Sudan University of Science and Technology

College of Graduate Studies

Assessment of the Effect of Green tea Consumption on Fibrinogen Among Adult Healthy Sudanese Volunteers.

تقييم تاثير إستهلاك الشاي الأخضر على الفيبرينوجين وسط المتطوعين السودانيين الأصحاء

"A Dissertation Submitted in partial Fulfillment of the Requirements of M. Sc Degree in Medical Laboratory Sciences (Hematology)"

Submitted By:

Abeer Ahmed Hmed Ali

BSc. Of Khartoum University

Supervisor:

Dr. Ibrahim Khider Ibrahim

(B.Sc.; M.Sc.; Ph.D.) in hematology

January-2019

الآيـــــــة

قال تعالي:

((.....يَرْفَع اللهُ الَّذِينَ ءَامَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا العِلمَ دَرَجَاتٍ وَاللهُ بِمَا تَعْمَلُونَ خَبِيْرٌ))

صدق الله العظيم

سورة المجادلة ــالاية ((11))

Dedication

To my mother

To The spirit of my father

To my dear husband and my children

To my friends

To my teachers

Acknowledgment

- All our thanks are to Allah who gave us health and strength to complete this research.

- Also particular thanks to our supervisor Dr. Ibrahim Khider for his unlimited support guidance throughout this research and his concentration on every little detail. It is difficult to find words to appreciate his effort. May Allah Reward him all good for what he did for me, and without him thus work would not have been accomplished.

- My sincere thanks extended to all volunteer participants that they participate effectively in this research. Finally special thanks to anyone who helped me in this study.

Abstract

Background

Camellia tea is a product made up from leaf and bud of a sinis plant. People around the world drink green tea as an everyday drink and as a therapeutic aid in Many illnesses. Fibrinogen is the main constituent that form at the blood clot. Elevated fibrinogen level raises heart disease risk by (60 to 90%). This study aimed to draw attention to importance of healthy green tea and explore the effect of green tea on fibrinogen level. This can be achieved through experimental study.

Materials and Methods

In this study venous blood sample 9:1 in trisodium citrate 3.2% were collected from healthy adult controls (n=30) (13male and 17female) randomly selected. The fibrinogen level was determined using the STA Compact Max.

Result

There was statistical significant difference (P.value <0.05) in the fibrinogen level before and after consumption of green tea (before 3.31±0.45) (after 2.29±0.41). Moreover there was statistically significante in levels of fibrinogen between males and females. The mean of fibrinogen level in male before and after were (3.12±0.49)(2.13± 0.44) respectively and in female were (3.46±0.35) (2.41±0.34) respectively. This study didn't obtained any statistically significance in fibrinogen levels between different age group.

Conclusion

This study concludes that drinking two cups of green tea per day reduce the level of fibrinogen. Also the present study obtained statistically significant difference between male and female with regard to fibrinogen level. However didn't revealed significant differences regard to age group.

الملخص

المقدمة:

الشاي الأخضر مصنوع من لحاء وأوراق نبات ميني (الكليليا), العديد من الاشخاص حول العالم يشربون الشاي الأخضر كعادة يوميه وأيضاً علاج للعديد من الأمراض. مادة (الفيبرينوجين) هي المكون الرئيسي للجلطة والمستويات العالية من هذه المادة تذيد من خطورة الإصابة بامراض القلب (60−90%) هذه الدراسة تهدف إلي تسليط الضوء علي اهمية الشاي الأخضر الصحى عبر دراسة تجريبية.

المواد والطرق المستخدمة :

في هذه الدراسة استخدم عينات دم وريدى محتوي علي مضاد تجلط (ثلاثي الصوديوم سترين تركيز3.2%)بنسبة 9 (من عينة دم المريض الي 1 (من مضاد التجلط) جمعت من أفراد أصحاء بالغين (ن=30)(13 من الرجال و 17من النساء) ثم اختبارهم عشوائياً. وثم قياس مستوى الفيبرينوجين.

النتائج:

تمت المقارنة بين مستوي الفيبرينوجين قبل وبعد استهلاك الشاي الأخضر لمدة شهر من قيل المتطوعين وكان للفروقات دلالة احصائيه في مستوي الفيبرينوجين.p<0.05

حيث كان المتوسط قبل الشرب(3.31±0.45) وبعد الشرب (2.29±0.41), وكان هنالك دلالة في الفروقات بين مستوى الفيبرينوجين بين الرجال والنساء حيث كان المتوسط في الفرق بين الرجال (3.12±0.49) (2.13±0.44) تباعاً , وفي النساء (3.46±0.35) (2.41±0.34) علي التوالي , ولم يكن هنالك فرق في الدلالة الإحصائيه بين الفئات العمرية المختلفه.

الملخص:

من هذه الدراسة استنتج أن بشرب كوبين من الشاي الأخضر الصحى يومياً يقلل نسبة تكوين (الفيبرينوجين) بشكل ملحوظ , وثم ملاحظة فرق واضح في مستويات (الفيبرينوجين) بين الجنسين , ولم يكن هناك فرق ملحوظ (يذكر) في الفئات العمرية المختلفه.

LIST OF CONTENT

List of Table

List of figure

List of Abbreviation

ADAMTS	A disintegrate associated metal proteases with thrombospondin
CAD	Coronary artery disease
Ca++	Calcium
CaCl2	Calcium chloride
DDAVP	1-deamineno -8-D-arginine vasopressin
FDPs	Fibrin degradation product
GPIIb/IIa	GlycoproteinIIb/IIa
HMWT	High molecular weight
M.WT	Molecular weight
MPV	Mean Platelet volume
PAI-1	Plasminogen activator inhibitors 1
PAI-2	Plasminogen activator inhibitors 2
PF3	Platelet factors3
PL	Phospholipids
RT	Prothrombin time
RNA	Ribonucleic acid
SD	Stander deviation
TAFI	Thrombin activator fibrinolysis inhibitor
TFPI	Tissue factor pathway inhibitors
T-PA	Tissue Plasminogen activator
VWF	Von will brand factor

Chapter One

Introduction and Literature review

1. Introduction and Literature review

1.1 Introduction

Green tea is a product made from the Camellia saneness plant and is commonly used as beverage worldwide (Gupta et al,2014 ; Jigsaw et al,2012). From the ancient time, green tea and its constituents show role in health management via modulation of biological process including molecular and biochemical pathways. Green tea shows health promoting effects mainly due to the polyphenol content (Cabrera et al,2006 ; Batten et al,2015) especially flavones, which constitutes 30% of fresh leaf dry weight (Hayat et al,2015 ; Wang et.al,2009). The chief constituents of green tea are catechism where (−)-epically catching gal late is one of the most effective types of catechism (Rowe et al,2010). However, the consumption of steamed green tea has various beneficial pharmacological effects (Babe et al,2008) According to the published researches, tea could be beneficial to one's health, such as reduction of the incidence of hyperlipidemia, atherosclerosis and anti-oxidant (Fernandez et al,2002) Black tea and green tea are powerful sources of flavonoids and other polyphenol antioxidants, which have a protective effect in coronary artery disease (CAD) (Lin et al,2003 ; Liu et al, 2015; Lambert et al 2007). It was also shown that catching contained in green tea prevents the cell proliferation of arterial wall muscle. (Lantana et al,2015). The protective effects of flavonoids contained in green tea are not only antioxidant, antithrombotic, and anti-inflammatory properties, but also additive to the rate of the coronary flow velocity reserve (Henning et al, 2004).

Fibrinogen (factor I) is a glycoprotein that in vertebrates circulates in the blood During tissue and vascular injury it is converted enzymatically by thrombin to fibrin- based blood clot. Fibrinogen functions primarily to occlude blood vessels and thereby stop excessive bleeding. However, fibrinogen's product, fibrin, binds and reduces the activity of thrombin. This activity, sometimes referred to as antithrombinI, serves to limit blood clotting. Loss or reduction in this antithrombin1 activity due to mutations in fibrinogen genes or hypo-fibrinogen conditions can lead to excessive blood clotting and thrombosis (Stomach et al,2009).

Fibrin also mediates blood platelet and endothelial cell spreading, tissue fibroblast proliferation, capillary tube formation, and angiogenesis and thereby functions to promote tissue revascularization, wound healing, and tissue repair (Clifford al, 2013).

Reduced and/or dysfunctional fibrinogens occur in various congenital and acquired human fibrinogen-related disorders. These disorders represent a clinically important group of rare conditions in which individuals may present with severe episodes of pathological bleeding and thrombosis; these conditions are treated by supplementing blood fibrinogen levels and inhibiting blood clotting, respectively (Lee et al ,1995) Certain of these disorders may also be the cause of liver and kidney diseases(Yang et al,1998) Fibrinogen is a "positive" acute-phase protein, i.e. its blood levels rise in response to systemic inflammation, tissue injury, and certain other events. It is also elevated in various cancers. Elevated levels of fibrinogen in inflammation as well as cancer and other conditions have been suggested to be the cause of thrombosis and vascular injury that accompanies these conditions (Chow et al, 2001).

1.2 Literature review

1.2.1 Hemostasis:

Normal hemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors. The efficient and rapid mechanism for stopping from sites blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and 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., 2011).

When a blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding:

1. Vessel wall contraction.
2. Platelet adhesion and aggregation (platelet plug formation).

3. Plasmatic coagulation to form a brim clot. All three mechanisms are essential for normal homeostasis.

Abnormal bleeding usually results from defects in one or more of these three mechanisms. For a better understanding of the pathogenesis of pathological bleeding, it is customary to divide hemostasis in to two stages (i.e., primary and secondary hemostasis) (hunker et al, 2007).

Component of normal homeostasis:-

-The hemostatic mechanisms have several important functions:

a) To maintain blood in allied state while it remains car collating within the vascular system;

b) To arrest bleeding at the site of injury or blood loss by formation of a hemostatic plug;

c) To ensure the eventual removal of the plug when healing in complete.

Normal physiology thus constitutes a delicate balance between these conflicting tend incise, and a deficiency or ex aggregation of any one may lead to either thrombosis or hemorrhage. There are at least five different components involved: blood vessels, platelets, plasma coagulation factors, and the fibrinolysis system (Mitchell et al; 2006).

-Coagulation involves both a cellular (plate late) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and, therefore, the best –understood. Coagulation is highly conserved throughout biology; in all mammals, coagulation begins almost instantly after an injury to the blood vessel which has damaged the endothelium (lining of the vessel), this releases phospholipids components called tissue factor and fibrinogen that initiate a chain reaction. Platelets immediately form a plug at the site of injury; this is called May hemostasis. Secondary hemostasis occurs simultaneously. Proteins in the blood plasma, called coagulation factors or clotting factors, respond in complex cascade to form fibrin strands, which string then the platelet. (Grimed and claim; 2001).

1.2.1.1 Primary hemostasis:

primary hemostasis is the term used for the instantaneous plug formation upon injury of the vessel wall, which is achieved by vasoconstnction, platelet adhesion, and aggregation. (Hunker et al; 2007)

1.2.1.1.1 Platelet production:

Platelets are produced predominantly by the bone morrow megakaryocytes as a result of budding of the cytoplasmic membrane. Megakaryocytes are derived from the haemopoetic stem cell, which is stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin. Once released from the bone marrow young platelets are trapped in the spleen for up to 36hours before entering the circulation, where they have a primary haemostatic role. Their normal lifespan is 7-10 days and the normal platelet count for all age groups is 150-450 x10/1. The mean platelet diameter is 1-2m and the normal range for all cell volume (MPV) is 8-11 fl. Although platelets are non-nucleated cells, those that have recently been released from the bone marrow contain RNA and are known as reticulated platelets. They normally represent 8-16% of the total count and they indirectly indicate the state of marrow production (Drew .P, 2003).

The main steps in platelet function are adhesim, activation with shape change and aggregation when the vessel wall is damaged. The sub endothelial structure including basement membrane collagen and meiro fibrils are exposed, surface bound VWF binds to Gelb on circulatory platelet resting in an initial Monday of adhesion platelet. Binding via Gelb initiate activation of the platelet via a G portion mechanism once activated platelet immediately change shape from a disk to a tiny sphere with numerous projecting pseudo pods (Casella; 2007).

1.2.1.1.2 Platelet function:

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) interaction.

The blood flow conditions determine the specific receptor ligand interactions (Hoff brand, et al., 2011).

1.2.1.1.3 Platelet adhesion and activation:

Following blood vessel injury, exposing the deeper structures of the vessel wall platelets not only adhere to the surface but also undergo the release reaction, facilitating further platelet aggregation and activating blood coagulation on their surfaces, Platelet adhesion to collagen type I and III requires the plasma protein von will brand factor (abbreviated FVIII: VWF), which acts as a link between the specific platelet glycoprotein receptor I and the sub endothelial cells (Anne .Steinem-Martine et al., 1992).

1.2.1.1.4 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 multiverse made up on average of 2-50 demerit subunits, with a molecular weight (MW) of 0.8-20 x 10^6. VWF is encoded by a gene on chromosome 12 and is synthesized both in endothelial cells and megakaryocytes, and stored in weibel-palade and platelet granules respectively (Hoff brand et al., 2011).

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, exercise, adrenaline and infusion of decompressing (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from weibel-palade bodies is in the form of 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., 2011).

1.2.1.2 Secondary hemostasis:

Intrinsic system is activated in vivo by the contact of certain coagulation proteins with sub endothelial connective tissue which sets the secondary hemostatic mechanism into motion extrinsic coagulation pathway in contrast is initiated with the release of tissue factor from injured vessels lumen. Tissue factors is high molecular

weight lipoprotein is found in most organs including lungs-kidneys-liver-brain-placenta-and spleen. As well as in large blood vessels such as vena cava and aorta, Both the intrinsic and the extrinsic coagulation path ways lead to the secondary hemostasis namely the formation of fibrin clot thus includes both fibrin formed in secondary hemostasis and the platelets plug formed in primary hemostasis (Anne .Steinem-Martin et al., 1992).

The intrinsic and extrinsic pathways are series of reactions that involve coagulation factors known as enzyme precursors (zymogen), non-enzymatic co factors and calcium. A fourth component is PL, All coagulation factors are present normally in plasma, with PL being provided by platelets. The zymogens are factors (II. VI. IX, X.XII and prekalikrein), The cofactors are (V-VII-tissue factor and HMWK). Zymogen are substrates that have no biologic activity until converted by enzyme to activate enzymes called serine proteases which have exposed serine-rich active enzyme sites. Serine proteases selectively hydrolyze arginine or lysine containing peptide bounds of other zymogens, thus converting them to serine proteases (Anne. Steinem-Martin et al., 1992).

The activation of zymogen factors X and II requires the presence of the non-enzymatic cofactors.VII and V respectively to perform their function. These cofactors must be activated (Via and VA) by small amounts of thrombin. Thrombin enhances their ability to assist in the activation of factor X, II respectively, although high concentrations of thrombin inhibit VII.V activity. Cofactors assist in the activation of zymogens by either altering zymogen conformation to permit more efficient cleavage by the serine protease or binding the zymogen and appropriate serine protease on platelets PL surface to enhance and accelerate the zymogen activation process or both (Anne. Steinem-Martin et al., 1992).

In contrast the hemostatic process also provides amplification of the control mechanisms that prevent excessive clotting and thrombosis. Inhibitors and thrombolytic factors maintain a balance in the system between clotting and clot lists. While tissue is be in repaired, the fibrinolysis system slowly dissolves the clot with the glycoprotein plasmin. Although plasmin is capable of digesting many proteins

(fibrin, fibrinogen, factor V, VIII) it is also held in check by several inhibitors (Anne, Steinem-Martine et al., 1992).

1.2.1.3 Classification of coagulation factors:

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

1. 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.

2. 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.

3. The contact group: factors XI, factors XII, prekalikrein, and high-molecular-weight kininogen (Cheesbrough, 2000).

Table (1.1) Coagulation factors:

Factors number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/cofactor
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart-prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Contact factor	Serine protease
XIII	Fibrin-stabilizing factor	Transglutaminase
	Prekalikrein	Serine protease
	HMWK	Cofactor

1.2.1.4 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 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 Via and tissue factor.

These two pathways meet at the common pathway, where they both generate factor Ax 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 (Cheesbrouge, 2000).

1.2.1.5 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 thromboplastic time (APTT), thrombin time (TT), fibrin degradation products (FDPs), and D-dimer. This section will focus on PT and 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. (Cheesbrough, 2000).

1.2.1.6 Pathways:

The Coagulation Cascade:-

The coagulation cascade of secondary hemostasis has tow pathways, the contact activation pathway (Formerly known as the intrinsic pathway), and the tissue factor pathway (formerly known as the extrinsic pathway), while lead to fibrin formation. It was previously thought that the coagulation cascade of two pathways of equal

importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the tissue factor pathway. The pathways are a series of reactions, in while a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-link eel fibrin. Coagulation factors are generally indicated by Roman numerals, with a lower case appended to indicate an active form (Casella; 2007).

-The coagulation factors are generally serine proteases (enzymes). There are some exceptions, for example, FVIII and FV are glycoprotein, and factor XIII is a Trans glutamines. Serine proteases act by cleaving other pretense at specie sites. The coagulation factors correlate as in active zymogens. The coagulation cascade is classically dwindled in to three pathways the tissue factor and contact activation pathways both activate the (final pathway) of factor X, thrombin and fibrin (Casella; 2007).

1.2.1.6 .1 Extrinsic pathways: "tissue factor pathway"

The main role of the tissue factor pathway is to generate a "thrombin burst" a process by while thrombin, the most important constituent of the coagulation cascade in terms at its feedback activation roles, is released instantaneously. Via circulates in a higher amount than any other activated coagulation factor (Casella; 2007).

-The extrinsic pathway is initiated by the release of tissue thromboplastic that has been expressed after damage to a vessel. Factor VII forms a complex with tissue thromboplastic and calcium. This complex converts factors X and Ax, which in turn converts prothrombin to thrombin, then thrombin activation other components of the coagulation cascade, including FV and FVII (which activates FXI, which in turn, activates FIX), and activates and releases FVIII from being bound to vows (Casella; 2007).

Then thrombin converts fibrinogen to fibrin this process takes between 10 and 15 seconds, The extrinsic pathway is initiated by the release of tissue thromboplastic that has been expressed after damage to a vessel. Factor VII forms a complex with tissue

thromboplastic and calcium. This complex converts factors X and Ax, which in turn converts prothrombin to thrombin.(Ciesla;2007).

1.2.1.6.2 Intrinsic System: "Contact activation pathway"

Contact activation is initiated by changes induced by vascular trauma. Prekalikrein is required as a cofactor for the auto activation of factor XIII by factor XIIa. XI is activation and required a factor of HMWK. XIa activation IX to IXa, which in the presence of VIIIa converts X to Xa. Also present are platelet phospholipids, Calcium is required for the activation of X to proceed rapidly. The reaction then enters the common pathway where both systems involve factors I, III, V, and X. this result in a fibrin monomer polymerizing into a fibrin clot. Factor XIII, or fibrin stabilizing factor, follows activation by thrombin. This wills covert initial weak hydrogen bonds, cross-linking fibrin polymers to a more stable covalent bond (Hoff brand et al., 2011).

1.2.1.6.3 Common Pathways:

Thrombin is present from the very beginning, already when platelets are making the plug. Thrombin has a large array of functions, not only the conversion of fibrinogen to fibrin, the building block of a haemostatic plug. In addition, it is the most important platelet activator and on top of that it activates VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates factor XIII, which forms covalent bonds that crosslink the fibrin polymers that from activated monomers (Palliser CJ, and Watson MS, 2010).

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tense complex, until it is down-regulated by the anticoagulant pathways (Palliser CJ, and Watson MS, 2010).

1.2.1.7 Co-Factors:

-Various substances are required for the proper functioning of the coagulation cascade: calcium and phospholipid (a platelet membrane constituent) are required for the tense and thrombinoase complexes to function. Calcium mediates the binding of

the complexes via the terminal gamma. Car boxy residues on Fax and Fix to the phospholipid surfaces expressed by platelets, as well as procoagulant microparicles or micro vesicles shed from them. Calcium is also required at other points in the coagulation cascade (Casella; 2007).

- Vitamin k is in essential factor to a hepatic gamma-glut a my L car boxy lase that adds a car boxy L group to glutamine allied residues on factor II, VII, IX, and X, as well as protein S, protein C and protein Z. In adding the gamma- car boxy L group to glutamate residues on the immature clotting factors vitamin K is itself oxidized. Another enzyme, vitamin K peroxide reeducates, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reeducates is pharmacologically important as a target for anticoagulant drugs warfarin and related coumarone sue has a cenocoumarol, phenprocoumon, and dicumarol.

These drugs create a deficiency of reduced vitamin K by blocking VKORC, there by inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g., in malabsorption), or disease (hepatic cellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (panties form eel in vitamin K absence); this causes partial or non-gamma carboxylation, and affects the coagulation factors ability to bind to expressed phospholipid (Casella; 2007).

-AS mention above hemostasis is achieved by a highly integrated process involving blood vessels together with the platelet and number of plasma protein which participate in the coagulation and fibrinolysis pathway the factors involved in the coagulation cascade are numbered I, II, and V through XIII. Factor I is fibrinogen, which factor II (Fibrinogen's immediate precursor) is called prothrombin. Most of the coagulation factors are made in the liver, which needs an adequate supply of vitamin K to manufacture the different clotting factors (Joel and Make; 2009).

1.2.1.8 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-Ixia 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 Ixia. 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 due hydrogen bonding.

Formation of fibrin occurs in three phases:

1. Proteolysis: protease enzyme thrombin cleaves fibrinogen resulting in a fibrin monomer, A and B fibrin peptide.

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

3. Stabilization: This occurs when the fibrin monomers are linked covalently by Ixia into fibrin polymers forming an insoluble fibrin clot, (figure 1.1) (Palliser CJ, and Watson MS, 2010).
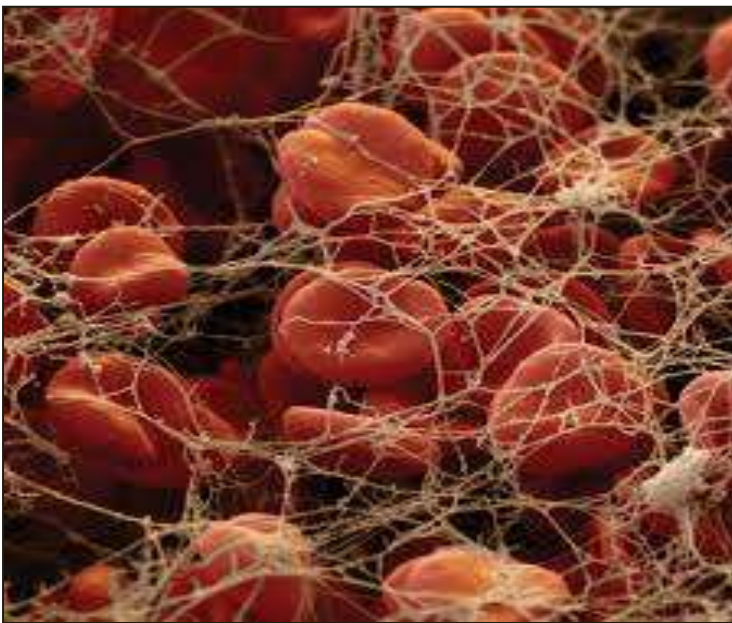

Figure 1.1 Fibrin Clot

1.2.1.9 Fibrinolysis:

Is process that prevents blood clots from growing and becoming problematic? This process has two types: primary fibrinolysis and secondary fibrinolysis. The primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause,

In fibrinolysis, a fibrin clot, the product of coagulation, is broken down. Its main enzyme plasmin cuts the fibrin mesh at various places, leading to the production of circulating fragments that are cleared by other proteases or by the kidney and liver.

Plasmin is produced in an inactive form, plasminogen, in the liver. Although plasminogen cannot cleave fibrin, it still has an affinity for it, and is incorporated into the clot when it is formed (Cesarman-Maus G and Haj jar KA 2005).

Tissue plasminogen activator (t-PA) and urokinase are the agents that covert plasminogen to the active plasmin, thus allowing fibrinolysis to occur. t-PA is released into the blood very slowly by the damaged endothelium of the blood vessels, such that, after several days (when the bleeding has stopped), the clot is broken down. This occurs because plasminogen became entrapped within the clot when it formed; as it is slowly activated, it breaks down the fibrin mesh (Contra, Razes, et al 2005).

T-PA and urokinase are themselves inhibited by plasminogen activator inhibitor-1 and plasminogen activator inhibitor-2 (PAI-1 and PAI-2). In contrast, plasmin further stimulates plasmin generation by producing more active forms of both tissue plasminogen activator (T-PA) and urokinase. Alpha 2-antiplasmin and alpha 2-macroglobulin inactivate plasmin.

Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor (TAFI), which modifies fibrin to make it more resistant to the tape-mediated plasminogen (Cot ran, Rami S.; et al 2005).

1.2.1.10 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.

These inhibitors are:

1. Protease inhibitors: inhibitors of coagulation factors, which include

- Anti-thrombin
- Heparin cofactor II
- Tissue factor pathway inhibitor
- Alpha-2-antiplasmin
- C1

2. The protein C pathway: inactivation of activated cofactors, which includes

- Protein C and Protein S (Hoff brand et al., 2011).

1.2.1.11 Fibrinogen:

-Fibrinogen is one of these plasma proteins it is a soluble glycoprotein that is synthesized by the liver. Process in the coagulation cascade activates the zymogene prothrombin to the serine protease thrombin, which is responsible for converting fibrinogen into fibrin. Fibrin is then cross linked. Factor XIII to form a clot, Fixit stafibrin (also called factor IA) is a fibrin protein involved in the clotting of blood, and is non-globular. It is a febrile protein that is polymerized to form a mesh that forms a hemostatic plug or clot (in conjunction with platelets) over a wound site (Hoff brand et al; 2001).

1.2.1.11.1 Fibrinogen Structure:-

The fibrinogen molecule is a 340-kDa homodimeric glycoprotein consisting of 2Aα, 2Bβ, and 2γ polypeptide chains linked by 29 disulfide bridges. Fibrinogen synthesis occurs primarily in hepatocytes . Assembly of the 6 chains takes place in a stepwise manner in which single chains assemble first into Aα-γ and Bβ-γ complexes, then into Aα/ Bβ/γ half-molecules, and finally into hexametric complexes (Aα/Bβ/γ) (Redman et al,2001) . 1 All 6 fibrinogen chains are assembled with their N termini

located in a central E nodule and extend outward in a coiled-coil arrangement. The Bβ and γ chains terminate in globular regions known as βC and γC modules, respectively. These regions collectively comprise the so-called D nodule. The Aα chains are the longest; at the end of the coiled-coil region, each chain extends into a highly flexible series of repeats followed by a globular αC region. Using high-resolution atomic force microscopy, Protopopova et al obtained striking images of fibrinogen that visualize each of these structural components. In healthy individuals, fibrinogen circulates in plasma at high concentrations (2–5 mg/mL). However, fibrinogen is an acute phase protein, and during acute inflammation, plasma fibrinogen levels can exceed 7 mg/mL. The fibrinogen chains are encoded in 3 genes that are thought to have arisen through gene duplication. Mechanisms that regulate expression of the fibrinogen genes are still largely undetermined. Genome wide association studies have identified single-nucleotide polymorphisms within the fibrinogen genes,3 as well as loci distinct from fibrinogen that implicate transcription factors (eg, hepatocyte nuclear factors 1 and 4 [TCF1 and HNF4], signal transducer and activator of transcription (Protopopova AD,2015), and inflammatory signaling pathways downstream of interleukin-65 in fibrinogen gene expression. In addition, microRNAs (miR) in the hsa-miR-29 family and hsa-miR-409-3p down regulate fibrinogen expression in hepatoma cells in vitro (Huffman et al,2015), revealing mechanisms that may fine-tune fibrinogen levels in response to environmental cues. Fibrin Formation, Structure, and Stability During coagulation, fibrinogen is converted into insoluble fibrin. Fibrin formation involves thrombin-mediated proteolytic cleavage and removal of N-terminal fibrin peptides from the Aα and Bβ chains. Insertion of these newly exposed α- and β-knobs into a- and b-holes in the γC and βC regions of the D nodule, respectively, on another fibrin monomer permits the half-staggered association of fibrin monomers into protofibrils. Subsequent aggregation of protofibrils into fibers yields a fibrin meshwork that is essential for blood clot stability. This process has been extensively reviewed (De Varies PS et al,2016; Fort A et al,2010); Clot formation, structure, and stability are strongly influenced by the conditions present during fibrin generation. These include the concentrations of

procoagulants, anticoagulants, fibrin(ogen)-binding proteins, molecules as well as contributions of blood and vascular cells, cell-derived microvesicles, (Machlus KR et al,2011); and presence of blood flow31,32. Many of these mechanisms have been reviewed (Whyte CS et al,2016). The contribution of thrombin concentration to fibrin formation and structure has received considerable attention. High thrombin concentrations produce dense networks of highly branched fibrin fibers, and these clots are relatively resistant to fibrinolysis. In contrast, low thrombin concentrations produce coarse networks of relatively unbranched fibrin fibers, and these clots are relatively susceptible to fibrinolysis (Handerson SJ et al,2016). Most studies have reported that compared with fibers formed from low thrombin concentrations, fibers generated by high thrombin concentrations are thinner. However, turbidimetry and microscopy studies of fully hydrated clots suggest that high thrombin concentrations decrease the average protofibril content per fiber but only slightly decrease the fiber size, leading to a generally less compact fiber (Wolberg AS et al.2002). Thus, the substantially thinner fibers observed at high thrombin concentrations in earlier studies may reflect fiber compaction or shrinkage that occurs during dehydration. Regardless, the association of fibrin clot structural parameters with clinical pathologies—dense networks of thinner/compact fibers with increased thrombotic risk (zubairova LD et al,2015); and coarse networks of thicker/ less compact fibers with increased bleeding risk suggests that fibrin structure is a critical determinant of hemostasis and thrombosis. (Whyte CS et al 2016);

1.2.1.11.2 Fibrinogen function:-

-Fibrinogen plays two essential roles in the body: it is a protein called an acute-phase reactant that becomes elevated with tissue inflammation or tissue destruction, and it is also a vital part of the "common pathway "of the coagulation process. In order for blood to clot, fibrinogen must be converted to fibrin by the action of an enzyme called thrombin. Fibrin molecules clump together to form long filaments, which trap blood cells to form a solid clot, The conversion of fibrinogen to fibrin is the last step of the "coagulation cascade ", a series of reactions in the blood triggered by tissue

injury and platelet activation. With each step in the cascade, a coagulation factor in the blood is converted from an inactive to an active form. The active form of the factor then activates several molecules of the next factor in the series, and so on, until the final step, when fibrinogen is converted into fibrin.

Fibrin (ogen) also participates in other biological functions including:

1. Molecular and cellular interactions of fibrin B 15- 42, which binds to heparin and also mediates platelet and endothelial cell spreading, as well as capillary tube formation via VE-Cadherin, an endothelial cell receptor ;

2. Leukocyte binding to fibrin (ogen) via integrin α MB2 (Mac-1), a receptor on stimulated monocyte and neutrophils;

3. Enhanced extracellular matrix interactions by binding to fibronectin;

4. Binding to the platelet α IIbB3 receptor, which facilitates platelet incorporation into a thrombus;

5. Enhanced plasminogen activation resulting from ternary tPA-plasminogen-fibrin complex formation;

6. Binding of inhibitors such as α 2-antiplasmuin, plasminogen activators inhibitor-2, lipoprotein (a), and histidine S. rich glycol protein, impairing fibrinolysis;

7. Down- regulation of factor XIII- mediated cross linking acuity via factor XIII A2 B2 complex binding to fibrin;

8. Anti thrombin I, a fibrin activity that inhibits thrombin generation in plasma by sequestering thrombin in the clot and by reducing the catalytic potential of fibrin-bound thrombin (Hoff brand et al; 2001).

1.2.1.11.3 Endogenous Mediators of Thrombin Generation and Fibrin

 Multiple mechanisms mediate thrombin generation and consequently the thrombin concentration present during fibrin formation. First, the levels of pro- and anticoagulants present during coagulation strongly influence procoagulant activity. For example, elevated levels of prothrombin are associated with increased thrombin generation (Gersh KC et al,2009), formation of dense fibrin networks, and increased venous thrombus weight in mice (Campbell RA et al,2008).These studies, designed to model the clinical situation in humans with the G20210A prothrombin mutation

associated with increased circulating prothrombin levels,44 suggest that increased thrombin generation enhances venous thrombosis risk in part by promoting abnormal fibrin deposition and structure. Second, the location of thrombin generation impacts fibrin network formation. Effective assembly and activity of the prothrombinase complex (factors Xa, Va, and prothrombin) requires a lipid surface (Jerome WG et al,2005). Localization of prothrombinase on a cell surface establishes a thrombin concentration gradient that influences both fibrin formation and network structure. In vitro experiments using in situ thrombin generation on fibroblasts and endothelial cells reveal a significantly denser fiber network proximal versus distal to the cell surface. These structural differences give rise to substantially different fibrinolytic susceptibilities in different regions of the clot; fibrin located near the cell surface is significantly more resistant to lysis than fibrin located distal to the cell surface. Third, blood flow (shear) present during fibrin formation influences local thrombin concentrations by (re)supplying procoagulant proteins and removing activated enzymes (Campbell et al,2010). Flow also aligns fibrin fibers, which may have profound effects on fibrin formation and mechanical and fibrinolytic stability (Crosby et al,2013). Furthermore, the shear rate affects clot formation triggered on tissue factor- plus collagen-coated plates, resulting in different fibrin deposition in different regions of a thrombus (Tucker EL et al,2009). Nanoindentation analysis to evaluate clot biophysical properties shows that this fibrin distribution pattern determines clot microelasticity, which may impact thrombus stability and risk of embolization. Fourth, thrombin movement through the thrombus is substantially influenced by solute transport mechanisms mediated by cell packing density; this may also influence the amount of fibrin deposition in different regions of the clot (Buller et al,2015).

1.2.1.11.4 Effects of Antithrombotic and Hemostatic Agents on Fibrin

Given the prominent role of thrombin concentration in determining fibrin network formation and structure, it is not surprising that antithrombotic agents that reduce thrombin activity reduce fibrin deposition and consequently thrombus formation. Because factor XI (FXI[a]) augments thrombin generation, in part by synergizing

tissue factor–mediated procoagulant activity, FXI inhibition strategies to reduce thrombosis have received considerable attention (Seligsohn U et al,2009). These approaches include conventional anti-FXI inhibitory antibodies, as well as technology in which antisense oligonucleotides (ASOs) result in the specific degradation of a target mRNA and corresponding reduction in target protein level. These studies reveal surprisingly specific effects of FXI inhibition in models of thrombosis and bleeding. Pharmacological FXI inhibition does not reduce local platelet adhesion in tissue factor and collagen-coated capillary tubes but reduces platelet activation and aggregation downstream of the growing thrombus (Asakai R et al,1991). Similarly, in an arteriovenous shunt model of thrombosis in nonhuman primates, neither anti-FXI antibodies nor ASOs alter platelet deposition on a collagen-rich segment of graft, but both decrease thrombus propagation (platelet accumulation and fibrin deposition) downstream of the collagenrich region (Zucker M et al,2014; Yakovlev S et al,2003). A promising phase I clinical trial demonstrated success of anti-FXI ASO treatment in humans undergoing elective total knee arthroplasty; ASO-mediated reduction of plasma FXI levels decreased symptomatic or asymptomatic venous thrombosis/thromboembolism (VTE) incidence, and the higher ASO dose tested was superior to enoxaparin (Yakovlev S et al,2003). In addition to its role in VTE, FXI also seems to contribute to atherogenesis in mice. Mice with deficiency in apolipoprotein E (Apoe−/−) spontaneously develop atherosclerotic lesions, but Apoe−/− mice with genetic FXI deficiency show reduced atherosclerosis progression (Yeromonahos C et al,2012). Moreover, anti-FXI ASO treatment reduces thrombus formation and fibrin deposition in a model of plaque rupture in Apoe−/− mice (Brummel-Ziedins KE et al,2014). Thus, FXI inhibition may also be effective for reducing arterial thrombosis in humans. Notably, FXI reduction has not been associated with increased bleeding in any of these studies, suggesting that FXI antagonism may be safer than current antithrombotics. However, given that bleeding occurs in a subset of patients with FXI deficiency (Kalathottukaren et al,2016); and the finding of altered structure and stability of plasma clots from these patients (Peyrou V et al,1999) the safety of FXI inhibition

should be carefully monitored in future trials. Regardless, these findings collectively support continued efforts to investigate and advance FXI inhibition strategies into the clinic. Heparin and heparin-like compounds are used to prevent thrombosis, presumably because of their ability to reduce thrombin activity. However, heparin binds to the central E nodule of fibrin (Horne MK III et al,2006) and both unfractioned heparin and low molecular weight heparin can also directly alter fibrin structure in an antithrombin-independent manner (Carvalho FA et al,2010). Observed changes include effects on fibrin fiber thickness, as well as porosity. These changes are not observed with the pentasaccharide, fondaparinux. Demonstration of these direct effects of unfractioned heparin and low molecular weight heparin on fibrin structure suggests tests that assess efficacy based solely on thrombin inhibition may not fully capture therapeutic effects of these drugs. Global assays that assess both thrombin generation and fibrin formation (Tutwiler V et al,2017) may more closely reflect therapeutic effects of these drugs. The common heparin reversal agent, protamine, also modulates fibrin network structure and stability. Protamine interacts directly with fibrinogen and is incorporated into clots, resulting in the production of thicker fibrin fibers in clots that are more susceptible to fibrinolysis.Recently, Kalathottukaren et al64 characterized a synthetic polycation they termed UHRA (universal heparin reversal agent) as an alternative to protamine. Universal heparin reversal agent can neutralize both heparin anticoagulant activity and polyphosphate procoagulant activity without the off-target effects on fibrin quality observed with protamine. Further studies to evaluate the therapeutic potential of universal heparin reversal agent are anticipated. (Fuchs TA et al,2010)

1.2.1.11.5 Clot Contraction

An essential function during coagulation is the platelet-mediated consolidation of clots in a process known as clot contraction (or retraction). This process involves fibrinogen binding to platelet integrin receptor αIIbB3 and is influenced by both platelet and fibrin(ogen) concentrations(Fuchs TA et al,2010). Although recognized as a fundamental process during coagulation, clot contraction has received little attention, particularly in a clinical setting. This gap is noteworthy given findings that

associate platelet aggregation and clot contraction with decreased clot permeability and increased resistance to fibrinolysis, 2 parameters thought to impact thrombosis risk.(Tutwiler et al,2010) evaluated the kinetics of clot contraction in blood samples collected from patients with recent acute ischemic stroke and correlated parameters with hemostatic and hematologic laboratory characteristics. Surprisingly, compared with clots from healthy individuals, whole blood clots from patients with recent ischemic stroke exhibit reduced clot contraction. However, because samples were collected after symptom onset, these changes may reflect a consequence, rather than cause, of the thrombotic event. Ischemic stroke patients had quantitative and qualitative defects in circulating platelets (decreased platelet count, shape change, and P-selectin exposure in unstimulated platelets and decreased fibrinogenbinding capacity of activated platelets), suggesting that the ischemic event may consume platelets and induce a refractory phenotype in circulating platelets that are not incorporated into the thrombus.(Tutwiler et al,2016).

1.2.1.11.6 Abnormal Fibrinogen and Fibrin Structure in Thrombosis

Production of clots with abnormal structure and stability has been demonstrated in plasma samples from patients with increased cardiovascular disease risk.38–40 After percutaneous coronary intervention, patients who develop in-stent thrombosis demonstrate abnormal plasma clot characteristics (eg, permeability, turbidity, and lysis time) compared with patients who did not develop in-stent thrombosis.(Tutwiler et al,2016). Similarly, compared with healthy controls, plasma clots from patients with abdominal aortic aneurysm have more densely packed fibrin networks with smaller pores and were more resistant to lysis (Collet JP et al,2001). Moreover, effects are aneurysm size dependent; patients with larger aneurysms have more densely packed fibers compared with patients with smaller aneurysms. In both in-stent thrombosis and abdominal aortic aneurysm patients, these effects on clot properties are independent of total fibrinogen levels but may be related to effects of other plasma proteins on fibrin formation. It remains unclear whether these fibrin clot abnormalities are only a biomarker for an operant pathophysiologic mechanism, or

whether abnormal fibrin clot structure is causative in the disease. (Tutwiler et al, 2016).

1.2.1.11.7 Fibrinogen Detection as a Diagnostic Tool:

VTE diagnosis includes imaging technologies such as Doppler ultrasound or computed tomography to detect deep vein thrombosis or pulmonary embolism, respectively (Kahn SR et al,2012). These technologies show vascular abnormalities and flow disturbances around the thrombus but do not reveal information about thrombus composition. Development of technologies that can detect thrombus composition may have clinical use. Notably, whereas early thrombi have substantial crosslinked fibrin content, this fibrin is replaced with collagen during thrombus resolution (Wolberg AS et al, 2002). Because fibrin-rich thrombi are more susceptible to fibrinolysis than collagen-rich thrombi, distinguishing early, fibrin-rich thrombi from older, collagen-rich thrombi may aid in identifying thrombi that are susceptible to fibrin-degrading thrombolytic therapy. Currently, assessment of thrombus age is highly subjective and only poorly able to identify patients who may respond to thrombolytic treatment.126 However, 2 recent studies of thrombosis detection in rodents have advanced methods to detect intravascular thrombi and reveal information about thrombus fibrin content. (Wakefield et al,2008). demonstrated the ability of a fibrin-binding probe,64 Cu-FBP8, to detect both venous and arterial thrombi in a single whole-body positron emission tomographic scan. Probe uptake correlated positively with fibrin content in both arterial and venous clots, distinguishing young (high probe uptake) from old (low probe uptake) thrombi. This positron emission tomography–based imaging method enables imaging of multiple thrombi in one examination and may be a noninvasive and sensitive approach to assess changes in thrombus composition over time. Similarly, the spatial and temporal uptake of a gadolinium-based fibrin-specific magnetic resonance imaging contrast agent, EP-2104R, also correlates positively with time-dependent changes in thrombus fibrin content. Furthermore, thrombi that exhibit high EP-2104R uptake are more susceptible to tissue-type plasminogen activator–mediated dissolution, suggesting that EP-2104R can be used to identify thrombi that are susceptible to

thrombolytic therapy (Patterson BO et al,2010). Additional studies are warranted to determine whether these methods can be used to identify human patients with greatest potential benefit of thrombolytic therapy. (Patterson BO et al,2010).

1.2.1.11.8 Normal Range:-

-Laboratory test results may vary depending on age, gender, health history, the method used for the test, and many other factors.

The following are considered to be normal results for this test.

Adults:150 — 400 mgldl (15 — 4gIL)

 Neonates: 125 — 300 mgldl (1.25 — 3gIL)

(William and Kern; 2002).

1.2.1.11.9 Fibrinogen Disorders:-

-Normal fibrinogen levels usually reflect normal blood clotting ability. Rarely, a person may have a sufficient quantity of fibrinogen, but the fibrinogen does not function normally. This is usually due to a rare inherited abnormality in the gene that produces fibrinogen, which leads to the production of an abnormal fibrinogen protein (dyes fibrinogenemia). If clinical finding suggest a fibrinogen problem, other specialized tests may be done to further evaluate fibrinogen function (William and Kern; 2002).

Fibrinogen is an acute phase reactant, meaning that fibrinogen concentration may rise sharply in any condition that causes inflammation or tissue damage. Elevated concentrations of fibrinogen are not specific. They do not tell the doctor the cause or location of the disturbance. Usually these elevations in the fibrinogen blood level are temporary; returning to normal after the underlying condition has been resolved. Elevated levels may be seen with: acute infections and cancer (William and Kern; 2002).

-Circulation, researchers discovered that high levels of this dangerous protein in the blood not only put individuals at a risk for heart disease, but can amplify the hazards of other standard heart attack risk factors such as smoking, obesity, and high cholesterol. Reduced fibrinogen levels can be found in liver disease, lung disease, bone marrow lesions, malnourishment, and certain bleeding disorders.

The low levels can be used to evaluate disseminated intravascular coagulation (DIS), a serious medical condition that develops when there is a disturbed balance between bleeding and clotting, other conditions related to decreased fibrinogen levels are those in which fibrinogen is completely absent (Congenital a fibrinogenemia), conditions in which levels are low (hypo fibrinogenemia), and conditions of abnormal fibrinogen (dys fibrinogenemia) obstetric complication or trauma may also cause low levels. Large – volume blood Trans fusions cause low levels because banked blood does not contain fibrinogen (William and Kern; 2002).

1.2.1.12 Green tea (camellia sinensis):

Tea is a popular drink worldwide. Cultivation of tea plants is economically important in many countries, and the tea plant, Camellia sinensis, is known to be grown in as many as 30 countries. Camellia sinensis grows best in certain tropical and subtropical regions (Guptan et al,2014). There are four main types of tea produced from this same plant, depending on how the tea leaves are processed. These teas are white, green, Oolong, and black tea. White tea is produced from very young leaves and buds that have not yet turned green, and the only processing is drying. Green tea is produced from mature leaves with minimal processing (only drying). Oolong tea is produced from partially fermented mature leaves, and black tea is produced from fully fermented mature leaves (Guptan et al,2014; Jigsaw et al,2012). Green tea, which makes up around 20% of tea production worldwide, is consumed most often in China, Korea, and Japan. Oolong tea is consumed most in China and Taiwan. Black tea (around 78% of tea production) is mostly consumed in the United States and the United Kingdom. Black tea contains up to three times the amount of caffeine as green tea (Cabrera et al, 2006; Batten et al,2015; Hayat et al,2015).

1.2.1.12.1 Chemistry of Green Tea:-

The components of green tea that are the most relevant medically are the polyphenols, with the flavonoids being the most important. The most pertinent flavonoids are the catechism, which make up 80%–90% of the flavonoids, and approximately 40% of the water-soluble solids in green tea(Wang Y et al,2009;Roowi S et al,2010;Babu et al,2008). The amount of catechism in the tea can

be affected by which leaves are harvested, how the leaves are processed, and how the tea is prepared. In addition, where the leaves are grown (geographically) and the growing conditions affect catching amounts(Cabrera et al, 2006;Fernandez et al,2002;Lin YS et al,2015;Liu M et al,2015; Lantana C et al,2017). Polyphenols are quickly oxidized after harvesting due to the enzyme polyphenol oxidase. To prevent loss of the polyphenols, green tea leaves are heated rapidly (most commonly by steaming or pan frying) to inactivate polyphenol oxidase. Black tea leaves are dried, then rolled and crushed, which promotes oxidation. Therefore, black tea has far fewer active catechism than green tea(Guptan et al,2014; Jigsaw et al,2012;Babu et al,2008). Green tea contains four main catechism: (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG). The most abundant of these in green tea is EGCG, which represents around 59% of total catechism. The next most abundant is EGC (around 19%), then ECG (around 14%), and EC (around 6%) (Jigsaw et al,2012; Cabrera et al, 2006; Babe et al,2008).

1.2.1.12.2 Origins and Nature of Tea:-

-Tea originated in china, Drinking water, boiled for reasons of hygiene, was made more platelet by the addition of leaves from the tea plant.

The world's most widely consumed beverage; tea has anecdotally been considered to have health-giving properties. The word "tea" has been used to describe the shrub camellia sinensis; the fresh leaves of this shrub picked as "two and abut" for processing (also termed "Flush" The processed Flush macerated and heat – dried in the case of green tea); and the beverage made by infusing the processed leaves in boiling water (peter et al; 2005).

1.2.1.12.3 Extracts of green tea and prepration:-

-Green tea extracts have been used in traditional Chinese and Indian medicine for a variety of uses. (Complementary and Alternative Medicine Guide. University of Mary land Medical Center. Retrieved 3May 2015)

 -Green tea leaves are initially processed by soaking in an alcohol solution, which may be further concentrated to various levels; by products of the process are also

packaged and used. Extracts may be sold in liquid, powder, capsule or tablet form (I.T.Johanson and G.William son; 2003).

-Green tea extract supplements are accessible over the counter in various forms. Standardized green tea extract is 90 percent total polyphone, and 1 capsule equals 5 cups of tea (A.H.Pressman and S.Buff; 1997, A.Bascom; 2002).

1.2.1.12.4 Some important properties of green tea:-

1. Antioxidant properties:-

Green tea and its supplements generally contain higher amounts of disease fighting anti. Oxidants called poly phenols. A plethora of evidence suggests strong antioxidant potentials of tea flavonoids in suppressing the production of excess free radicals. Major catechism present in green tea I e epicatechin (EC), epically catching gall ate (EGCG), epically catechism (EGC) and epicatechin gall ate (ECG) have strong antioxidant potentials. The higher antioxidant activity of green tea makes it more beneficial in protecting the body from oxidative damage due to free radical (Hamilton M; 2001).

2. Nano particles:-

-Nano technology has emerged as a promising technology that has been advocated for the delivery of antimicrobial phenolie compound extracts. There have been some recent efforts to enhance its bioavailability by delivering EGCG using lipid nano capsules and liposome encapsulation, suggesting the possibility of this molecule being developed further by medicinal chemists (Barras et al; 2009).

Phenolie compounds can be used as natural and safer alternative to chemical disinfectants in food systems and delivery of antimicrobial agents using nano particles to better control pathogens for commercial food safety applications (Ravichandran et al; 2011).

1.2.1.12.5 Biological activity of tea component:-

Health benefits of green tea:

health benefits of green tea depend on its bioavailability after consumption. In the body, the components in green tea may undergo metabolic processing such as glucuronidation, methylation, and sulfation, which produces active metabolites

(Lambert JD et al,2007). The catechism and their metabolites may be detected in blood plasma, urine, and various tissues. Studies on bioavailability are often conducted collecting specimens at timed intervals (after ingestion). Various studies have been conducted using normally prepared green tea beverages,ingested green tea extract (total catechism)(Henning SM et al,2004; Lee MJ et al,1995;Yang CS et al 1998) , or ingestion of specific catechism (Chow et al,2001); These studies have shown that ECG and EGCG, and metabolites of EC and EGC can be detected and measured in blood plasma. In urine, only metabolites of EC and EGC can be detected. Peak concentrations of components in blood plasma generally occur about 2 h after ingestion. Peak concentrations of components in urine generally occur between 4–6 h after ingestion. Certain studies have been conducted using various concentrations of catching, and generally show that the bioavailability of these substances is in proportion to the amount ingested, It has been suggested that levels of EC and ECG detected are too low to be of any therapeutic value, so most research considers only EGC and EGC. (Yang et al, 1998; Chow et al, 2001).

1. Effectiveness in skin damages:

Green tea is effective in the area of skin care, particularly in alleviating the symptoms of acne and eczyema. When used in a combination with sunscreen, green tea enhances sun protection. Due to the presence of anti-oxidants, green tea is also effective in slowing down the process of aging.

Green tea extract has proved to be effective for the treatment of patients who have suffered from skin damage following radio therapy for cancer. In a study can dueted at university of Rochester Medical centre, VSA, it has been shown that green tea acts at the cellular level and reduces inflammation by inhibiting the inflammatory pathways (Pajonk et al; 2006).

2. Antiviral potentials:

Green tea blocks viral attachment and entry into cells. It protects RNA and DNA integrity to reduce mutations that can lead to drug resistance. With the frequent consumption of green tea, AIDs – related ctementia may be protected (Brain et al; 2006).

-Green tea also suppresses the adenovirus, Epstein-Barr, herpes simplex, and influenza viruses. ECGC binds to the hem agglutinin of the influenza virus, which blocks it from attaching to (and infecting) target receptor cells. Alters the virus cell membrane, which further in hibits its ability to infect other cells. Effects of green tea catechism and theanine are effective in preventing influenza (Matsumoto et al; 2011).

3. Helps combat obesity:

-Green tea has recently become the latest weapon in fighting over weight conditions. It appears to fight obesity by increasing the rate of calories burning, reducing body fat levels and preventing excess weight gain.

The consumption of green tea extract is associated with a statistically significant reduction in total and low density lipoprotein cholesterol levels (Kim et al; 2011).

Green tea cate chins enhance exercise induced abdominal fat loss in over weight and obese adult (Maki et al; 2009).also green tea catechism and epically catching gallate (EGCG) have been shown to reduce adipoaytes differentiation and proliferation (Wolfram et al; 2006).

4. Good vision:

-Green tea "catechism" are among a number of antioxidants such as vitamin C, vitamin E, lutein, and zeaxanthin through capable of protecting the eye (Chu et al; 2010).

-Green tea antioxidant EGCG provided protection agarist UV damage in cultured human retinal pigmented epithelial cells (Yang et al; 2007).

5. Prevents Hair Loss:

-Green tea polyphenols are only recently understood as positive factors in hair growth and follicle health (Patil et al; 2008).

6. Effective in Renal Failures:

Decreased kidney function due to aging and kidney failure are a frequent cause of death. A Preliminary study in Mansoura University in Egypt has explored the possibility kidney function from life threatening failure with the frequent use of green tea (Mowafy et al; 2011).

7. Improves Insulin Sensitivity:

The green tea has an ant diabetic. Green tea consumption may help prevent type 2diabetes. It improves glucose tolerance and insulin sensitivity in individuals with 2 diabetes. In a study, after receiving green tea for 12 weeks, diabetic rats had lower fasting blood levels of glucose, insulin, triglycerides of their adipocytes to respond to insulin and absorbs blood sugar greatly increased (Fiorino et al; 2012).

8. Synergism with antibiotics:

Treatment of many infections in hindered due to resistace of pathogenic micro – organism against several antibiotics. A recent investigation reported that the antibacterial activity obtained using boiled water green tea extract is enhanced in combination with penicillin G against Bacillus Subtilis bacterium (Smeeton; 2011).

9. Therapeutic potentials against Parkinson's and Alzheimer's disease:

-Green tea has revealed considerable health promoting qualities for nerve degenerative diseases such as Parkinson's and Alzheimer's disease. Synergistic effects of green tea with anti-Parkinson's drug "rasagiline" were observed (Reznichenko; 2010).

10. Rheumatoid Arthritis and Osteoarthritis:

Green tea poly phenols offer a promising new option for the development of more effective strategies for the treatment and prevention of inflammation and rheumatoid arthritis (Akhtar and Haggi; 2011).

11. Anticarcinogenic Properties of Green Tea:

The main component of green tea that has been studied in cancer research is EGCG. There are several cancer related mechanisms attributed to EGCG. These include: inhibition of angiogenesis, DNA hypermethylation, NF-B, telomerase activity, and tumor cell proliferation and metastasis; induction of tumor suppressor genes; and promotion of tumor cell apoptosis (Granja et al,2016). Inhibition of angiogenesis is suggested to occur through a decrease in RNA and peptide levels of vascular endothelial growth factor (VEGF), and by disrupting the dimerization of VEGF with the vascular endothelial growth factor receptor 2 (VEFR2) (Yang et al,2011). Another suggested way in which green tea catechism may generally inhibit

carcinogenesis is through increasing levels of glutathione S-transferase pi (GST-pi), which catalyzes detoxification reactions that inhibit carcinogen-induced DNA damage(Yang et al,2009). Analysis of studies performed using human oral consumption of green tea to assess cancer risk showed that case-control studies gave the most consistent results and were positive for reduced cancer risk in breast, cardiac, colorectal, esophageal, gastric, lung, ovarian, pancreatic, and prostate cancers (Boehm et al,2009). A recent large study showed a relationship between breast cancer risk and tea consumption, with the risk being highest in the groups that did not consume tea and lowest in the groups that consumed the most cups per day. Number of cups were assessed in five categories (0.1–1.0 cups, 1.1–2.0 cups, 2.1–3.0 cups, 3.1–5.0 cups, >5.0 cups) (Bhoo-Papathy et al,2010). Analysis of the types of green tea beverage or extracts used in studies suggests that green tea beverage or a supplement containing mixed catechism may be more effective than using single catching (e.g., EGCG) supplements (Bode et al,2009). The potential molecular mechanisms and targets that might explain how green tea catechism possess ant carcinogenic properties have been widely studied (using various cell cultures, etc.), especially in breast cancers. These include interaction with specific proteins, anti-angiogenesis mechanisms, targets for inhibition of enzyme activities and cell signaling pathways, and induction of cell cycle arrest and apoptosis (Li et al, 2014).

12. Cardiovascular Disease Health Benefits

Cardiovascular disease (CVD) is a complex disorder involving multiple factors. Among those factors are inflammation, oxidative stress, platelet aggregation, and lipid metabolism. There have been an umber of studies over the years assessing green tea consumption in respect to CVD risk (Jochmann et al,2008). Two studies from Japan that included nearly 50,000 people found a decreased mortality rate due to CVD based on consumption of various numbers of cups per day. One study showed a 28% decrease in CVD death between those who consumed 3 cups and those who consumed 10 cups. The other study showed a 14% decrease in CVD mortality between those who consumed <1 cup and those who consumed 5 cups (Koriyama et al,2006). Other studies in Japan using a green tea extract found that, after 12 weeks,

the subjects had reductions in body fat (10%), blood pressure (6.5%), and low-density lipoprotein (LDL) levels (2.6%), suggesting reduced risk of CVD. After two months, diabetic patients also had reduced fasting blood glucose levels (from 135 to 128.8 mg/dL), and hemoglobin A1c (HBA1c) levels (from 6.2% to 6.0%)(Nagao et al,2007). A large meta-analysis of 17 studies from over 30 years, including data from Europe, the UK, and the U.S., found that increasing consumption of green tea by three cups per day decreased the risk of myocardial infarction (MI) death by 11%(Peters et al,2001). Another study showed a decreased risk of mortality in patients who had an acute MI and a history of regular green tea consumption for at least a year prior to the MI. Participants who did not drink green tea had a 14% rate of death due to the MI; participants who drank up to 14 cups per week had an 11% rate of MI death; and participants who drank more than 14 cups per week had a 10% rate of MI death (Mukamal et al,2002). An interesting study in patients with CVD showed that consumption of EGCG resulted in a rapid improvement of vascular endothelial function. Participants who ingested an initial dose of 300 mg of EGCG had an improved brachial artery flow-mediated dilation from 7.1% to 8.6% after 2 h (Widlanski et al, 2007). Another recent study found that increased intake of dietary flavonoids was associated with a decreased risk of CVD. The participants were divided into three groups based on average daily consumption of flavonoids. The first tertile consumed 89 mg/day, the second tertial consumed 251 mg/day, and the third tertile consumed 532 mg/day. The number of deaths due to CVD in the first tertile was 8.6%; in the second tertile, 6.4%; and in the third tertile, 5.0% (Ponzo et al,2015).

> **Inflammation**

Besides CVD, inflammation is also involved in arthritis, aging, cancer, etc. Many of the anti-inflammatory effects when using green tea have been studied in rheumatoid arthritis (RA) and osteoarthritis (OA), and are pertinent to CVD as well. Some general anti-inflammatory mechanisms of green tea components are: increased production of the anti-inflammatory cytokine, IL-10; regulation of IL-6 synthesis and signaling; decreased production of destructive matrix metalloproteinases via TNF-

induced phosphorylation of mitogen-activated protein kinases (MAPKs); and decreased expression of the chemokine receptor CCR2 and decreased levels of the proin flammatory cytokines IL-1 and TNF- (Serafini et al,2011). The specific studies on inflammation can be roughly categorized into: inhibition of neutrophil-endothelium interaction, modulation of neutrophil functions and death, and regulation of inflammation factors. Neutrophil migration and function is an integral part of the inflammatory response, so controlling neutrophils is vital in decreasing inflammation. Studies have shown that green tea catechism cause a reduction in the number of leukocyte-endothelial cell adhesion molecules (CAMs), such as ICAM-1, VCAM-1, and E-selection, expressed on the endothelial cell surface. This restricts the ability of the neutrophils to migrate to sites of infection(liu et al,2016). Other studies have shown that factors known to regulate neutrophil function, such as IL-1, IL-2, TNF- and granulocyte-macrophage colony-stimulating factor (GM-CSF), are suppressed by consumption of green tea or EGCG, resulting in inhibition of inflammation (Aktar et al,2011); Studies on the inhibition of pro-inflammatory factors have shown that green tea catechism down regulate many inflammatory chemokines, cytokines, and inflammatory markers such as: IL-1 IL-1 , IL-6, IL-8, Interferon gamma (INF-), and C-reactive protein (CRP) (Akhtar et al,2011).

➢ Oxidative Stress

Oxidative stress in the body is closely tied to inflammation and CVD, and is the result of the damaging effects of reactive oxygen species (ROS). These ROS are capable of causing chronic inflammation through induction of inflammatory cytokines and chemokines, and pro-inflammatory transcription factors. In general, green tea catechism have been found to have antioxidant activity through: inhibiting redox sensitive transcription factors and pro-oxidant enzymes, scavenging ROS, and inducing anti-oxidant enzymes (Babe PV et al,2008). Studies to determine the antioxidant capabilities of green tea may measure a variety of substances. Tests may measure the presence of known ROS or their metabolites, such as hydroxyl radical, peroxides,superoxide, and singlet oxygen. Other measurements may be for known antioxidant substances such as superoxide dismutase (SOD) and glutathione

peroxidase, or substances that indicate inflammation such as high-sensitivity C-reactive protein (hs-CRP) and TNF- . Another type of testing assesses total antioxidant capacity (TAC), also known as total antioxidant status (TAS), which measures the amount of oxidants that are neutralized in the body (e.g., moles of oxidant neutralized by 1 L of plasma), with a lower number translating into a higher risk of disease (Bhardwaj et al,2013). The results from recent studies have shown that green tea catechism can affect levels of ROS, Whyte et al,2016; Smith et al,2015), increase levels of antioxidants(Henderson SJ et al,2016; Wolberg AS et al,2002), decrease levels of inflammatory substances (Son et al,2017; Jin et al,2008), and increase TAC (TAS)(Wolberg AS et al,2002;Cines DB et al,2014). An excellent summary of earlier studies that measured ROS and TAC can be found in a chapter by Serafini et al. 2011.

> **Platelet Aggregation**

Platelet activation and subsequent aggregation play an important role in CVD. When the vascular endothelium is damaged, platelets usually respond rapidly and aggregate to form plugs at the damage site, and may also form clots that could lead to vessel occlusion (Babe PV et al,2008; Son DJ et al,2004). Many of the studies on platelet aggregation have been carried out using various animal platelets. In addition to showing that green tea catechism were involved in inhibition of platelet aggregation, studies suggested that catechism may affect several cellular targets that are related to platelet activation, including: through the arachadonic acid pathway, inhibition of a cytoplasmic increase in calcium, decreased thrombaxane A2 (TXA2) production, and inhibition of cyclooxygenase-1 (COX)-1 (Jin et al,2008; Ok et al,2012; Lee et al,2013; Iida et al,2014). A study using human platelets concluded that EGCG was able to inhibit platelet activation by adenosine diphosphate (ADP) stimulation, and suppressed the p38 MAPK phosphorylation of heat shock protein 27 (HSP27), which would inhibit the release of pro-thrombotic contents from platelets (Jain et al,2007).

1.2.2 Prervious studies:

- A study done by hussam MA et at (2017)is sudan which study the effect of green tea consumption on coagulation profile and fibrinogen level reported that after reglular consumption of two cup of green tea for one month they was

Significantly decrease in level of fibrinogen after consumption of green tea also where was statistically significance in levels of fibrinogen between males and females this study didn't obtain any statistical significance in fibrinogen level ; between different age groups (Hussam et al,2017).

- Another study was done in sudan by Ayat F, et al studies the effect of green tea consumption on fibrinogen level among healthy Sudanese's volunteers trees and result was significantly decrease in level of fibrinogen (Ayat et al, 2014).

- A study done by F.Jalali MD et al (2008) cement the effect of green tea on serum lipids, antioxidant, and coagulation test in stable coronary artery disease and the result was significantly desrease in fibrinogen level after regular consumptive of 4gld green tea for one month.(.Jalali et al;2008).

- Another study done by MPM de Maat et al oct (2000), which study the consumption of black and green tea has no effect on inflammation, haemostasis and endothelial markers in smoking health individuals in Eur leiden , the nether lands reported that after regular consumptive green tea for one month there was no effect of green tea in the level of the inflammation , fibrinogen and endothelial cardiovascular risk faetors measure (Maat et al;2000).

Joe and yousef (1998) studies the effect of green tea lipids, lipid oxidation and fibrinogen in the hamster the result green tea was significantly more effective than the black tea. These result show in the hamster model that black and green tea improve the risk factor for heart disease by both hypoliemic and antioxidant mechanisms and possibly a fibrinolytic effect (joe and yousef; (1998)).

1.3 Justification

-Sudanese populations in general have a special climate. These factors play a major role in the tradition and habits of the society and one of the most common habits is drinking tea almost after every meal but green tea is still rarely used for daily consumption in spite of their beneficial effects.

-The importance of this study is to drew attention to the others benefits of green tee consumption because usually people taken green tea for certain benefits, these include, improved brain function, fat loss, a lower risk of cancer and many other impressive benefits, and also to explore the effect of green tea on fibrinogen level because fibrinogen is the main constituent that format the blood clot, elevated fibrinogen level raises heart disease risk by (60 to 90%). And also to sheel alight and drew attention to high light the importance of healthy green tea over other kinds of tea.

1.4 Objectives

1.4.1 General Objective:

To assess the effect of green tea consumption on fibrinogen level among healthy adult Sudanese individuals.

1.4.2 Specific objective:

1. To determine the fibrinogen level in the target population before and after intake of green tea.
2. To correlate fibrinogen level in regard to age and gender.

Chapter Tow

Materials and Methods

2. Materials and Methods

2.1 Study design:

This study is experimental study (inter ventional) design.

2. 2 Study area:

This study was conducted in Khartoum state.

2.3 Study period:

This study was carried during the period between March; 2018 ──May; 2018.

2.4 Study population:

Thirty healthy adult Sudanese volunteers were enrolled in this study, their age ranged between 20 ── 45 years old; 13 of them were males and 17 of them were females, they were instructed to drink two cups of green tea, Appendix (1). per day for 30 consecutive days.

2.4.1 Inclusion criteria:

All adult participants in this study were selected according to normal fibrinogen level.

2.4.2 Exclusion Criteria:

People have previous history of thrombosis and undertake of warfarin or heparin or have heart diseases were exclucled from this study.

2.5 Sampling:

2. 5.1 Sample Collection:

Blood samples, two and half milliliter (ML) of venous blood was collected in 3.2% tri-sodium citrate containers from each participant before and after the consumption of green tea, samples were collected by vein puncture.

Platelet poor plasma was prepared centerfugation at 2000 ── 4000 round / min for 15 minutes.

2. 5.2 Sample size:

A total of sixty samples (n=60), (30 befor consumption of green tea and 30 after consumption)) were enrolled in this study.

2.6 Data collation:

2. 6.1 Data Collection tool:

Personal and clinical data from all participants was collected using special form of questionnaire, Appendix (2).

2. 6.2 Statistical Analysis:

Date were entered and analyzed by using statistical package for social sciences (Spss) for windows version 16.

The mean and standard deviation (SD) were calculated for each test before and after drink of tea . Paired T.test was applied to analyze the changes in the Fibrinogen level and also used independent T.test for corrlate Fibrinogen level regard to age and gender, The p-value less than 0.05 were considered significant.

2.7 Fibrinogen Test:

2.7.1 Principle and Procedures:

Fibrinogen level was measured by using STA compact MAX appendix (3) and procedure in appendix (4).

2.8 Ethical Considerations:

Procedure of blood sampling was explained to the participants; all participants were informed about the research objectives and procedures; oral consent was taken from all participants.

Chapter Three

Results

3. Results

-This study was done in Khartoum state at Sudan University of science and technology in the period from March to May 2018 to evaluate the effect of green tea consumption on fibrinogen level.30 healthy Sudanese volunteers aged between 20 – 45 years were enrolled to participate in this study, 13 of them were males and 17 of them were females.

The studied subjects consumed two cups of green tea per day for 30 days, then fibrinogen level was measured from pre and post samples collected from them.

Figure (3.1) shows distribution of study group according to gender. The results showed that of 30 green tea drinkers, 13 (43%) were male while 17 (57%) were female.

Figure (3.2) distribution of study group according to age group, the results showed that, 14 (47%) of tea drinkers were within age range between 20-32 years and 16 (53%) of them were within age range between 33-45 Years.

Table (3.1) The statistical analysis of the result showed that there is asignificant difference (P.value = 0.000) among participant′s fibrinogen level between before (3.31±0.45) and after (2.29±0.41) green tea consumption samples.

Table (3.2) When we compared the fibrinogen level in both males and females the mean of pre green tea consumption sample among females was (3.46±0.35) and among males was(3.12±0.49) whereas the mean of fibrinogen level in post green tea consumption samples collected from females was (2.41±0.34) while it was (2.13±0.44) in samples collected from males. The result showed that there is asignificant difference between males and females in the pre and post green tea consumption samples (P.value =0.036 and 0.060 respectively).

Table (3.3) When we compared the fibrinogen level in the different aged group, the mean of fibrinogen for pre green tea consumption sample among group one (20-32) years was (3.45± 0.38) and among group two (33-45) years was (3.19±0.47) while the mean of pre fibrinogen level of post green tea consumption samples among group one (2.42± 0.38) and it was (2.18±0.41) in samples collected from group two. The

result showed that there is no significant difference between the two age group in the pre and post samples(P.value = 0.104 and 0.112 respectively).
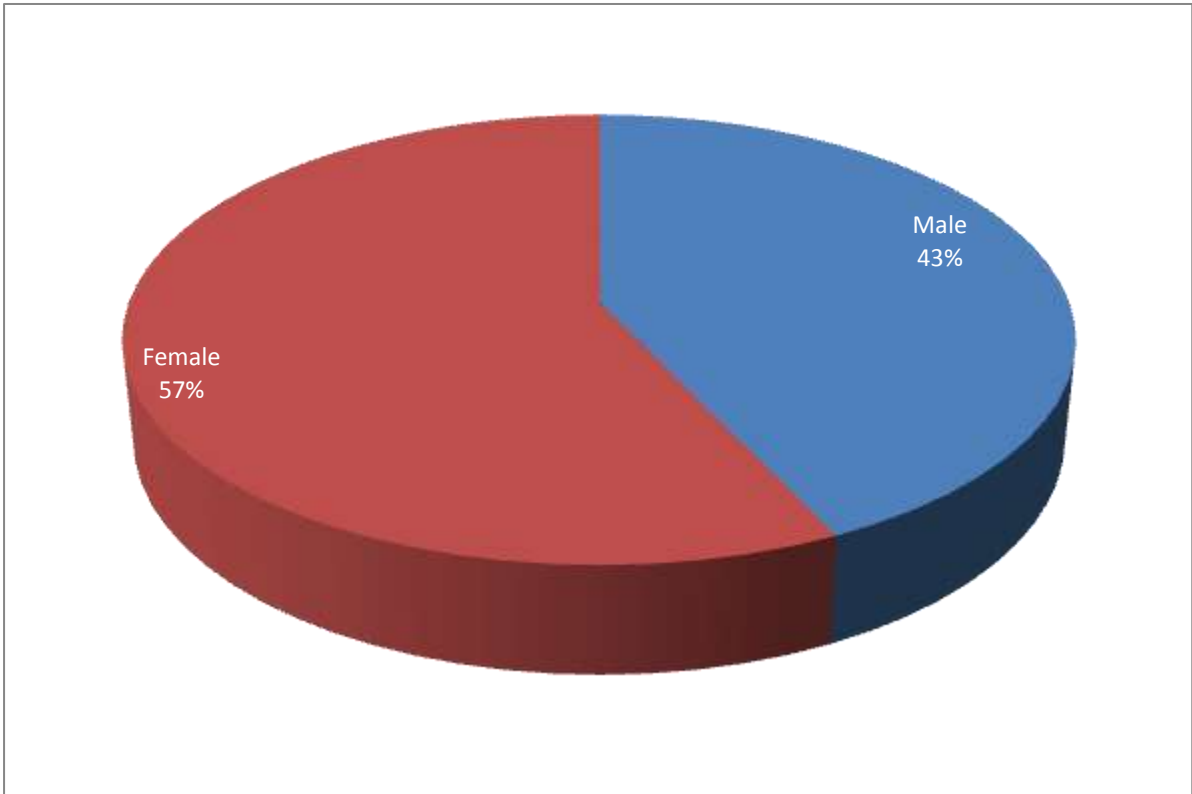
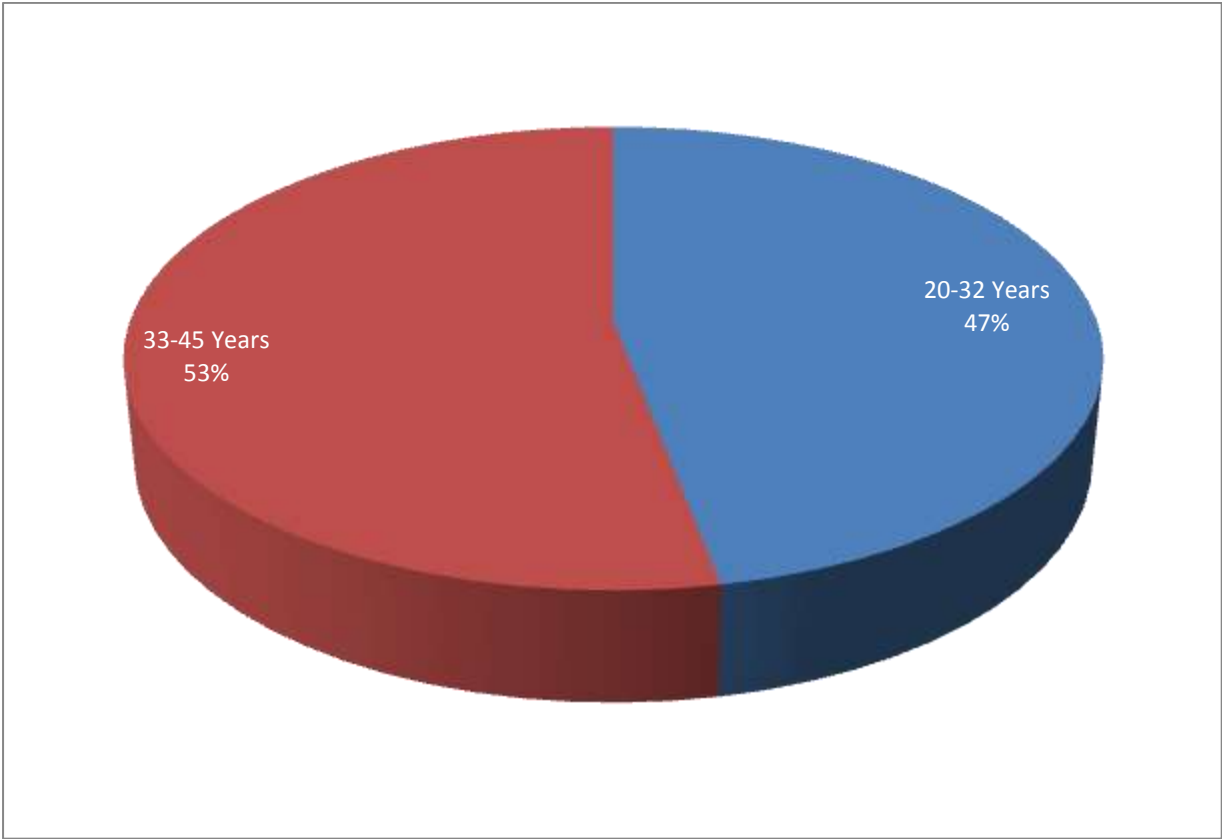**Figure (3.1) Distribution of study group according to gender**

56

Figure (3.2) Distribution of study group according to age group

Table (3.1) Mean of fibrinogen before and after drinking green tea

Parameter	Mean±SD	P-value
Before Fibrinogen (g/L)	3.31±0.45	0.000
After Fibrinogen (g/L)	2.29±0.41	

Table (3.2) Mean of fibrinogenbefore and after drinking green tea across gender

Parameters	Male (Mean±SD)	Female (Mean±SD)	P-value
Before Fibrinogen (g/L)	3.12±0.49	3.46±0.35	0.036
After Fibrinogen (g/L)	2.13±0.44	2.41±0.34	0.060

Table (3.3) Mean of fibrinogen before and after drinking green tea across age

Parameters	20-32 Years (Mean±SD)	33-45 Years (Mean±SD)	P-value
Before Fibrinogen (g/L)	3.45±0.38	3.19±0.47	0.104
After Fibrinogen (g/L)	2.42±0.38	2.18±0.41	0.112

Chapter Four

Discussion, Conclusion, and Recommendations

4.1 Discussion

- Fibrinogen is a 340KD a plasma glycoprotein that plays a key role in coagulation. Upon cleavage by thrombin the fibrinogen is converted to fibrin which polymerizes into a fibrin network or clot. This study was carryout to evaluate the effect of green tea consumption on fibrinogen level in apparently healthy Sudanese people aged between 20 – 45years.

- In this study, the mean of fibrinogen level in the samples collected after green tea consumption was significantly lower than of the samples collected before the green tea consumption. These findings were in agreement with the findings of study done by (Jalali et al., 2008, Ayat et al., 2014 And Hussam et al., 2017). Which they were reported the same findings.

- Another study conducted by de Maat et al (2000) in Netherlands among adult smokers reported that there was no effect of green tea on the level of fibrinogen, the difference between our results and this findings may be due to difference in the studies populations because de Maat et al. study was conducted on smokers while all others previous studies were conducted on non-smokers.

- This study revealed significant difference in the level of fibrinogen between males and females. Similar findings were obtained by Hussam et al (2017) which revealed significant increase in fibrinogen level in female compared to male and this is could be attributed to body mass index and heavy exercise. Thus heavy exercise enhance fibrinogen lysis which decrease level of plasma fibrinogen in male, these findings were not in agreement with Ayat et al (2014) that mentioned no statistical significant difference in fibrinogen level between males and females.

-More over, this study didn't obtained statistical significant difference in fibrinogen level with regards to age group and this similarly obtained by Ay at et al (2014), Hussam et al (2017).

4.2 Conclusion

-This study concludes that drinking two cups of healthy green tea per day for 30days significantly reduce the level of fibrinogen among healthy Sudanese volunteers. Also the present statistically significant difference between male and female with regard to fibrinogen level.

- This study didn't revealed any statistical significant difference in fibrinogen with regard to different age groups.

4.3 Recommendations

-Further study should be conducted in patient with coronary artery disease by using the same parameter that have been used in this study.

-New study with larger sample size that will consider age, ethnic group, sex of Sudanese individual from different parts of Sudan should be conducted and different number of cup per day.

-Comparative study should be conducted to compare the effect of healthy green tea with other kinds of tea.

References

References

A.Bascom (2002). Incorporating herbal medicine into clinical practice, Philadelphia: F.A. Davis Company, P.153.

A.H.Pressman and S. Buff (1997). The complete idiot's guide to vitamins and minerals, New York: New York Alpha Books, P.283.

Agric. Food Chem 2010; 58 (3): 1523-34.an effective therapy in hair loss – A review. RJPBCS 2010; 1 (2): 773-

Anne, Stiene-martin, Cheryl, A, Lotspeich-Steininger, John A. Koepke., (1992). Clinical hematology, Second Edition, J.B.Lippincott company, (58) (664-667)

Asakai R, Chung DW, Davie EW, Seligsohn U;(1991). Factor XI deficiency in Ashkenazi Jews in Israel. N Engl J Med;325:153–158.

Ayat, F,S.,Esraa, S;Ragaa, A; Hiba, B; Elshazali,W;Ibrahim, Kh (2014): the effect of green tea consumption on fibrinogen level among healthy Sudanese volunteers.MLS AL Neelain University, Khartoum, Sudan.

Babe, P.V.; Liu, D., (2008): Green tea catechism and cardiovascular health: An update. Curr. Med. Chem. 15, 1840–1850.

Barras A, Mezzetti A, Richard A, lazzaroni S, Roux, Melhy kp, Betbeder D, Monfi 11iette – DuPont N(2009). Formulation and characterization of poly phenol loaded lipid nano capsules. Int pharm; 379:270-277.

BhooPathy,N.;Peeters,P.H.;vanGils,C.;Beulens,J.W.;vanderGraaf,Y.;Bueno-de-Mesquita,B.;Bulgiba,A.;Uiterwaal, C.S.,(2010): Coffee and tea intake and risk of breast . Breast Cancer Res. Treat. 121,461–467.

Bode, A.M.; Dong, Z., (2009): Epigallocatechin 3-gallate and green tea catechism: United they work, divided they fall. Cancer Prev. Res. (Phila.) 2, 514–517.

Boehm, K.; Borelli, F.; Ernst, E.; Habacher, G.; Hung, S.K.; Milazzo, S.; Hornebar, M., (2009): Green tea (Camellia sinensis) for the prevention of cancer. Cochrane Database Syst. Rev. 3. [Beverages 2017, 3, 6 10 of 14

Batten, D.; Fugalio, G.; Fraternali, F.; Molteni, C.(2015): Structural properties of green tea catechism. J.Phys. Chem. B 119, 12860–12867.

Brain Giunta, Demian Obregon, Hauyan Hou, Jin zeng, Nan sun, Veljko Nikolic, Jared Ehrhart, Douglas shytle, Francisco Fernandez, Jun Tan (2006), EGCG mitigates neurotoxicity mediated by HIV-1 protevnsgpl20 and tat in the presence of IFN-Y:Role of JAK/STATI Sig nalling and implications for HIV- associated dementia. Brain Research; 1123(1):216-225.Cardiometabolic Alterations Induced by Experimental Diabetes.

Brummel-Ziedins KE, Wolberg AS., (2014): Global assays of hemostasis. Curr Opin Hematol. 21:395–403.

Büller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, Segers A, Verhamme P, Weitz JI; FXI-ASO TKA Investigators., (2015): Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med. 372:232–240.

Cabrera, C.; Artacho, R.; Gimenez, R., (2006): Beneficial effects of green tea—A review. J. Am. Coll. Nutr. 25, 79–99.

Campbell RA, Aleman M, Gray LD, Falvo MR, Wolberg AS., (2010): Flow profoundly influences fibrin network structure: implications for fibrin formation and clot stability in haemostasis. Thromb Haemost. 104:1281–1284.

Campbell RA, Overmyer KA, Bagnell CR, Wolberg AS., (2008): Cellular procoagulant nactivity dictates clot structure and stability as a function of distance from the cell surface. Arterioscler Thromb Vasc Biol. 28:2247–2254.

Carvalho FA, Connell S, Miltenberger-Miltenyi G, Pereira SV, Tavares A, Ariëns RA, Santos NC., (2010): Atomic force microscopy-based molecular recognition of a fibrinogen receptor on human erythrocytes. ACS Nano. 4:4609–4620.

Cesarman-Maus, G, Hajjar, KA., (May 2005). "Molecular mechanisms of fibrinolysis". British journal of hematology 129 (3): 307-321.

Cheesbrough , M (2000) , Distric laboratory practice in tropical countries , part 2 , second Edition ., (343 – 344) .

Chow, H.H.; Cai, Y.; Alberts, D.S.; Hakim, I.; Dorr, R.; Shahi, F.; Crowell, J.A.; Yang, C.S.; Hara, Y., (2001): Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol. Biomark. Prev. 10, 53–58.

Chu Kai On, Chan Kwok Ping, Wang Chi Chiu, Chu Ching Yan, Li

Wai Ying, Choy Kwong Wai, Rogers Michael Scott and Pang Chi Pui;(2010).

Green tea catechism and their oxidative protection in the Rat Eye. J.

Agric. Food Chem; 58 (3): 1523-34.

Ciesla, B. (2007). Haematology in practice Philadelphia, United state of America F. A. Davis compary publishing Ltd. (15):229-244.

Clarke, K.A.; Dew, T.P.; Watson, R.E.; Farrar, M.D.; Bennett, S.; Nicolaou, A.; Rhodes, L.E.; Williamson, G., (2014): High performance liquid chromatography tandem mass spectrometry dual extraction method for identification of green tea catching metabolites excreted in human urine. J.Chromatogr. BAnalyt. Techonol. Biomed. Life Sci. 1, 29–37.

Clifford, M.N.; van der Hooft, J.J.; Crozier, A., (2013): Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols. Am. J. Clin. Nutr. 98, 1619S–1630S.

Cotran, Ramzi, S.; Kumar, Vinay; Fausto, Nelson; NelsonFausto; Robbins, Stanley L.; Abbas, Abul K. (2005). Robbins and Cotran pathologic basis of disease. St.Louis, Mo: Elsevier Saunders. P. (125).

Crosby JR, Marzec U, Revenko AS, Zhao C, Gao D, Matafonov A, Gailani D, MacLeod AR, Tucker EI, Gruber A, Hanson SR, Monia BP.,(2013): Antithrombotic effect of antisense factor XI oligonucleotide treatment in primates. Arterioscler Thromb Vasc Biol. 33:1670–1678.

De Vries PS, Chasman DI, Sabater-Lleal M, et al., (2016): A meta-analysis of 120 246 individuals identifies 18 new loci for fibrinogen concentration. Hum Mol Genet. 25:358–370.

Drew.P, (2003), Abc of clinical hematology, second edition, BMJ Books, (28).

Fernandez, P.L.; Pablos, F.; Martin, M.J.; Gonzalez, A.G;(2002). Study of catching and xanthine tea profiles as geographical tracers. J. Agric. Food Chem. 59, 1833–1839.

Fiorino Patricia, Evangelista Fabian a Sant, Anna, Santos Fernando, Magn F antima Maria a Motter, Jan Carlo Morais O. B-Delorenzi, Ginoza Milton, and Farah Vera (2012). The Effects of green tea consumption on cardio metabolic Alterations Induced by Experimental Diabetes. Experimental Diabetes Research; Article ID 309231:7.

Fort A, Borel C, Migliavacca E, Antonarakis SE, Fish RJ, Neerman- Arbez M., (2010): Regulation of fibrinogen production by microRNAs. Blood. 116:2608–2615.

Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, Wrobleski SK, Wakefield TW, Hartwig JH, Wagner DD., (2010): Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci USA. 107:15880–15885.

Gersh KC, Nagaswami C, Weisel JW., (2009): Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes. Thromb Haemost. 102:1169–1175.

Granja, A.; Pinheiro, M.; Reis, S., (2016): Epigallocatechin gallate nanodelivery systems for cancer therapy. Nutrients 8, E307.

Grimaudo, R; Craxim, F; (2001). Haematology and haemostasis, 5th edition, ox Ford, UK black well publishing Ltd. (1):1-33.

Gupta, D.A.; Bhaskar, D.J.; Gupta, R.K.; Karim, B.; Jain, A.; Dalai, D.R., (2014): Green tea: A review on its natural anti-oxidant therapy and cariostatic benefits. Biol. Sci. Pharm. Res. 2, 8–12.

Hamilton-Miller JMT (2001). Anti carcinogenic properties of tea (camellia sinensis), Journal of medical microbiology.50:299-302.

Henderson SJ, Xia J, Wu H, Stafford AR, Leslie BA, Fredenburgh JC, Weitz DA, Weitz JI., (2016):Zinc promotes clot stability by accelerating clot formation and modifying fibrin structure. Thromb Haemost. 115:533– 542.

Henning, S.M.; Niu, Y.; Lee, N.H.; Thames, G.D.; Minutti, R.R.; Wang, H.; Go, V.L.; Heber, D., (2004): Bioavailability and antioxidant activity of tea flavanoids after consumption of green tea, black tea, or a green tea extract supplement. Am. J. Clin. Nutr. 80, 1558–1564.

Hoff brand, A, V., Moss, P, A, H., and Pettit, I, E., (2011). Essential hematology, 6the edition, WILEY-BLACKWLL, (247-283) .

Hoff brand, A. V; Lewis, S.M and Tuddenham, G.D. (2001).Post graduate haematology, 4th edition, Ox ford university press, New York. (26):550- 580.

 Horne MK III, Cullinane AM, Merryman PK, Hoddeson EK., (2006): The effect of red blood cells on thrombin generation. Br J Haematol. 133:403– 408.

Huffman JE, de Vries PS, Morrison AC, et al., (2015): Rare and low-frequency variants and their association with plasma levels of fibrinogen, FVII, FVIII, and vWF. Blood. 126:e19–e29.

Hussam MA; Remah, S; Fatima, M; Esraa, M ;Esraa,M; Abubaker,A; Mubark,H (2017) The effect of green tea consumption on coagulation profile among adult healthy Sudanese, International Journal of Applied Research 3(3): 703-705.

I.T. Johnson and G. Williamson (2003), photochemical functional foods, Cambridge, UK: Wood head publishing, PP.135-145.

Iida, Y.; Doi, T.; Matsushima-Nishiwaki, R.; Tokuda, H.; Ogura, S.; Kozawa, O.; Iida, H., (2014): (−)-Epigallocatechin gallate selectively inhibits adenosine diphosphate-stimulated human platelet activation: Suppression of heat shock protein 27 phosphorylation via p38 mitogen-activated protein kinase. Mol. Med. Rep. 10, 1383–1388.

Jain, K.S.; Kathiravan, M.K.; Somani, R.S.; Shisloo, C.J.,(2007): The biology and chemistry of hyperlipidemia. Bioorg. Med. Chem. 15, 4674–4699. [CrossRef] [PubMed]

Jalali, M, D., et. al., (2008): Qualified Health Claim Petition — Green tea and Reduced Risk of cardiovascular disease in Iran. Iranian heart journal (3):47-52.

Jerome WG, Handt S, Hantgan RR.,(2005): Endothelial cells organize fibrin clots into structures that are more resistant to lysis. Microsc Microanal. 11:268–277.

Jigsaw, A.; Nishant, R.; Navin, K.; Pankaj, G., (2012): Green tea: A magical herb with miraculous outcomes. Int. Res. J. Pharm. 3, 139–148.

Jin, Y.R.; Im, J.H.; Park, E.S.; Cho, M.R.; Han, X.H.; Lee, J.J.; Lim, Y.; Kim, T.J.; Yun, Y.P., (2008): Antiplatelet activity of epigallocatechin gallate is mediated by the inhibition of PLCγ2 phosphorylation, elevation of PGD2 production, and maintaining calcium-ATPase activity. J.Cardiovasc. Pharmacol. 51, 45–54.

Jochmann,N.;Baumann,G.;Stangl,V.,(2008):Greenteaandcardiovasculardisease: From molecular targets to wards human health. Curr. Opin. Clin. Nutr. Metab. Care 11, 758–765. [CrossRef] [PubMed].

Joe A and yousef A;(1998): studies the effect of green tea lipids, lipid oxidation and fibrinogen in the hamster: mechanisms for the epidemiological benefits of tea drinking 433:44-46.

Joel. Moake, MD; (2009): Introduction haemstasis, Journal of haematology, 112. (18):34-78.

Kahn SR, Lim W, Dunn AS, et al., (2012): Prevention of VTE in nonsurgical patients: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 141(suppl 2):e195S–226S.

Kalathottukaren MT, Abraham L, Kapopara PR, et al.,(2016): Alteration of blood clotting and lung damage by protamine are avoided using the heparin and polyphosphate inhibitor UHRA [published online ahead of print December 29,]. Blood.

Kuriyama, S.; Shimazu, T.; Ohmori, K.; Kikuchi, N.; Nakaya, N.; Nishino, Y.; Tsubono, Y.; Tsuji, I.,(2006): Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: The Ohsaki study. JAMA 296, 1255–1265.

Lambert, J.D.; Sang, S.; Yang, C.S., (2007): Biotransformation of green tea polyphenols and the biological activities of those metabolites. Mol. Pharm. 4, 819–825.

Lantana, C.; Rinaldi, M.; Cavazza, A.; Barbanti, D.; Corradini, C.,(2017): Effects of alternative steeping methods on composition, antioxidant property and colour of green, black and oolong tea infusions. J. Food Sci. Technol, 52, 8276–8283. Beverages 3, 6 9 of 14.

Lee, D.H.; Kim, Y.J.; Kim, H.H.; Cho, H.J.; Ryu, J.H.; Rhee, M.H.; Park, H.J., (2013): Inhibitory effects of epigallocatechin-3-gallate on microsomal cyclooxygenase-1 activity in platelets. Biomol. Ther. 21, 54–59.

Lee, M.J.; Wang, Z.Y.; Li, H.; Chen, L.; Sun, Y.; Gobbo, S.; Balentine, D.A.; Yang, C.S., (1995): Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol. Biomark. Prev. 4, 393–399.

Li, M.J.; Yin, Y.C.; Wang, J.; Jiang, Y.F., (2014): Green tea compounds in breast cancer prevention and treatment. World J. Clin. Oncol. 5, 520–528.

Lin, Y.S.; Tsai, Y.J.; Tsay, J.S.; Lin, J.K., (2003): Factors affecting the levels of tea polyphenols and caffeine in tea leaves. J. Agric. Food Chem. 51, 1864–1873.

Liu, D.; Perkins, J.T.; Hennig, B., (2016): EGCG prevents PCB-126-induced endothelial cell inflammation via epigenetic modifications of NF-κB target genes in human endothelial cells. J. Nutr. Biochem. 28, 164–170.

Liu, M.; Tian, H.I.; Wu, J.H.; Cao, R.R.; Wang, R.X.; Qi, X.H.; Xu, Q.; Chen, X.H., (2015): Relationship between gene expression and the accumulation of catching during spring and autumn in tea plants (Camellia sinensis L.). Hortic. Res. 2, 15011.

Maat, Mpm H, Pijl., C, Kluft, et. al., (2000) consumption of black and green tea has no effect on haemostasis on smoking healthy individual, European Journal of clinical Nutrition, (54) 10, 757-763.

Machlus KR, Cardenas JC, Church FC, Wolberg AS., (2011): Causal relationship between hyperfibrinogenemia, thrombosis, and resistance to thrombolysis in mice. Blood. 117:4953–4963.

Matsumoto K, Yamada H, Takuma N, Niino H, Sagesaka YM (2011). Effects of green tea catechism and Theanine on preventing influenza infection among healthcare worker: a randomized controlled trail, BMC Complement ALtern Med; 21; 11:15.

Mitchell Lewis, S; Barbara, J.B and Imelda B. (2006), Practical Hematology, 10th edition. Philadelphia, Churchill living stone, an Imprint of Elsevier publishing Ltd. (16):380-437.

Mowafy AM, Salem HA, Gayyar MM, Mesery ME, Azab MF;(2011).

Evaluation of renal protective effects of the green-tea (EGCG) and red

grape resveratrol: role of oxidative stress and inflammatory cytokines.

Nat Prod Res; 25(8):850-6.

Mukamal, K.J.; Maclure, M.; Muller, J.E.; Sherwood, J.B.; Mittleman, M.A., (2002): Tea consumption and mortality after acute myocardial infarction. Circulation 105, 2476–2481. [CrossRef] [PubMed]

Munker, M; Erhard, H; Jonathan, G;Ronald P.(2007) modern Hematology. 2nd edittion. New Jersey United state of America Humana press publishing Ltd. (19): 327-345.

Nagao, T.; Hase, T.; Tokimitsu, I., (2007): A green tea extract high in catechism reduces body fat and cardiovascular risks in humans. Obesity (Silver Spring) 15, 1473–1483. Nat Prod Res 2011; 25(8):850-6.

Ok, W.J.; Cho, H.J.; Kim, H.H.; Lee, D.H.; Kang, H.Y.; Kwon, H.W.; Rhee, M.H.; Kim, M.; Park, H.J., (2012): Epigallocatechin-3-gallate has an anti-platelet effect in a cyclic AMP-dependent manner. J. Atheroscler. Thromb. 19, 337–348.

Pajonk F, Riedisser A, Henke M, et al (2006). The effects of tea extracts on proinflammatory signaling. BMC Med; 4:28.

Pallister, CJ, and Watson, MS., (2010). Haematology. Scion Publishing. P(11).336-347.

Patil SM, Sapkale GN, Surwase US, Bhombe BT;(2010). Herbal medicines as

an effective therapy in hair loss – A review. RJPBCS; 1 (2): 773-

81.

Patterson BO, Hinchliffe R, Loftus IM, Thompson MM, Holt PJ.,(2010): Indications for catheter-directed thrombolysis in the management of acute proximal deep venous thrombosis. Arterioscler Thromb Vasc Biol. 30:669–674.

Peter W. Taylor, Jeremy M.T. Hamilton- Miller, Paul D and Stapleton. (2005). Antimicrobial properties of green tea catechism, Food Sci techno L Bull. 2:71-8.

Peters, U.; Poole, C.; Arab, L.,(2001): Does tea affect cardiovascular disease? A meta-analysis. Am. J.Epidemiol. 154, 495–503.

Peyrou V, Lormeau JC, Hérault JP, Gaich C, Pfliegger AM, Herbert JM.,(1999): Contribution of erythrocytes to thrombin generation in whole blood. Thromb Haemost. 81:400–406.

Ponzo, V.; Goitre, I.; Fadda, M.; Gambino, R.; De Francesco, A.; Soldati, L.; Gentile, L.; Magistroni, P.; Cassader, M.; Bo, S.,(2015): Dietary flavonoid intake and cardiovascular risk: A population-based cohort study. J. Transl. Med. 13, 218.

Protopopova AD, Barinov NA, Zavyalova EG, Kopylov AM, Sergienko VI, Klinov DV.,(2015): Visualization of fibrinogen αC regions and their arrangement during fibrin network formation by high-resolution AFM. J Thromb Haemost. 13:570–579. doi: 10.1111/jth.12785.

Ravichandran Mad huram, Navam S Hetti arachchy, vijayalak shim Ganesh, Steven C Ricke, Surrender Singh (2011). Enhancement of antimicrobial activities of naturally occurring phenolic compounds by nanoscle delivery against listeria mono aytenes, Escherichia coli 0157:h7 and salmonella typhimuriumb in broth and chicken meat system. Journal of food safety; 31(4):462-471.

Redman CM, Xia H.,(2001): Fibrinogen biosynthesis. Assembly, intracellular degradation, and association with lipid synthesis and secretion. Ann N Y Acad Sci. 936:480–495.

Reznichenko L, Kalfon L, Amit T, You dim MB, Mandel SA (2010). Low dosage of rasagiline and epically catching gallate synergistically restored the nigrostriatal axis in MPTP- induced Parkinsonism. Neuro degener Dis; 7(4):219-31.

Rowe, S.; Stalmach, A.; Mullen, W.; Lean, M.E.; Edwards, C.A.; Crozier, A.,(2010): Green tea flavan-3-ols: Colonic degradation and urinary excretion of catabolites by humans. J. Agric. Food Chem. 58, 1296–1304.

Seligsohn U.,(2009): Factor XI deficiency in humans. J Thromb Haemost. 7(suppl 1):84–87.

Serafini,M.;DelRio,D.;Yao, D. N.;Bettuzzi,S.;Peluso,I.,(2011):Chapter12: Health benefit soft tea. In Herbal Medicine: Biomolecular, and Clinical Aspects, 2nd ed.; Benzie, I.F.F., Wachtel-Galor, S., Eds.; CRC Press: Boca Raton, FL, USA, pp. 239–262.

Smeeton Benjamin (2011). The synergy of green tea and pencillin G against Bacillus Subtilis.J APP Pharma; 02(03):197-200. Wai Ying, Choy Kwong Wai, Rogers Michael Scott and Pang Chi Pui.

Smith SA, Morrissey JH.,(2015): scientific sessions Sol Sherry distinguished lecture in thrombosis: polyphosphate: a novel modulator of hemostasis and thrombosis. Arterioscler Thromb Vasc Biol. 35:1298–1305.

Son,D.J.;Cho,M.R.;Jin,Y.R.;Kim,S.Y.;Park,Y.H.;Lee,S.H.;Akiba,S.;Sato,T.;Yun,Y.P (2004).Antiplateleteffectof green tea catechism: A possible mechanism through arachidonic acid pathway. Prostaglandins Leukot. Essent. Fatty Acids, 71, 25–31. Beverages 2017, 3, 6 13 of 14

Stalmach, A.; Troufflard, S.; Serafini, M.; Crozier, A.(2009): Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. Mol. Nutr. Food Res. 53, S44–S53.

Tucker EI, Marzec UM, White TC, Hurst S, Rugonyi S, McCarty OJ, Gailani D, Gruber A, Hanson SR.,(2009): Prevention of vascular graft occlusion and thrombus-associated thrombin generation by inhibition of factor XI. Blood. 113:936–944.

Tutwiler V, Litvinov RI, Lozhkin AP, Peshkova AD, Lebedeva T, Ataullakhanov FI, Spiller KL, Cines DB, Weisel JW.,(2016): Kinetics and mechanics of clot contraction are governed by the molecular and cellular composition of the blood. Blood. 127:149–159.

Tutwiler V, Peshkova AD, Andrianova IA, Khasanova DR, Weisel JW Litvinov RI.,(2017): Contraction of blood clots is impaired in acute ischemic stroke. Arterioscler Thromb Vasc Biol. 37:271–279.

Wakefield TW, Myers DD, Henke PK.,(2008): Mechanisms of venous thrombosis and resolution. Arterioscler Thromb Vasc Biol. 28:387–391. doi:10.1161/ATVBAHA.108.162289.

 Wang, Y.; Ho, C.T.,(2009): Polyphenolic chemistry of tea and coffee: A century of progress. J. Agric. Food Chem. 57, 8109–8114.

Whyte CS, Chernysh IN, Domingues MM, Connell S, Weisel JW, Ariens RA, Mutch NJ.,(2016): Polyphosphate delays fibrin polymerisation and alters the mechanical properties of the fibrin network. Thromb Haemost. 116:897–903.

Widlanski, M.E.; Hamburg, N.M.; Anter, E.; Holbrook, M.; Kahn, D.F.; Elliott, J.G.; Keaney, J.F., Jr.; Vita, J.A.,(2007): Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. J. Am. Coll. Nutr. 26, 95–102.

William, F.Kern. (2002). PDQ haematology, Hamilton Ontario Canada BC Decker Inc. (20):381-429.

Wolberg AS, Gabriel DA, Hoffman M.,(2002): Analyzing fibrin clot structure

using a microplate reader. Blood Coagul Fibrinolysis. 13:533–539.

Wolberg AS, Rosendaal FR, Weitz JI, Jaffer IH, Agnelli G, Baglin T,Mackman N.,(2015): Venous thrombosis. Nat Rev Dis Primers. 1:15006.

Wolfram S, Raeder storff D, Preller M, Wang Y, Teixeira S R, Ricgger Cand Weber P (2006). Epigallo catching gallate supplementation alleviates diabetes ain J Nuts; 136:3512-3518.

Yakovlev S, Gorlatov S, Ingham K, Medved L.,(2003): Interaction of fibrin(ogen) with heparin: further characterization and localization of the heparin-binding site. Biochemistry. 42:7709–7716.

Yang, C.S.,(2009): Antioxidant and anti-carcinogenic activities of tea polyphenols. Arch. Toxicol. 83, 11–21.

Yang, C.S.; Chen, L.; Lee, M.J.; Balentine, D.; Kuo, M.C.; Schantz, S.P.,(1998): Blood and urine levels of tea catechism after ingestion of different amounts of green tea by human volunteers. CancerEpidemiol. Biomark. Prev. 7, 351–354.

Yang, C.S.; Wang, H.; Li, G.X.; Yang, Z.; Guan, F.; Jin, H.,(2011): Cancer prevention by tea: Evidence from laboratory studies. Pharmacol. Res. 64, 113–122.

Yeromonahos C, Marlu R, Polack B, Caton F.,(2012): Antithrombin-independent effects of heparins on fibrin clot nanostructure. Arterioscler Thromb Vasc Biol. 32:1320–1324.

Zucker M, Seligsohn U, Salomon O, Wolberg AS;(2014). Abnormal plasma clot structure and stability distinguish bleeding risk in patients with severe factor XI deficiency. J Thromb Haemost. 12:1121–1130.

Appendix

Appendix No(1): Green tea

بسم الله الرحمن الرحيم

University of science and technology

Faculty of Medical Laboratory Science

Questionnaire about the effect consumption of green tea on
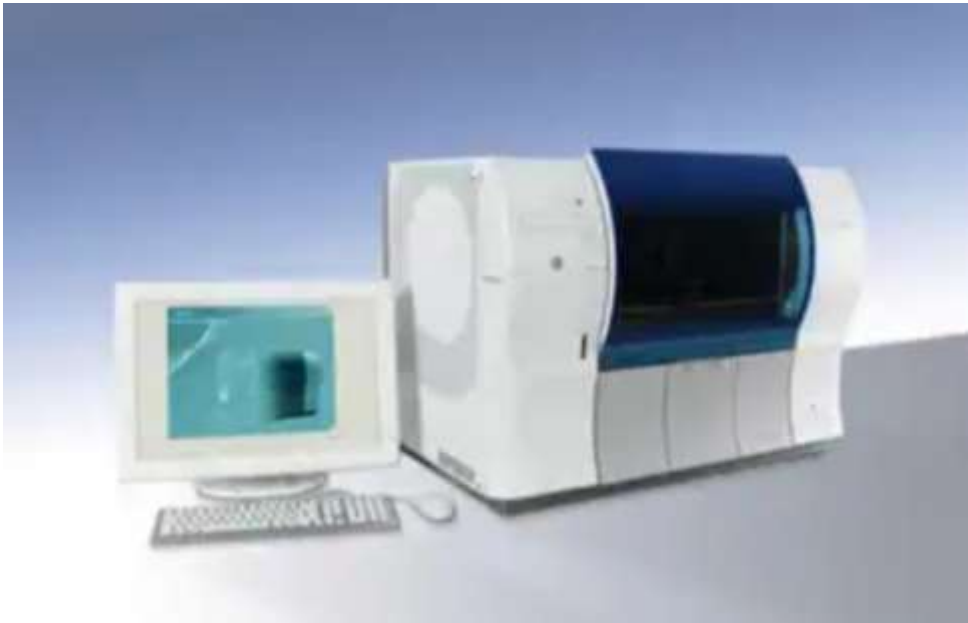
Fibrinogen Level Among Adult Healthy Sudanese

No(   )

1. ID :…………………………………………………
2. Age : (   )
3. Gender : a/ male   (   )   b/ Female (   )
4. Do you have and of this disease:

a- Heart disease (   )   b- liver Disease (   )   c- Others (   )

d- Not found   (   )

Fibrinogen Level before: …………………………………………

Fibrinogen Level After: …………………………………………

Appendix No(2): Questionnaire

Appendix No(3): STA Compact MAX

Appendix No(4)