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Effects of Camellia Sinensis (Green Tea) on Prothrombin Time and International Normalized Ratio

A dissertation submitted in partial fulfillment of the M.Sc. degree in Hematology

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الآيــة

بسم الله الرحمن الرحيم

قال تعالى:

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

سوره البقرة: الآية ٣٢

Dedication

To my dear parents:

Thinking of you today with love in every though.

Thinking all the joy we have thought, the happiness you have brought.

Thinking of just have nice and how like you, your guidance meant more than your even guess.

To my brother, sisters, and husband.

Acknowledgment

All my thanks are to Allah who gave me health and strength to complete this research.

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My sincere thanks extended to all volunteer participants that they participate effectively in this research. Finally special thanks to all those who contributed to the production of this research.

List of abbreviation

Abbreviation	Mean
APC	Activated protein c
APTT	Activated partial thromboplastin time
CAD	Coronary artery disease
CNS	Central nervous system
DDAVP	Deamieno-8-D-arginine vasopressin
DIC	Disseminated intravascular coagulation
EGCG	Epigallo catechin -3 gallate
LDL	Low –density lipoprotein
MPV	Mean platelet volume
MW	Molecular weight
PAI	Plasminogen activator inhibitor
PF3	Platelet phospholipids
PL	Phospholipid
PT	Prothrombin time
TAFI	Thrombin –activatable Fibrinolysis inhibitor
TF	Tissue Factor
TFPI	Tissue factor pathway inhibitor
TG's	Triglyceride
t-PA	Tissue plasminogen activator
TT	Thrombin time
VIII-Rag	VIII related antigen
VWF	Von will brand factor

ملخص البحث

المقدمة: الشاي الأخضر مصنوع من لحاء وأوراق نبات صيني (الكاميليا)، العديد من الأشخاص حول العالم يشربون الشاي الأخضر كعادة يومية وأيضاً كعلاج للعديد من الأمراض. هدفت هذه الدراسة إلى تسليط الضوء على أهمية الشاي الأخضر الصحية عبر دراسة وصفية وتحديد مدي تأثيره علي زمن البروثرمبين و INR .

منهجيه البحث: تم أخذ عينات دم وريدي محتوي على مضاد التجلط (ثلاثي الصوديوم ستريت تركيز ٢,٣%) بنسبة ١:٩ وجمعت العينات من أفراد أصحاء بالغين (ن=٢٠) (١٠من الرجال و ١٠ من النساء) اللذين لا يشربون الشاي الأخضر كعينة ضابطة، وتم جمع عينات أخري في نفس مضاد التجلط (ن=٥٠) (٢٤ من الرجال و ٢٦ من النساء) اللذين يشربون الشاي الأخضر كعينة اختبار. وتم قياس وقت البروثرومبين بواسطة جهاز قياس زمن تجلط الدم.

النتائج: أوضحت النتائج أن متوسط زمن البروثرومبين في العينة الضابطة كان (14.21 ثانية) وكان متوسط نسبة ال INR (١,٠٠٢) , بينما كان متوسط زمن البروثرومبين في عينه الاختبار (١٣,٣٩ ثانيه) وكان متوسط نسبة ال INR (٠,٩٨),بينما أوضحت نتائج التحليل الإحصائي عدم وجود فروق ذات دلالة احصائية.

وأيضا أوضحت النتائج أن متوسط زمن البروثرومبين عند الذكور بلغ (١٣,٩ثانيه) بينما كان متوسط نسبة ال INR كان (٠,٩٨٥٧) ، أما في الإناث فقد كان متوسط زمن البروثرومبين (١٣,٨ ثانية) بينما كان متوسط INR (٠,٩٨٥١) , كذالك لم يكن هنالك فروق ذات دلاله إحصائية بين الذكور والإناث.

الخلاصة: أوضحت نتائج الدراسة أن الشاي الأخضر ليس له تأثير علي زمن البروثرومبين إلا عند تناوله لفترات ليست طويلة (شهور) .

Abstract

Background: Camellia Tea is a product made up from leaf and bud of sinensis plant .People around the world drink green tea as an everyday drink and as a therapeutic aid in many illnesses. This study aimed to drew attention to the importance of healthy green tea over other kinds of tea and to explore the effect of green tea on prothrombin time (PT) and International Normalized Ratio (INR). This can be a chived through descriptive case control study.

Methodology: In this study venous blood sample were collected on trisodium citrate 3.2% with ratio (9:1), from healthy adult (n=20) (10 male and 10 female) who do not drink green tea as control group, and other samples were collected from healthy adult (n=50) (24 male and 26 female) who drink green tea as test. The prothrombin time (PT) was performed by the Operation Manual (coatron M1- Soft ware C1.20) and INR was calculated.

Results:

The results of current study revealed that the mean of PT and INR in study group was (14.21 second and 1.002) ,while it was (13.89 second and 0.98) in Control group . Statistical analysis show that there was no significant difference between case and control in PT and INR with P.value of (0.189 and 0.653) .

More over the mean of PT and INR in male group was (13.9 second and 0.9857) , while it was (13.8 second and 0.9851) in female group .Also there was no statistical significant difference between males and females group in PT and INR with P.value of (0.968 and 0.96).

Conclusion:

From the current study we conclude that there was no effect of green tea drinking on prothrombin time except that the consuming of green tea for period of months.

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Chapter One

Introduction
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1.1 Introduction

Camellia Tea a product made up from leaf and bud of the plant sinensis. It is the second most consumed beverage in the world, well a head of coffee, beer, wine and carbonated soft drinks. [1,2]

Originating from china, tea has gained the world's taste in the past 2000 years. The economic and social interest of tea is clear and its consumption is part of many people daily routine, as an everyday drink and as a therapeutic aid in many illnesses. Depending on the manufacturing process, teas are classified into three major types:

Non-fermented green tea (produced by drying and steaming the fresh leaves to inactivate the polyphenol oxides and thus, non-oxidation occurs). Semi-fermented oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying).

Fermented black and red (Pu-Frh) teas which undergo a post harvest fermentation stage before drying and steaming, although the fermentation of black tea is due to an oxidation catalyzed by polyphenol oxidase, and that of Pu-Erh tea is attained by using microorganisms . [3,4]

Approximately 76 – 78% of the tea produced and consumed is black tea, 20 – 22% is green tea and less than 2% is oolong tea.[5]

Black tea is consumed principally in Europe, North America and North Africa (except Morocco) while green tea is widely drunk in China, Japan, Korea and Morocco; oolong tea is popular in China and Taiwan; in USA, the 80% of tea consumed is black ice tea. [5,6,7]

Green tea is prepared without the process of fermentation and heating. The process of fermentation of black tea leads to the activation of various enzymes and intensive changes with respect to color, aroma, and flavor. These changes are usually desirable for taste. Since green tea is heated before the process of fermentation and the fermentation is not carried out for green tea as it is for black tea, it is not usually suitable for taste with respect to aroma and flavor.

However, the consumption of steamed green tea has various beneficial pharmacological effects .[8]

black tea and green tea are powerful sources of flavonoids and other polyphenolic antioxidants, which have a protective effect in coronary artery disease (CAD).[9,10]

The prothrombin time was described by Quick in 1935 and the test was often referred to as 'Quick's Prothrombin Time.' The prothrombin time was developed to measure Prothrombin (Factor II) and hence its name. However, it subsequently became clear that it was sensitive to abnormalities of factors VII, X,V,II and fibrinogen.

The Prothrombin Time (PT) in contrast to the APTT measures the activity of the so-called extrinsic and common pathways of coagulation. The division of the clotting cascade into the intrinsic, extrinsic and common pathways is medieval and has little *in vivo* validity but nevertheless remains a useful concept for interpreting the results of laboratory investigation.[11]

1.2 Rationale

The coagulation parameters in terms of practical aspect need to some extent pre analytical preparation for patients to achieve reliability of result like some drugs taken e.g. heparin and others drinks. Sudanese populations in general have a special characteristic due to the diversity of the atmosphere and 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.

The importance of this study is to shed alight and drew attention to high light the importance of healthy green tea over other kinds of tea. Moreover ferret out secrets of drinking green tea.

In addition this study was never done before in Shendi, so it's a chance to add a knowledge about the effect of drinking of green tea on coagulation system

1.3 Objectives

1.3.1 General objectives

To evaluate the effect of green tea drinking in secondary heamostasis

1.3.2 Specific objectives

1- To determine the effect of green tea drinking on prothrombin time.

2- To evaluate the effect of green tea drinking on prothrombin time according to gender.

3- To evaluate the effect of duration of green tea drinking on prothrombin time.

4- To compare the effect between drinking of green alone and green tea with other types of tea on prothrombin time.

Chapter Two

Literature Review

2 Literature review

2.1 Haemostasis

Normal haemostatic 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 bleeding from sites of blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired.

The haemostatic system thus represents a delicate balance between procogulant and anticoagulant mechanisms allied to a process fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels. [12]

2.1.1 Primary haemostasis

2.1.1.1 Platelet production

Platelets are produced predominantly by the bone marrow 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 inluuence of various cytokines, including thrombopoietin. Once released from the bone marrow young platelets are trapped in the spleen for up to 36 hours before entering the circulation, where they have a primary haemostatic role. Their normal life span is $7 - 10$ days and the normal platelet count for all age groups is $150 - 450x10/L$. the mean platelet diameter is 1-2m and the normal range for 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 indirectly indicate the state of marrow production. [13]

2.1.1.2 Platelet function

The main function of platelets is the formation of mechanical plugs during the normal hemostatic response to vascular injury. In the absence of platelets,

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spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet- platelet (aggregation) interactions. The blood flow condition determine the specific receptor ligand interactions. [12]

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 Ib and the sub endothelial cells. [14]

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 multimers made up on average of 2-50 dimeric subunits, with a molecular weight (MW) of $0.8 - 20 \times 10^6$. VEF is encoded by a gene on chromosome 12 and is synthesized both in endothelial cells and megakarocytes, and stored in weibel-Palade bodies and platelet a granules respectively. [12]

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-Palde bodies. The stored VWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of desmopressin (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from weibel-Palade bodies is in the form of large and ultra large multiverse, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to monomeric VWF and smaller multiverse by the specific plasma metalloprotease, ADAMTS-1. [12]

2.1.1.5 Platelet aggregation

It is characterized by cross-linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has about 50 – 80 000 GPIIb/IIIa receptors, which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/IIIa molecules, due to binding of alpha-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb/IIIa, enabling platelet cross-linking with fibrinogen bridges. [12]

2.1.2 Secondary haemostasis

Intrinsic system is activated in vivo by the contact of certain coagulation proteins with sub endothelial connective tissue which sets the secondary haemostatic mechanism into motion extrinsic coagulation pathway in contrast is initiated with the release of tissue factor from injured vessel endothelial cells and sub endothelium into vessel 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 haemostasis namely the formation of fibrin clot the clot thus includes both fibrin formed in secondary haemostasis and the platelet plug formed in primary haemostasis. [14]

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 subsrates that have no biologic activity until converted by enzyme to activate enzymes called serine protease 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. [14]

The activation of zymogen factor X and II requires the presence of the non-enzymatic cofactors. VII and V respectively to perform their function. These cofactors must be activated (VIIa and Va) by small amounts of thrombin. Thrombin enhances their ability to assist in the activation of factors 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 seine protease or binding the zymogen and appropriate serine prorease on platelets PL surface to enhance and accelerate the zymogen activation process or both. [14]

In contrast the haemostatic 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 lysis. While tissue is be in repaired, the fibrinolytic 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. [14]

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: Factor XI, factor XII, prekallikerin, and high-molecular-weight kininogen (HMWK) are involved in the intrinsic pathway, moderately stable, and not consumed during coagulation. [15]

Table (2.1) Coagulation Factors

Factor 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
	Prekallikrein	Serine protease
	HMWK	Cofactor

2.1.4 Physiological Coagulation (In Vivo)

The original theory of coagulation used a cascade or waterfall theory. This description depicted the generation of thrombin by the soluble coagulation factors and the initiation of coagulation. This theory identified two starting points for the generation of thrombin: the initiation of the intrinsic pathway with factor XII and surface contact, and the extrinsic pathway with factor VIIa and tissue factor.

These two pathways meet at the common pathway, where they both generate factor Xa from X, leading to a common pathway of thrombin from Prothrombin and the conversion of fibrinogen to fibrin. This process holds true under laboratory conditions. The discovery of a naturally occurring inhibitor of hemostasis, tissue factor pathway inhibitor (TFPI), is able to block the activity of the tissue factor VIIa complex, soon after it becomes active. [15]

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2.1.5 Laboratory Model of Coagulation

Laboratory testing looks at the in vitro effect of the coagulation process which is measured by the Prothrobin time (PT), activated partial thromboplasmin time (APTT), thrombin time (TT), fibrin degradation products (FDPs), and D-dimer. This section will focus on PT and a PTT. While the coagulation cascade does not reflect what goes on in vivo, it provides a model in which the laboratory relates to for testing. However, the coagulation cascade reflects the mechanisms that the laboratory uses for results. The screening tests provide a tremendous amount of information to the physician. They can be performed both quickly and accurately . [15]

2.1.6 Coagulation Pathways

2.1.6.1 Extrinsic Pathway

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

2.1.6.2 Intrinsic pathway

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

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

2.1.6.3 Common Pathway

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 no top of that it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates factor XIII, which forms covalent bounds that crosslink the fibrin polymers that from activated monomers. [16]

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 tenase complex, until it is down – regulated by the anticoagulant pathways. [16]

2.1.7 Formation of Thrombin

When plasma fibrinogen is activated by thrombin, this conversion results in a stable fibrin clot. This clot is a visible result that the action of the protease enzyme thrombin has achieved fibrin formation. Thrombin is also involved in the XIII-XIIIa activation due to the reaction of thrombin cleaving a peptide bond from each of two alpha chains. Inactive XIII along with Ca^{+2} ions enables XIII to dissociate to XIIIa. If thrombin were allowed to circulate in its active form (Ia), uncontrollable clotting would occur. As a result thrombin circulation in its inactive form Prothrombin (II). Thrombin, a protease enzyme, cleaves fibrinogen (factor I) which results in a fibrin monomer and fibrinogen peptides A and B. These initial monomers polymerize end to end due to hydrogen bonding.

Formation of fibrin occurs in three phases:

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 XIIIa into fibrin polymers forming an insoluble fibrin clot.[16]

2.1.8 Fibrinolysis

Is a 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.[17]

In fibrinolysis, a fibrin clot, the product of coagulation, is broken down. Its main enzymeplasmin 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.

Plasma 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. [18]

Tissue plasminogen activator (t-PA) and urokinase are the agents that convert 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.[19]

T-PA and urokinase are themselves inhibited by Plasminogen activator inhibitor-1 and Plasminogen activator inhibitor-2 (PAI-1and 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 fibtinolysis inhibitor (TAFI), which modifies fibrin to make it more resistant to the tPA-mediated Plasminogen. [19]

2.1.9 Coagulation Inhibitors

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

These inhibitors are:

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

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

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

- Protein C and protein S. [12]

2.2 Prothrombin time (PT)

To as 'Quick's Prothrombin The prothrombin time was described by Quick in 1935 and the test was often referred Time.' The prothrombin time was developed to measure Prothrombin (pt Factor II) and hence its name. However, it subsequently became clear that it was sensitive to abnormalities of factors VII, X, V, II and fibrinogen.

The Prothrombin Time (PT) in contrast to the APTT measures the activity of the so-called extrinsic and common pathways of coagulation. The division of the clotting cascade into the intrinsic, extrinsic and common pathways is medieval and has little *in vivo* validity but nevertheless remains a useful concept for interpreting the results of laboratory investigations.

The prothrombin time is a one-stage test based upon on the time required for a fibrin clot to form after the addition of Tissue Factor (TF) (historically known as tissue thromboplastin), phospholipid and calcium to decalcified, platelet poor plasma.

The term 'Thromboplastin' was originally used to describe a substance in plasma that converted prothrombin to thrombin. Historically thromboplastins were

extracted from brain and other organs and these contained significant amounts of tissue factor [TF] and phospholipid [PL]. TF is species specific and most laboratories now use a recombinant human TF with an ISI close to 1 and which is relipidated to provide a source of phospholipid. Animal thromboplastins are usually derived from rabbit brain.

TF was originally designated Factor III when the nomenclature of the clotting proteins was undertaken. [11]

2.2.1 Prothrombin Time Blood Test-PT

1. This test is done to evaluate the blood for its ability to clot. It is often done before surgery to evaluate how likely the patient is to have a bleeding or clotting problem during or after surgery.

2. Normal PT Values: 10-12 seconds (this can vary slightly from lab to lab)

3. Common causes of a prolonged PT include vitamin K deficiency, hormone drugs including hormone replacements and oral contraceptives, disseminated intravascular coagulation (a serious clotting problem that requires immediate intervention), liver disease, and the use of the anti-coagulant drug warfarin. Additionally, the PT result can be altered by a diet high in vitamin K, liver, green tea, dark green vegetables, and soybeans.[11]

2.3 Disorder of coagulation

A balance between clotting and bleeding is always maintained in the body under normal physiology. However any pathological scenario will tilt this balance to either haemorrhagic or thrombotic complications. Hence as a corollary disorders of haemostasis can be categorised into those that lead to abnormal bleeding and those that lead to abnormal clotting.

Table (2.2): Classification of disorders of coagulation.

Bleeding disorders	Thrombotic disorder (thrombophilia)
Hereditary	Hereditary
Von Willebrand disease	Hereditary thrombphilia
Haemophilia A	Antithrombin III deficiency
Haemophilia B	Protein C deficiency
Haemophilia C	Protein S deficiency
Factor V deficiency	Factor V leiden (factor V mutation)
Factor X deficiency	Prothrombin mutation (Gene 201210A mutation)
Factor VII deficiency	
Factor XIII deficiency	
Prothrombin deficiency	
Afibrinogenemia	
Acquired	Acquired
Consumptive coagulophies	Antiphospholipid antibody syndrome
DIC	Increased levels of factors VIII, IX, XI, or fibrinogen
Microangiopathic haemolytic anemias	Fibrinolysis defects
Vitamin K deficiency liver disease	Homozygous homocystinuria

2.3.1 Bleeding Disorders

2.3.1.1 Haemophilias

Haemophilia A, the most common form of haemophilia is associated with the deficiency of factor VIII and B (Christmas disease) is secondary to deficiency of factor IX. C is only found in 1% of the population and is due to deficiency of factor XI. Haemophilia's are X linked recessive disorders and occur in males. The severity of the bleeding tendency is directly related to the levels of the coagulation factors. The levels of factor VIII need to be assessed in the preoperative period and human or recombinant factor VIII concentrates are transfused to keep the factor VIII levels 100% in the perioperative period. [20]

Von Willebr and disease is the most common autosomal dominant inherited disorder of coagulation due to abnormality in the production of vWF, which may be qualitative or quantitative. vWF acts as a carrier molecule for factor VIII coagulant protein and the deficiency of former has a profound effect on the stability of latter. The clinical presentation may be variable and complete deficiency, though rare leads to severe bleeding. The diagnosis is usually made by prolonged bleeding time in the presence of adequate platelets. Antifibrinolytic agent and tranexamic acid may be used for minor bleeding. Desmopressin D arginine vasopressin administered in the preoperative period is expected to raise the concentration of factor VIII in patients with quantitative disord.[20]

2.3.1.2 Fibrinogen deficiency

Total or partial is an extremely rare inherited bleeding disorder. Afibrinogenemia is rather well tolerated and may manifest as subcutaneous haematoma or umbilical haematoma at birth. The clinical findings are variable in childhood and adults .[21] DIC is the most common consumptive coagulopathy that maybe observed secondary to numerous causes. It often presents as diffuse bleeding associated with consumption of coagulation factors and thrombocytopenia secondary to widespread small vessel thrombosis. Common causes include sepsis/infections, obstetric causes, incompatible blood transfusion, shock, trauma and embolism.

2.3.1.3 Liver disease

Majority of clotting factors are synthesized in liver therefore severe liver disease is associated with coagulopathy. Since liver is also involved in the clearance of activated clotting factors and fibrinolytic products, it may predispose to DIC. Management of bleeding secondary to liver disease is based on the laboratory values of various coagulation tests.

Hypothermia is also associated with anticoagulatory effects, which are more pronounced in the presence of acidosis. The effects may result from platelet

dysfunction in mild hypothermia (below 35°C) to decreased synthesis of clotting enzymes and plasminogen activator inhibitors when temperatures is <33°C [.22]

The availability of newer oral anticoagulants, targeting either thrombin (dabigatran etexilate) or factor Xa (rivaroxaban or apixaban) exhibit rapid onset/offset and minimal drug interactions with more predictable pharmacokinetics thus eliminating the need for frequent coagulation monitoring. All these features give newer oral anticoagulants a major pharmacological benefits over vitamin K antagonists. [23]

2.3.2 Thrombotic Disorders

Plasma concentration of certain coagulation factors (factor V, VII, VIII, IX, fibrinogen) increase progressively with ag . [24] Same is true for vWF, a key protein in platelet vessel wall interaction . [21] High incidence of cardiovascular events seen in elderly may be due to increased levels of plasma fibrinogen, which enhance the bridging of the platelets via glycoprotein IIb-IIIa receptor and act as a direct substrate for clot and/or by increasing the blood viscosity . [24]

The constitutive or acquired disorders of thrombosis are termed as thrombophillia. There are number of factors that are associated with the hypercoaguable states. In addition to the genetic and hereditary disorders that predispose to thrombosis, several risk factors such as smoking, obesity, pregnancy, immobility, malignancy, surgery, females on oral contraceptives may also contribute to its development . [25] Disorders of ATIII deficiency, reduced protein C and Protein S are inherited in autosomal dominant fashion and are associated with increased risk of thrombosis. Acquired Protein C and Protien S deficiency may be observed in vitamin K deficiency, warfarin therapy, pregnancy, liver cirrhosis and sepsis . [26] Numbers of observational studies have shown decreased levels of APC in critically ill-patients that may have a direct correlation with the mortality. [27]

The risk of thromboembolism in the perioperative period is well recognized. Therefore, patients with herditary thrombophillia should be given thromboprophylaxis.

During pregnancy stasis due to obstruction of inferior vene cava by gravid uterus along with increase in the majority of clotting factors, fibrinogen and vWF is observed. Activity of Protein S decreases with simultaneous resistance of protein C. In addition, fibrinolytic system is also impaird thus contributing to a hypercoaguable state that makes the parturient more prone to thromboembolism. [28]

During surgery and trauma, prolonged immobility promotes stasis which results in local hypoxia. Physical disruption leads to exposure of TF thus triggering thrombosis . [21] Furthermore during the first hours of surgery, there is increase in TF, tissue plasminogen activator (tPA) and vWF, leading to hypercoaguable state thus promoting venous thrombosis. Even a venepuncture cause vascular wall injury thus, predisposing to thrombus formation. Since lower limb is associated with stasis and immobilization during surgery, venepuncture preferably should be avoided in the lower limb.

2.4 Fibrinolytic System

Fibrinolytic system is a parallel system which is activated along with activation of coagulation cascade and serves to limit the size of clot. Fibrinolysis is an enzymatic process that dissolves the fibrin clot into fibrin degradation products (FDPs) by plasmin originating from fibrin bound plasminogen in liver. This reaction is catalysed by tPA or urokinase plasminogen activator (u-PA) released from vascular endothelium. The release of t-PA is stimulated by tissue occlusion, thrombin, epinephrine, vasopressin and strenuous exercise.

Plasmin activity is tightly regulated by its inhibitor (α-2 antiplasmin) thus preventing widespread fibrinolysis. [29] In vivo activity of the fibrinolytic system is assessed clinically by measuring the FDP's. D dimers are produced by digestion of cross linked fibrin and are specific indicators of fibrinolysis used in the assessment and diagnosis of pulmonary embolism, DIC or deep vein thrombosis. [29]
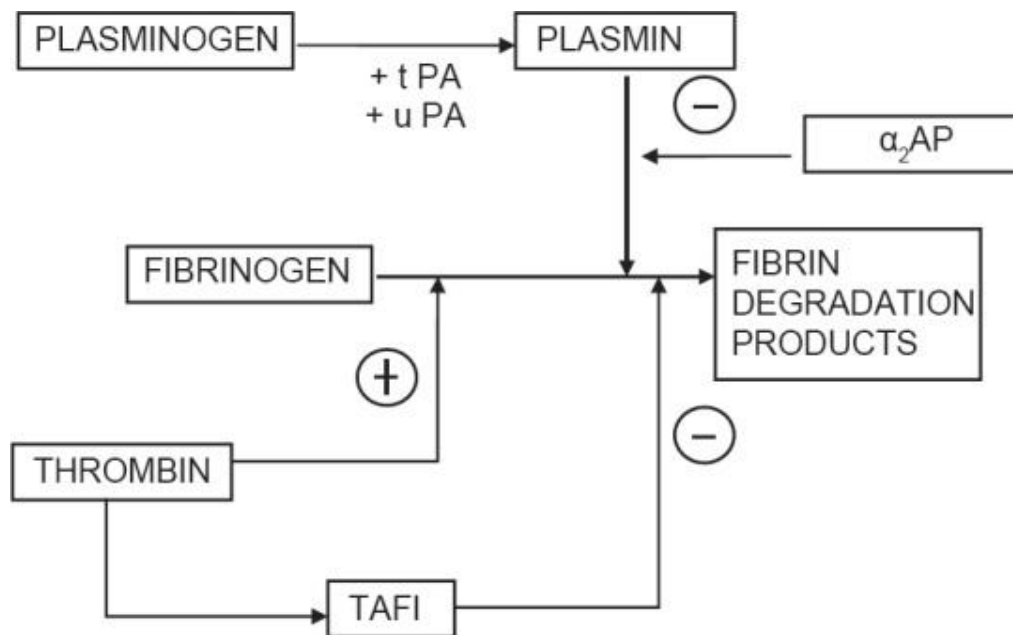
Figure (2.1) fibrinolytic system.

2.4.1 Regulation of the fibrinolytic system

Since plasmin has the potential to degrade fibrinogen leading to deleterious consequences, the fibrinolytic activity is limited by following factors:

Plasminogen activator inhibitor - It is the main physiological inhibitor of fibriolysis and acts by inhibiting t-PA and u-PA irreversibly AFI - It is a plasma proenzyme synthesized by liver and activated by thrombin. It decreases the affinity of plasminogen to fibrin and augments the action of anti-trypsin in inhibiting plasmin.

Plasmin inhibitors - α2 antiplasmin and α2Macroglobulin are the glycoproteins that exert action by virtue of plasmin inhibition. [30]

2.4.2 Disorders of Fibrinolysis

Congenital disorders pertaining to fibrinolytic system are rare. Although the hyperfibrinolytic state is associated with increased tendency to bleed, deficiency of the same predisposes to thromboembolism. [31] Excessive activation of fibrinolysis may be observed during cardiopulmonary bypass, hence antifibrinolytics have a beneficial role in the prevention of same. Acquired hyperfibrinolysis may be encountered in trauma, liver cirrhosis, amniotic fluid

embolism, multiple myeloma, snake bite and conditions associated with massive activation of t-PA, which can lead to DIC and haemorrhage. [31]

2.5 Green tea

Scientific Names: Camellia sinensis, Camellia thea, Camillia theifera, Thea sinensis, thea bohea, Thea viridis [32]

Common Names: Green tea, Black Tea, Chinese Tea, Unfermented Tea, Sencha.[33]

Active Ingredients: [33-34]

1. Polyphenols (Catechins) (10-25%): a. epigallocatechin-3-gallate (EGCG), epicatechin, epicatechin-3-gallate, epigallocatechin.

2. Purine Alkaloids (Methyl Xanthines): a. caffeine (2.9-4.2%), theobromine (0.15-0.2%), theophylline (0.02-0.04%).

3. Anorganic Ions: a. fluoride (130-160 mg/kg), potassium, aluminum.

4. Other Ingredients: a. flavonoids (e.g. quercetin), caffeic acid derivatives, triterpene saponins, volatile oils.

2.5.1 Mechanisms of Action

1. Anticarcinogenic = antioxidant activity promotes inhibition of biochemical markers of tumor initiation and promotion, induction of apoptosis, and inhibition of cell replication rates thus retarding the growth of neoplasms .[35]

2. CNS Effects= caffeine is centrally stimulating and causes antidepressant Effect. [33]

3. Diuresis = adenosine antagonism by caffeine leads to dilation of the renal vessels with a consecutive increase in the rate of filtration. [33]

4. Antidiarrheal = tannin effect and polyphenols promote the growth of Lactobacillis and Bifidobacter while inhibiting the growth of C. perfingens and C. difficile. [33]

5. Decreased Cholesterol = antioxidants have a direct effect on lowering LDL and TG's. [32]

6. Anti-inflammatory = bradykinin and prostaglandin antagonism causes a capillary sealing. [33]

7. Dental Hygiene = large amount of fluoride and inhibition of the growth of cavity-associated bacteria such as Streptococcus mutans and E. coli. [32-34]

2.5.2 Indications and Efficacy

1- Cancer prevention = Likely effective.

According to a review of the evidence for the efficacy of unconventional therapies used in cancer patients,several studies have demonstrated green tea polyphenols preventative and inhibitory effects against tumor formation and growth ,While the studies are not conclusive ,green tea polyphenols ,particularly EGCG,my be effective in preventing cancer of prostate breast esophagus, stomach, pancreas and colon .Thus much of the research in to the effects of green tea has focused on it's potential to prevent cancer; however, there has been limited research in to its role in the actual treatment of disease.[35]

2-A ntioxidant Application =Iikely effective.

Many chronic disease states and inflammatory conditions are aresult of oxidative stress and generation of free radicals .Such disease include heart disease, renal failure, cancer, skin exposure damage ,and several diseases associated with aging.

Green tea polyphenols are potent free radical scavengers due to the hydroxyl groups in their chemical structure. Hydroxyl groups can form complexes with freeradicals and neutralize them, preventing the progression of the disease process. [33]

There have been conflicting studies in this area, however. [36]

3-Dental Hygiene=likely effective.

Various components of green tea have properties to suggest an anti-cariogenic activity. Such activities include a direct bactericidal effect against Streptococcus mutans and S. sobrinus, prevention of adherence to teeth, inhibition of glucosyl transferase which limits the biosynthesis of sticky glucan, and inhibition of human and bacterial amylases. Studies in animal models show that these in-vitro effects can translate into dental caries prevention. In addition, there have been a few clinical trials in humans that suggest similar findings. [37]

Inhibition of plaque deposition in the tea group vs. placebo.

4-Obesity & Weight Loss = possibly effective.

Recent studies on green tea's thermogenic properties have demonstrated a synergistic interaction between caffeine and catechin polyphenols that appear to prolong sympathetic stimulation of thermogenesis. [33]

2.5.3 Drug-Food Interactions [32-33, 38-39]

1-Acid-Inhibiting Drugs = caffeine increases stomach acid and can interfere with antacids, H2-Antagonists, and proton pump inhibitors.

2-Adenosine = concomitant use might inhibit the hemodynamic effects of adenosine.

3-Aspirin = caffeine can increase the effectiveness by as much as 40% by increasing overall pain reduction.

4-Acetaminophen = caffeine can increase the effectiveness by as much as 40% by increasing overall pain reduction.

5-Antipsychotic Agents = caffeine can decrease the metabolism of these agents.

6-Barbiturates = can decrease the effects of caffeine.

7-Beta-Agonists = caffeine can increase the cardiac inotropic effect of these drugs.

8-Benzodiazepines = concomitant use might reduce sedative effects of benzodiazepines.

9-Beta-Blockers = concomitant use can increase blood pressure.

10-Caffeine = concomitant use can increase the risk of adverse effects.

11-Chlorpromazine = concomitant use inhibits the effects of this drug.

12-Cimetidine = decreases caffeine clearance by 30-50%.

13-Clozapine = caffeine can increase the effects and toxicity of this agent

14-CNS Depressants = can increase the toxic effects of caffeine.

15-Diabetic Drugs = monitor blood glucose closely due to claims that caffeine has hyperglycemic effects.

16-Disulfiram = decreases clearance and metabolism of caffeine.

17-Ephedrine = concomitant use enhances side effects of agitation, tremors, and insomnia.

18-Ergotamine = caffeine can increase the GI absorption of this drug.

19-Fluconazole = inhibits the metabolism of caffeine.

20-Grapefruit Juice = can increase caffeine levels, activity, and the risk of adverse events.

21-Iron = concomitant use may reduce the absorption of iron.

22-Lithium = abrupt caffeine withdrawal can increase serum lithium levels.

23-MAO Inhibitors = concomitant use with large amounts of caffeine can cause a hypertensive crisis.

24-Milk = may decrease the antioxidant effect.

25-Oral Contraceptives = can decrease caffeine clearance by 40-65% .

26-Quinolones = can decrease or increase caffeine clearance.

27-Phenytoin = concomitant use might enhance metabolism and excretion of caffeine.

28-Theophylline = caffeine can increase theophylline levels.

29-Verapamil = can increase plasma caffeine levels by 25%.

30-Warfarin = concomitant use can cause bleeding.

2.5.4 Drug-Disease Interactions

1. Gastric, Duodenal Ulcers = Caffeine can aggravate these conditions.

2. Heart Conditions = Caffeine can induce cardiac arrhythmias in sensitive individuals.

3. Depression, Anxiety Disorders = Caffeine can aggravate these conditions.

4. Anemia = caffeine may reduce the absorption of already depleted iron stores .

2.5.5 Other Safety Issues [32]

1. Pregnancy = possibly safe in moderation although caffeine crosses the placenta.

2. Lactation = caffeine should be avoided because it can cause sleep disturbances in breast-fed infants.

2.6 Previous studies

Effect of green tea on (coagulation profile) was found in many previous data ; study done by F. Jalali and his colleagues in 2008 in Iran on 100 pation who given brewed green tea (4gper day in divided doses) for one month.This study conducted to document the effect of green tea on Serum Lipids, Antioxidants, and coagulation test in stable coronary Artery Disease .Also the result was significantly decrease in fibrinogen, PT and APTT after one month's consumption of green tea (P< 0.001). Also, there was a significant decrease in fibrinogen and homocysteine levels. There was an increase in HDL and antioxidant levels after the consumption of green tea (P<0.001). In addition, average PT and PTT measurements were decreased significantly (P = 0.001 and P= 0.012, respectively). Conclusion- Regular consumption of 4g/d green tea for one month had beneficial effects on serum lipid parameters and antioxidant levels. [8]

Another study done in Sudan by Hussam MA Ibrahim and his colleagues and bulished in International Journal of Applied Research at (2017) in page 703-705 .The research studied the effect of green tea consumption on coagulation profile among adult healthy Sudanese, this study aimed to draw attention to the importance of healthy green tea over other kinds of tea and to explore the effect of green tea on coagulation profile.This study aimed to assess the effect of green tea consumption on coagulation profile among apparently healthy adult Sudanese individuals. The prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen test were performed by the coagulometer .There was statistical significant difference (p.<0.05) in the fibrinogen level before and after consumption of green tea (before 339.9+-62.5) (after 310.6+_47.9).While there was no statistical significant difference in other coagulation tests. Moreover there was statistically significance in levels of fibrinogen between males and females. The mean of fibrinogen level in male before and after were(306+_55) (275+_28) respectively and in female were

(362+_57) (343+_44) respectively .This study didn't obtain any statistical significance in fibrinogen levels between different age group. [40]

Also study done by Kannan Eagappan, Mamatha G.philip, Deenac .Sangeetha the objective of the study was to asses the effect of green tea on the pharmacodynamics of single oral dose of warfarin in healthy volunteers .15 healthy male volunteers in the age group of (18-40)years were selected and given 20 mg warfarin orally on Day 1 of phase1 .PTand INRwere measured for 7 day .After a wash out period of 7 days ,green tea was given thrice daily (6mg/day)for 10 days .On the 4th day of green tea administration 20 mg warfarin was given in phase II and PT and INR were measured for 7 days . Day –wise and phase –wise means were calculated, along with other pharmacodynamic parameters like INR_{max}, PT_{max}, PT_{AUC}, INR_{AUC} and time to reach PT_{max} and INR_{max}. Tow tailed t test was used to analyze the differences .There was a statistically significant decrease in the mean INR (p=0.042) and PT(p=0.021)in the treatment phase (warfarin +green tea)when compared with the control phase (warfarin alone). When the mean INR and PT for each day was analyzed separately ,this deference was statistically significant only on Day 5 and 6.As the antagonism of warfarin by vitamin K is competitive in nature . This finding assumes significance and high lights a need for caution when green tea is consumed by patients on warfarin. [41]

Chapter Three

Material and Method

3 Material and Methods

3-1 Study Design

This descriptive prospective Case control study which conducted in Shendi locality-River Nile state during the period of (March to May2018)and aimed to evaluate the effect of drinking of green tea on pt and INR.

3-2 Study Area

This study was conducted at Shendi city. Located in River Nile State, Shendi is the centre of the Ja'aliin tribe and important historic trading center. It is bounded by Elddmer locality northern of River Nile State ,Khartoum state to the north , River Nile to west and Gadarif State to the east .Geographically it lies between line 360 east to 310 west longitudinal and line 190 north to line 150 south latitudinal in the arid Zone of Sudan .The Rural areas of the Shendi locality are composed of about 96 villages, 63 of these are at southern side of the locality.

3-3 Study Population

A total of (50) venous blood sample were collected from healthy individuals who drinking green tea as test group and (20)venous blood samples were collected from healthy individuals who don't drink green tea as control group.

3-4 Inclusion Criteria

Healthy Individuals who drink green tea from both sex with different age.

3-5 Exclusion criteria

Individual who don't drink green tea.

3-6 Data Collection tools

Data collected using self–administrated pre-coded questionnaire which specifically designed to obtain information that helped in study.

3-7 Blood Sampling

Venous blood collected using sterile disposable plastic syringe after cleaning the vien puncture area with (70%) ethanol, the blood added to the anticoagulant and gently mixed. The sample centerfuged at 1000 rcf for 15 min to obtain plasma for (PT test).

3-8 Methods Prothrombin time

3-8-1 Principle

The one stage prothrombin time measures the clotting time of test plasma after the addition of Thromboplastin reagent containing calcium chloride.

The reagent supplies asource of "tissue thromboplastin", activating factor vII, and is therefore sensitive to all stage II and III Factors .Deficiencies of stage 1 Factors (VII,IX ,XI and XII) are not detected by the test.

3-8-2 International Sensitivity Index (ISI)

International committee for standardization in Hematology and the International committee on Thrombosis and Hemostasis have agreed on recommendations for the reporting of prothrombin Time results based up on an international sensitivity Index (ISI) of Thromboplastin reagents and an International Normalized Ratio (INR).Thromboplastin reagents are assigned an ISI value by calibration against an International Reference preparation,   (IRP,67/40) which by definition has an ISI =1.0.The ISI value assigned to commercial Thromboplastin reagents therefore defines a comparative slope ,or relative sensitivity ,in comparison to the Reference Thromboplastin . The lower the ISI value ,the more "sensitive" the reagent. By knowing the ISI of aparticular Thromboplastin reagent , the ratio can be calculated which wouid have been found if the IRP 67/40 had been used as the reagent .

This is termed the international Normalized Ratio (INR), and is determined by:

$$INR = R^{ISI} = Ratio^{ISI} = \left(\frac{Patient\ PT\ (S)}{Normal\ PT\ (S)} \right)^{ISI}$$

Reference Range:

(11-14) second

3-8-3 Components and Reagents

Components:

   1- Operation Manual for Coatron M1 Rev.C1.20.

   2- 2-Anticoagulant :use buffered sodium citrate ,3.8% or 3.2%.

   3-Otomatic pipette 100 micro liter.

   4-Test tubes.

3-8-4 Test Procedure

A-1-Obtain venous blood by clean venipuncture.

2-Immediately mix 9 parts blood wih 1 part anticoagulant (buffered sodium citrate , 3.8% or 3.2%, mix well by inversion of tube against the stopper .

3-centrifuge the specimen at 1000 rcf for 15 min.

4-Remove plasma from the tube with in 60 min .using a plastic pipette and store in aplastic tube .

5-Test plasma sample with in 2 hr, other wise store frozen and thaw just prior to use.

B-Coatron M1 Preparation

1-Turn on instrument and waituntil the ready LED is lighting.

2-Turn on printer if connected

3-Select "PT" as active test

4-Check Calibration

5-Allow reagent to prewam at least 5min

Procedure on Coatron M1

1-pipette 25ML plasma into cuvette.

2-prewarm plasma for 1 min.

3-Transfer cuvette to measuring position.

4-Activate optic (press key "optic".

5-Add 50 MLprewarmed Thromboplastin and simultaneous start the optic .press Key"optic " again).

6-The instrument will read maximal 300 second . If No colt detected the display wil lread "+ + + s"

7-The result is displayed in seconds and INR.

3-9 Ethical Consideration

The informed consent of the selected individuals of the study was taken after being informed with all detailed objectives of the study and it is health emphasis in the future.

3-10 Data Analysis

The collected data code in master sheet and proceed for analysis using (SPSS) program. (Mean and p.value by using independent T test).

3-11 Data Presentation

This results of this research shown in tables.

Chapter Four

Results

4 Results

This prospective cross sectional study was conducted to evaluate the effect of green tea drinking on the value of PT and INR.

In the present study the mean of PT and INR value in test group was (13.89 second and 0.98), while in control group was (14.21 second and 1.002) as noted in table (4-1).

Also the current study revealed that the mean of PT and INR in male group was (13.9 second and 1.002), while in female was (13.8second and 0.9851) as demonstrated in table (4-2) .

According to the table (4-3) the results showed that the mean of PT and INR in those individual who drink green tea only was (13.9 second and 0.988), while in those who drink green tea with black tea was (13.78 second and 0.977).

Also the results of present study revealed that the mean of PT and INR in individual who drink green tea for months was (14.78 second and 1.01), while the mean of PT and INR in individuals who drink green tea for years was (13.27 second and 0.977) as shown in table (4-4).

Regarding the age ; the mean of PT and INR in age group of (15-24years) was (14.18 second and 1.00),while it was (13.86 second and 0.98) in age group of (25-35 years), in addition the mean of PT and INR was (13.67 second and 0.97) according to age group of (36-45years), further more the mean of PT and INR was (14.02 second and 0.99)in age group of (46 years and more) as shown in table (4-5).

Also according to green tea drinking times per day, the mean of PT and INR in individuals how drink green tea once time a day was (13.92 second and 0.987), while it was (13.85second and 0.982) in individuals how drink green tea twice a day, in addition the mean of PT and INR was (13.87 second and 0.983) in the others how drink green tea three times a day as demonstrated in table (4-6).

Table (4-1): Showed the mean of PT and INR in test and control group

Type of sample	Mean of PT	p.value	Mean of INR	p.value
Control	14.21	0.189	1.002	0.653
Cases	13.89		0.98	

Table (4-2): Showed the mean of PT and INR according to gender in Cases:-

Sex	Mean of Pt	p.value	Mean of INR	p.value
Male	13.9	0.968	0.9857	0.96
Female	13.8		0.9851	

Table (4-3): Showed the mean of PT and INR according to Type of tea

Type of tea	Mean of Pt	p.value	Mean of INR	p.value
Green tea only	13.93	0.582	0.988	0.58
Green and Black tea	13.78		0.977	

Table (4-4): Showed the mean of PTand INR according to drinking tea period

Drinking tea period	Mean of Pt	p.value	Mean of INR	p.value
Months	14.78	0.081	1.01	0.044
Years	13.27		0.977	

Table (4-5) Showed the mean of PT and INR according to age groups

level of age	Mean of Pt	p.value	Mean of INR	p.value
15-24years	14.18		1.00	
25-35years	13.86	0.411	0.98	0.411
36-45years	13.67		0.97	
46 and more	14.02		0.99	

Table (4-6) Showed the mean of PT and INR according to green tea drinking times per day

Number of time	Mean of Pt	p.value	Mean of INR	p.value
Once time aday	13.92		0.987	
Twice aday	13.85	0.838	0.982	0.838
Three times aday	13.87		0.983	

Chapter Five

Discussion
Conclusion
Recommendations

5-1 Discussion

The drinking tea was became traditional habits in our community; some of people drink green tea only and others preferred it in combination with black tea. So this study was conducted to assess the effect of green drinking on PT and INR.

The current study revealed that the mean of PT in control group was (14.21second), while in study group was (13.89 second). Statistical analysis showed that there was no significant variation with P.value of (0.653). This result was similar to the result of study done by Hussam in Khartoum –Sudan, which reveal also there was no statistical significant variation in PT before and after consumption of green tea.[40] Also this results were disagree with results of study done by Jalali and his colleagues; which found a significantly decreased in PT. [8] These finding of Jalali my be disagreed with our finding due to using large sample size for study (100 case) and taking them fixed dose of brewed green tea (4g per day in divided doses) for one month. Also the results revealed that the mean of INR in control group was (1.002), while in study group was (0.98). Statistical analysis show there was insignificant variation with p.value of (0.653).

This also the results of present study show that the mean of PT in male and female was (13.9 second, 13.8 second). Statistical analysis show there was insignificant variation with p.value of (0.968).

The mean of PT in individuals who drink green tea only was (13.9 second), while in individuals who drinks both black and green tea was (13.7 second).Statistical analysis noted that the difference is not significant with p.value of (0.58).

The results of current study revealed that the mean of (PT) in individuals who consume green tea for months was (14.78 second), while it was (13.27second) in others who consume green tea for years. Statistical analysis showed that the difference was significant with p.value of (0.044).This maybe due to the fact

that green tea has effect on fibrinogen by decreasing the concentration of fibrinogen protein in human plasma which effect in blood coagulation process. Also according to level of age ,the mean of PT and INR in age group of (15-24 years),(25-35 years),(36-45 years) and (46 years and more) was (14.18 second and 1.00), (13.86 second and 0.98), (13.67 second and 0.97) and (14.02 second, 0.99). Statistical analysis showed that there was insignificant variation with p.value of (0.411). Further more current study demonstrated that the mean of PT and INR in individuals how drink green tea once time a day was (13.92 second and 0.987), while it was (13.85 second and 0.982) in individuals how consume green tea twice a day, more over it was (13.87 second and 0.983) in the others how drink green tea three times a day. Statistical analysis demonstrated that there was insignificant variation in PT and INR with p.value of (0.838) according to drinking times per day.

5-2 Conclusion

By the end of this study we conclude that:

- Green tea has effect on prothrombin time PT and international normalize ratio INR according to drinking green tea period.

- The green tea has no effect on PT and INR according to gender, type of tea, age and times of drinking green tea per day.

5-3 Recommendations

1- The research in this topic should be continued with increasing sample size and area of research and by addition other laboratory parameter to obtain accurate results and introduce knowledge.

2- Further study should be conducted in patient with Coronary artery disease by using the same coagulation parameter which used in this study.

3- Experimental study should be conducted on animals as a case control because it is easy to control and to ensure the ideal doses of effective agent was taken.

Chapter Six

References

Appendix

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Appendix I

Shendi University

Faculty of medical laboratory sciences

College of Graduate Studies

Questionnaire about the effect of green tea on prothrombin time

Name:…………………………………………………………………………

State:…………………………………………………………………………

City:…………………………………………………………………………

Sample number:

    1- **Age:** a - 15 – 24   (……).   b- 25 – 35 (……).   c- 36 – 45 (……...).

         d- 46 and more (……)

    2- **If you drinking tea, which type you drink?**

        a- Green tea only (……).   b-Green and Black tea (……).

    3- **Drinking tea period:**

        a- Month   (……)   b- Years   (….…)

    4- **How many times do you drink green tea during the day ?**

        a-once time a day   (……..)   b- Twice a days   (……..)

        C-Three times a day (…..….)

Appendix ii

إقـرار بالمــوافقة

الاسم...

العمر...   العنوان.........................

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة تأثير شـــرب الـــشاي الأخضر على زمن البروثرومبين PT و INR.

هند محمود إبراهيم علي

بعد أن شرحت له بأنه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر على صحتي بأي حال من الأحوال كما انه يحق لي بدون إبــداء أســباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف:

د. محمد عثمان على

التوقيع..........................   التاريخ..............

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Appendix III

Rabbit brain theomboplastin for PT determination
ISI = 1.05