin Time
TT	Thrombin Time
FDPs	Fibrin Degradation Products
DVT	Deep Vein Thrombosis
PE	Pulmonary Embolism
INR	International Normalize Ratio
ISI	International Sensitivity Index
TF	Tissue Factor
ECs	Endothelial Cells
Ca^{2++}	Ionized Calcium
TFPI	Tissue Factor Pathway Inhibitor
PF_3	Platelet Phospholipid
BK	Brady Kinin
$Cacl_2$	Calcium choloride
tPA	tissue-Plasminogen Activator
PAI-1	Plasminogen Activator Inhibitor - 1
CT	Clotting Time
PC	Platelet Count
CR	Clot Retraction
BT	Bleeding Time

Abstract

This is expermintal study which conducted in Shendi town to determine the effect of garlic on the prothrombin time and INR during the period from April to July 2018.

Total of 60 blood samples were collected from health population before and after eating garlic with different age and sex,2.5ml of citrated platelet poor plasma sample were collected and tested for prothrombin time by using manual method and then calculated INR. The result show that mean of PT before eating garlic 14.0 seconds and after eating garlic was 14.5 seconds, and mean of INR before eating garlic 1.1 and after eating garlic 1.2. also the results show that mean of PT in both sex male, female before eating garlic were 14.2, 14.0 and after eating garlic were 14.6, 14.4 respectively and mean of INR in both sex before eating garlic 1.1 and after eating garlic 1.2 .

According to age (20-25 , 26-31 , 32-37) mean of PT before eating garlic (14.3, 14.1, 13.8) and after eating garlic (14.6, 14.4, 14.0) respectively.and mean of INR before eating garlic (1.14 , 1.13, 1.10) and after eating garlic (1.17, 1.16, 1.12) respectively.

Statistical analysis shows that there significant variation between prothrombin time and INR before and after eating garlic. and no variation in INR between both sex before and after eating garlic . Prothrombin Time and INR were decrease according to increase of age before and after eating garlic. This study was concluded that the prothrombin time and INR effected when eating garlic.

ملخص البحث

أجريت هذه الدراسة بمدينة شندي خلال الفترة من ابريل إلى يوليو ٢٠١٨م وهـدفت هذه الدراسة إلى معرفة تأثير الثوم على الثرومبين. تم أخذ٦٠ عينة من أفراد أصـحاء قبـل وبعد تناول الثوم بمقدار ٢،٥ من الدم الوريدي في مانع تجلط tri sodium citrate بمقـدار ٢٥, وتم إرسال العينات تحت ظروف مثالية لحمايتها من التلوث ليتم تحليلها يدويا. وجد فـي الدراسة بعد تحليل النتائج إحصائيا بواسطة SPSS أن الوسط الحسابي للثرومبين قبل تنـاول الثوم ١٤,٠ ثانية وبعد تناول الثوم ١٤,٥ ثانية. كما وجد في الدراسة أن الوسط الحـسابي ل INR قبل تناول الثوم ١,١وبعد تناول الثوم ١,٢. وأن الوسط الحسابي للثرومبين للذكور قبل تناول الثوم ١٤,٢ وبعد تناول الثوم ١٤,٦وللاناث قبل تناول الثوم ١٤,٠ وبعد تناول الثـوم ١٤,٤.كما وجد أن الوسط الحسابي ل INR لكل من الجنسين ١,١ قبل تناول الثوم و ١,٢ بعد تناول الثوم. و الوسط الحسابي للثرومبين لكل من الأعمار (٢٠-٢٥،٢٦-٣١،٣٢-٣٧) قبل تناول الثوم (١٤,٣،١٤,١،١٣,١) وبعد تنـاول الثـوم (١٤,٤،١٤,١،١٤,٠) علـى التـوالي والوسط الحسابي ل INR قبـل تنـاول الثـوم(١,١٤،١,١٣،١,١٠) وبعـد تنـاول الثـوم (١,١٧،١,١٦،١,١٢).

خلصت الدراسة إلى أن الثوم يؤثر علي زمن الثرومبين.

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1.1 Introduction

Blood coagulation is the process by which free-flowing blood forms semi-solid, gel-like clots to limit further bleeding and begin repairing the damaged vessel. However, disorders of blood coagulation lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis). At the macromolecular level, blood clotting is a series of proteolytic events in which zymogens of serine proteases are converted into active enzymes, ultimately causing fibrin clot formation and platelet activation [1,2].

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). [3]

The prothrombin time (PT) is a screening test for the extrinsic clotting system [3] measures factors VII, X, V, prothrombin and fibrinogen. Tissue thrombo- plastin (a brain extract) and calcium are added to citrated plasma. The normal time for clotting is 10-14 s. It may be expressed as the international normalized ratio (INR).[4]

The activated partial thromboplastin time (APTT) is a screening test of the intrinsic clotting system [3] 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. The normal time for clotting is approximately 30- 40s. [4]

Garlic (Allium sativum) is a popular vegetable with a variety of medicinal properties. Garlic bulbs are edible, inexpensive and are readily available. 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 immunomodulatory [5-7]. Garlic consumption is an alternative thrombolysis medicine, which has been used for many years in different cultures [8]. Allicin, one of the garlic components, could have therapeutic effects, including anti-microbial effect, immunostimulating properties, improve fibrinolytic activity, inhibit platelet aggregation and adhesion and also reduce blood pressure [8].

1-2 Rationale

The INR is specific measure of the body clotting ability. However, disorders of blood coagulation lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis). Garlic (Allium sativum) is a popular vegetable with a variety of medicinal properties and is used as food appetizer in our daily life. 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. So that in this study we attempt to determine the effect of garlic on the Prothrombin Time and INR.

1-3 Objectives

General objective

To determine the effect of Allium Sativum (garlic) intake on prothrombin time and international normalize ratio.

Specific objective

1. To determine the effect of garlic on prothrombin time and INR among both sex.
2. To determine the effect of garlic on prothrombin time and INR according to age.

Chapter Tow

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. [9]

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 . [10]

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.[10]

2.1.1.2.Platelets:

Platelet production:

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast arises by a process of differentiation from the haemopoietic stem cell the megakaryocyte matures active, by end mitotic synchronous replication enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets. The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days. [(9)]

Platelet function:

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, the main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions.

Platelet adhesion and activation:

Following blood vessel injury, platelets adhere to exposed sub endothelial matrix proteins via specific adhesive glycoproteins (GP). Under condition high shear, e.g. arterioles, the exposed subendotrlial Mahdi is it usually coated with VWF mealtime .The platelets than make contact with VWF via the GPIb-XI-V complex on platelets. This initiates platelet rolling in the direction of blood flow over the exposed VWF with activation of GPIIb/IIIa receptor. Firm adhesion is established by the slower stronger interaction of other glycoproteins inclutlg

activated GPIIb/IIIa with VWF and GPVI and integrin (alpha1/beta2) with collagen and other composer of the sub endothelial matrix. Under static or low shear conditions, platelets adhere predominantly to collagen of the sub endothelium. Collagen binds initially to GPIa/IIa, crosslinks many of these ingrain molecules, and in this way activates platelets This ligand receptor binding results in a complex cascade of signals which result in platelet activation The events that follow are shape change and spreading, activation of GPIIb/IIIa and granule section. Platelets become more spherical and extrude long pseudopodia which enhance platelet vessel wall and platelet-platelet interaction. The end result of spreading is a flattened spread out platelet with granules and organelles in the Centre, resulting in a characteristic fried egg appearance. These changes are brought about by the actin cytoske.

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 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. [9]

Platelet aggregation:

It is characterized by cross-linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has about 50-80 000 GPIIb/IIIa receptors, which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/Illa molecules, due to binding of alpha-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb/IIIa, enabling platelet cross-linking with fibrinogen bridges. Binding brings about molecular conformational changes resulting in a firm connection and further activation of the platelet. [9]

Clot formation and retraction:

The highly localized enhancement of ongoing platelet activation by ADP and TXA2 results in a platelet plug large enough to plug the area of endothelial injury. In this platelet plug the platelets are completely de- granulated and adherent to each other. This is followed by clot retraction which is mediated by GPIIb/IIIa receptors which link the cytoplasmic actin filaments to the surface bound fibrin polymer. [9]

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. [11]

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, moderately stable, and not consumed during coagulation. [12]

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 a vitamin 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. [12]

Prekallikrein, Fletcher Factor:

This is a surface contact activator, in which 75% is bound to HMWK. [12]

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. (12)

2.1.5. Laboratory Model of Coagulation:

Laboratory testing looks at the in vitro effect of the coagulation process which is measured by the Prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrin degradation products (FDPs), and D-dimer. This section will focus on PT and a PTT. While the coagulation cascade does not reflect what goes on in vivo, it provides a model in which the laboratory relates to for testing. However, the coagulation cascade reflects the mechanisms that the laboratory uses for results. The screening tests provide a tremendous amount of information to the physician. They can be performed both quickly and accurately. (12)

2.1.6. Extrinsic Pathways:

The extrinsic pathway is initiated by the release of tissue thromboplastin that has been expressed after damage to a vessel. Factor VII forms a complex with tissue thromboplastin and calcium. This complex converts factors X and Xa, which in turn converts Prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin. This process takes between 10 and 15 seconds.

-Prothrombin time (PT): developed by Armand Quick in 1935 measures the extrinsic system of coagulation. It is dependent upon the addition of calcium chloride and tissue factor. It uses a lipoprotein extract from rabbit brain and lung. PT uses citrate anti coagulated plasma. After the addition of an optimum concentration of calcium and an excess of thromboplastin, clot detection is measured by an automated device for fibrin clot detection. The result is reported in seconds. PT is exclusive for factor VII, but this test is also sensitive to decreases in the common pathway factors. Therefore, if a patient presents with a prolonged PT and there is no other clinical abnormality or medication, the patient is most likely

factor VII deficient. The PT is also used to monitor oral anticoagulation or warfarin therapy used to treat and prevent blood clots. In many instances, patients are placed on life-long therapy and the dosage is monitored by the PT test. The attempt in anticoagulant therapy is to impede thrombus formation without the threat of morbidity or mortality from hemorrhage. Warfarin is an oral anticoagulant, which means it must be ingested. It was discovered in 1939 at the University of Wisconsin quite by accident. It seems that a farmer found that his cattle were hemorrhaging to death, for what appeared to be no reason. The cattle were grazing in a field eating sweet clover. This contains dicumarol, actually bus hydroxyl Coumadin, which caused the cattle to bleed.

There are several compounds of Coumadin:

Dicumarol, indanedione, and warfarin. Dicumarol works too slowly, and indanedione has too many side effects. Warfarin or 4-oxycoumarin is the mostcommonly used oral anticoagulant. Coumadin works by inhibiting the y-carboxylation step of clotting and the vitamin K–dependent factors.[12]

2.1.7. Intrinsic Pathway:

Contact activation is initiated by changes induced by vascular trauma. Prekallikrein is required as a cofactor for the auto activation of factor XII by factor XIIa. XI is activated and requires a cofactor of HMWK. XIa activates IX to IXa, which in the presence of VIIIa converts X to Xa. Also present are platelet phospholipids PF3.

Calcium is required for the activation of X to proceed rapidly. The reaction then enters the common pathway where both systems involve factors I, II, V, and X. This results in a fibrin monomer polymerizing into a fibrin clot. Factor XIII, or fibrin stabilizing factor, follows activation by thrombin. This will convert initial weak hydrogen bonds, cross-linking fibrin polymers to a more stable covalent bond. [12]

-Activated Partial Thromboplastin Time :APTT measures the intrinsic pathway. The test consists of decalcifying plasma in the presence of a standardized amount of platelet-like phosphatides and an activator of the contact factors. It will detect abnormalities to factors VIII, IX, XI, and XII. The APTT is also used to monitor heparin therapy. Heparin is an anticoagulant used to treat and or prevent acute thrombotic events such as deep vein thrombosis (DVT), pulmonary embolism (PE), or acute coronary syndromes. The action of heparin is to inactivate factors XII, XI, and IX in the presence of anti-thrombin.[12]

2.1.8.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 at not only the formation of the clot but also if the clot lazes after 24 hours. [12]

2.1.9. 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.[12]

2.1.10. 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.

2.1.11. Fibrinolysis:

The fibrinolysis system is responsible for the dissolution of a clot. Fibrin clots are not intended to be permanent. The purpose of the clot is to stop the flow of blood until the damaged vessel can be repaired. The presence or absence of hemorrhage or thrombosis depends on a balance between the procoagulant and the fibrinolysis

system. The key components of the system are plasminogen, plasminogen activators, plasmin, fibrin, fibrin/ FDP, and inhibitors of plasminogen activators and plasmin.6 Fibrinolysis is the process by which the hydrolytic enzyme plasmin digests fibrin and fibrinogen, resulting in progressively reduced clots. This system is activation of the contact factors. Plasmin is capable of digesting either fibrin or fibrinogen as well as other factors in the cascade (V, VIII, IX, and XI). Normal plasma contains the inactive form of plasmin in a precursor called plasminogen. This precursor remains dormant until it is activated by proteolysis enzymes, the kinases, or plasminogen activators. Fibrinolysis is controlled by the plasminogen activator system. The components of this system are found in tissues, urine, plasma, and lysosome.

Granules, and vascular endothelium. An activator, tissue-plasminogen activator (tPA) results in the activation of plasminogen to plasmin resulting in the degradation of fibrin. The fibrinolysis system includes several inhibitors. Alpha-2-antiplasmin is a rapid inhibitor of plasmin activity and alpha- 2 macroglobulin is an effective slow inhibit.

of plasmin activity. This system is in turn controlled by inhibitors to tPA and plasmin-plasminogen activator inhibitor 1 (PAI-1) and alpha-2-antiplasmin. Reduced fibrinolytic activity may result in increased risk for cardiovascular events and thrombosis. Pharmacologic activators are currently used for therapeutic thrombolysis, including streptokinase, urokinase, and tPA. Urokinase directly activates plasminogen into plasmin, and streptokinase forms a streptokinase plasminogen complex, which then converts plasminogen into plasmin. [9]

2.1.12. Coagulation Inhibitors:

Inhibitors are soluble plasma proteins that are natural anticoagulants. They prevent the initiation of the clotting cascade. There are two major inhibitors in plasma that keep the activation of coagulation under control.[13]

These inhibitors are:

- Protease inhibitors: inhibitors of coagulation factors, which include:

• Anti thrombin.

• Heparin cofactor II.

• Tissue factor pathway inhibitor.

• Alpha-2-antiplasmin.

• C1.

- The protein C pathway: inactivation of activated cofactors, which include:

• Protein C and protein S.

2.1.13. 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.[12]

2.2. Garlic

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 [14]. These effects are primarily attributed to the 20ulphur-containing compounds, particularly allicin and its transformation products. Commercial garlic preparations may be standardized to a fixed alliin and allicin content [15].

Garlic inhibits platelet aggregation in vivo in a dose-dependent fashion [16,17]. 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 [18]. Although these effects have not been consistently demonstrated in clinical trials [19], there are several cases in the literature on excessive dietary garlic intake or use of garlic as a medicine associated with coagulation alterations [20]. One case report showed an interaction between garlic and warfarin, resulting in an increased INR [21]. 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.[22]

In an early study in rats, alliin was absorbed quickly after oral administration and eliminated after 6 h. Allicin was absorbed slowly after oral administration, and its plasma peak level appeared between 0.5 h and 2 h. Even four days later, allicin could still be detected in the rats [21]. Although in one clinical study garlic oil selectively inhibited CYP2E1 activity [23], it is still difficult to predict drug interactions with garlic [24,25].

2-3 Previous study:

Garlic was reported by many studies as anti hypertension and reducing thrombus formation and serum lipid and cholesterol levels [14]. and inhibit platelet aggregation [16-17].beside this study conducted by M.A Yeganeh, R Khojir Yeganeh Rad in 2007, which reported that garlic has no significant effect on prothrombin time.[26] and 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 ($p<0.001$).[27]

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Study design:

This is expermintal study, conducted in Shendi Town during the period from April to July 2018 aimed to determine the effect of garlic on prothrombin time and INR.

3.2 Study area:

The study was done in Shendi Town which is located in the north of Sudan and north of the capital Khartoum and for about 173km, and to the south of Aldamer for about 127km, located in the east side of the river Nile, and covering area about 30km, most of people are farming.

It contain three hospitals and health centers, also there is Shendi University with various faculties like faculty of medicine and health science.

3.3 Study Population:

Health people to measure PT and calculate INR before and after eating garlic.

Inclusion criteria:

-adult male and female from 20 to 37 years.

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

Exclusion criteria:

-children and elderly.

-anyone who have disease such as hypertension and heart disease.

-anyone take medication such as warfarin and heparin.

3.4 Sampling:

Sixty venous blood samples were collected into tubes containing 0.25 ml of 3.2% trisodium citrate ratio(9:1). The sample centrifuged at 3000g for 10 minutes to prepare platelet poor plasma.

3.5 Ethical considerations:

Informed consent was attached to each questionnaire to be obtained from the patient verbally. There was full commitment precaution sample taken and privacy and confidentiality.

3.6 Data collection tools:

The primary data will be collected by using questionnaire.

3.7 Method:

3.7.1 Prothrombin time (PT):

3.7.1.1 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 ($CaCl_2$) which indicates over all the efficiency of the extrinsic clotting system.

3.7.1.2 Reagents and materials:

Phospholipid + ca^{2+} + tissue factor.

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

3.7.1.3 Assay procedure:

Firstly collect blood sample from health people before eating garlic and measure pt and then calculate the INR, then about (2-3)g of garlic must be eaten by the volunteer and then collect other blood sample after (3-4)hour from eating garlic and also measure pt and calculate INR.

-prothrombin time procedure: Pipette 0.1ml plasma in to clean test tube and pre warm within 2-3 min at $37c^{o}$ add 0.2ml of thromboplastin (pre warm 2-3 min in $37c^{o}$) and start the time observe clot formation and stop watch at the appearance of the first fibrin web get the main of the dabble reading .

3.7.1.4 Normal value:

10 – 15 seconds (depend on PT reagent)

3.7.0.1.5 Calculation:

The result may be reported directly in terms of the mean of the double determination of PT of the test plasma in seconds.

Or as a ratio "R": R = mean of the patient plasma PT in second / MNPT for the reagent.

Or as International Normalized Ratio (INR), $INR = R^{ISI}$

Chapter Four

Results

4-Results

Table (4-1): Mean of Prothrombin Time before and after eating garlic.

Test	Mean	N	P value
PT before	14.0	60	0.000
PT after	14.5	60	

The mean of prothrombin time before was 14.0 and after was 14.5, the study found that highly significant b/w tow mean at p value 0.000.

Table (4-2): Mean of INR before and after eating garlic:

Test	Mean	N	P value
INR before	1.1	60	0.000
INR after	1.2	60	

The mean of INR before was 1.1 and after was 1.2, the study found that highly significant b/w tow mean at p value 0.000.

Table (4-3): Mean of Prothrombin Time in both sex before and after eating garlic:

Sex	Before	After
Male	14.2	14.6
Female	14.0	14.4
P value	0.278	0.219

The mean of prothrobin time in male before was 14.2 and in female before was 14.0, and the mean of prothrombin time in male after was 14.6 and in female was 14.4, the study found that no clinical significant variation in prothrombin time b/w both sex .

Table (4-4): Mean of INR in both sex before and after eating garlic:

Sex	Before	After
Male	1.1	1.2
Female	1.1	1.2
P value	0.539	0.397

The mean of INR in male before was 1.1 and in female before was 1.1, and the mean of INR in male after was 1.2 and in female after was 1.2, the study found that no clinical significant variation in INR b/w both sex.

Table (4-5): Mean of Prothrombin Time before and after eating garlic according to age:

Age	Before	After	N
20 – 25	14.3	14.6	18
26 – 31	14.1	14.4	30
32 – 37	13.8	14.0	12
Total			60

The mean of prothrombin time at age (20-25 , 26-31 , 32-37) before were (14.3, 14.1, 13.8) respectively, and after were (14.6, 14.4, 14.0) respectively, the study found that prothrombin time was decrease according to increase of age.

Table (4-6): Mean of INR before and after eating garlic according to age:

Age	Before	After	N
20 – 25	1.14	1.17	18
26 – 31	1.13	1.16	30
32 – 37	1.10	1.12	12
Total			60

The mean of INR at age (20-25 , 26-31, 32-37) before were (1.14, 1.13, 1.10) respectively, and after were (1.17, 1.16, 1.12) respectively, the study found that INR was decrease according to increase of age.

Chapter Five

Discussion

Conclusion

Recommendations

5.1 Discussion

This expermintal study conducted in Shendi Town during the period from April to July 2018. This study was aimed to know the effect of garlic on the prothrombin time and INR.

The result of study reveals that the prolongation of prothrombin time and INR in health population after eating garlic is highly significant ($p<0.001$) Prothrombin Time before eating garlic 14.0 and after 14.5 sec as shown in table (4-1) and INR before eating garlic 1.1 and after 1.2 as shown in table (4-2) the present result disagree with a previous study conducted by M.A Yeganeh , R Khojir Yeganeh Rad in 2007, which reported that garlic has no significant effect on prothrombin time[26],when compared with other previous 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 ($p<0.001$).[27]

Regarding the results of similar tests according to gender Prothrombin Time before eating garlic was 14.2 in male and in female 14.0 and after eating garlic male 14.6, female 14.4 as shown in table (4.3) the results show no clinical significant variation in PT b/w both sex, and Prothrombin time was increase in both sex after eating garlic male before eating garlic 14.2 and after 14.6,and in female before eating garlic 14.0 and after eatin 14.4 as shown in table (4.3), and INR in both sex before eating garlic 1.1 and after 1.2 as shown in table (4.4) this result show that no different in INR according to sex before eating garlic and no different after eating garlic. And INR was increase in both sex after eating garlic in male before eating garlic was 1.1 and after 1.2, and in female before eating garlic was 1.1 and after 1.2 as shown in table (4.4).

The results of the study reveals that Prothrombin Time and INR were decrease according to increase of age (20-25, 26-31, 32-37) years Prothrombin time

before eating garlic (14.3, 14.1, 13.8) and after eating garlic (14.6, 14.3, 14.0) respectively as shown in table (4.5) . and INR before eating garlic (1.14 , 1.13 , 1.10) and after eating garlic (1.17, 1.16, 1.12) respectively as shown in table (4.6).and the results show that increase of PT after eating garlic in all age, PT in age (20-25) before eating garlic 14.3 and after 14.6,and in age (26-31) before eating garlic 14.1 and after 14.3,and in age (32-37) before eating garlic 13.8 and after 14.0 as shown in table(4.5). The results also show increase of INR in all age, INR in age (20-25) before eating garlic 1.14 and after 1.17, and in age (26-31) before eating garlic 1.13 and after 1.16, and in age (32-37) before eating garlic 1.10 and after 1.12 as shown in table (4-6).

5.2 Conclusion

It can be concluded that:

1. Garlic affect Prothrombin Time and INR.
2. No variation on the Prothrombin Time according to sex before and after eating garlic.
3. No variation on the INR according to sex before and after eating garlic.
4. Decrease on the Prothrombin Time according to increase of age before and after eating garlic.
5. Decrease on the INR according to increase of age before and after eating garlic.
6. Garlic can be useful as antithrombotic agent.

5.3 Recommendations

1. Perform intensive study on this topic in different study area with increase sample size.
2. Perform other study to evaluate effect of garlic on other coagulation parameter such as (APTT – CT – TT – CR – PC – BT).
3. Perform intensive effort about using garlic as antithrombotic agent**.**

Chapter Six

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Appendix

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Questionnaire

Shendi University

Assessment of effect of Allium sativum (garlic) intak on prothrombin Time and International Normalize Ratio

Questionnaire No ( ) :

1-Name: ……

2-Age: ……

3-Gender:

Male ( ) Female ( )

4-Residence: ……

5-Underling disease: ……

a. Yes ( ) b. No ( )

If yes mention: ..…….

6-Do you consume any type of drug:

a. Yes ( ) b. No ( )

If yes mention: ……

investigations:

1). PT (pre): ……

2).PT (post): …… **Date: / /**