Sudan University of Science & Technology
College of Graduate Studies

Effect of Green Tea Consumption on Prothrombin Time among Healthy Sudanese Volunteers

تأثير استهلاك الشاي الأخضر علي زمن البروثرومبي في المتطوعين السودانيين الأصحاء

A dissertation submitted for partial fulfillment for the requirement of MSc degree in Medical Laboratory Sciences (Hematology and Immunohematology)

By

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November 2018
الأية
قال تعالى:

(قل لو كان بحرٌ مدادًا لكلمتarti ربي لنفد بحرٍ قبل أن تنفد كلمتarti ربي ولو جئنا بمثلي مدادًا)

صدق الله العظيم
سورة الكهف الآية (109)
Dedication

Thanks first and finally to Allah, Thanks to my mother who means a lot to me, to my brother and sisters who support me all time, Special thanks to my dear husband for encouraging and helping me, to my lovely kids.

Finally great thanks to kind supervisor

Dr. Ibrahim Khider
Acknowledgment

First praise to almighty Allah who gave me health and ability to complete this work.

I would like to express my deep appreciation to Mr. Ibrahim Khider for his encouragement, patience, guidance and helpful supervision throughout all stages of this work.

I am indebted to Bashair Hospital and all of staff Team work in it.

I thank all of participants who donate to give me the samples.

I wish to thank the staff of haematology department colleague of medical laboratory sciences in Sudan University.
Abstract

This study is an experimental intervention study aimed to study the effect of the consumption of green tea on pro thrombin time level among apparently healthy Sudanese volunteers. A total of 40 healthy Sudanese volunteers participate, aged between 20-45 years, twenty-one of them were females and nineteen were males.

Each subject recruited into the study instructed to consume steamed green tea for one month (two cups per day). Two and half milliliter (ml) of venous blood was collected in 3.2% tri-sodium citrate from each participant before and after the consumption of green tea; platelet poor plasma was prepared and used for measurement of (PT). Determination of (PT) was done by a semi automated coagulometer (Stago &France).

The data were analyzed using statistical package for social sciences (SPSS), version16.

A paired sample t-test was used to compare the mean PT before and after consumption of green tea. A P-value less than 0.05 were considered significant.

The mean of pro thrombin time level was found to be shorter in samples collected after green tea consumption than in the samples collected before green tea consumption (14.33 ± 0.96 sec, 14.06 ± 0.90) sec respectively, the difference was Statistically significant (p. value =0.000) statistically significant difference was found in the mean of pro thrombin

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time level between males and females in both pre and post green tea consumption samples \( (p.\text{value} = 0.000, \ 0.005) \) respectively. No statistically significant difference was found in the two aged group \( (p.\text{value} = 0.09 \text{ and } 0.06) \) respectively.

In conclusion, consumption of two cups of green tea per day slightly decreased the level of pro thrombin time level among healthy Sudanese volunteers.
الملخص

هذه دراسة من النوع التجاري التدخلي هدفت إلى دراسة تأثير استهلاك الشاي الأخضر على معدل زمن البروثرومبيين بين متطوعين سودانيين اصحاء.

شارك في هذه الدراسة مجموع عدد أربعون من المتطوعين السودانيين الأصحاء اعمارهم بين عشرون الي خمسة وأربعون عاما. واحد وعشرون منهم كانوا نساء وتسعة عشر كانوا رجال. قمنا بالاشارة الى كل المتطوعين بشرب كوبين من الشاي الأخضر لمدة شهر واحد ومن ثم جمعنا انتين ونص مل عينات دم وريدية في مائع التجلط سترات الصوديوم الثلاثية قبل وبعد استهلاك الشاي الأخضر. تم تجهيز البلازما فقيرة الصفائح الدموية لقياس مستوى زمن البروثرومبيين وتم جمع البيانات مباشرة من المشاركين وتحليلها باستعمال الحزم الإحصائية لعلم الاجتماع spss. ووجد اقل في العينات التي جمعت بعد استهلاك الشاي الأخضر من تلك العينات التي جمعت قبل استهلاك الشاي الأخضر (قيمة 14.33 اس دي 5.46 و14.06 اس دي 09.94). على التوالي اختلاف هام بشكل احصائي وجد في مستوى البروثرومبيين تايم (قيمة P=0.00).

(اختلاف هام بشكل احصائي وجد في متوسط مستوى البروثرومبيين تايم بين الاناث و الذكور في كلتا قبل و بعد عينات استهلاك الشاي الاخضر (قيمة P=0.000
0.05) علي التوالي لا اختلاف هام بشكل احصائي وجد في مستوى البروثرومبيين في الفئات العمرية المختلفة (قيمة p=0.06). علي التوالي.

في الخلاصة نستنتج أن شرب كوبين من الشاي الأخضر يخفض مستوى زمن البروثرومبيين بصورة طفيفة بين المتطوعين الأصحاء.

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<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AT</td>
<td>Anti-Thrombin</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>EC</td>
<td>Epicatechin</td>
</tr>
<tr>
<td>ECG</td>
<td>Catechinsepiallocapicatechingallate</td>
</tr>
<tr>
<td>EGC</td>
<td>Epicatechingallate</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin-3gallate</td>
</tr>
<tr>
<td>GPIb</td>
<td>Glyco protein 1b</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoproteins</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National committee for clinical laboratory</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin Time</td>
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<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue factor plasminogen inhibitor</td>
</tr>
<tr>
<td>VWD</td>
<td>Von well brand disease</td>
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<tr>
<td>VWF</td>
<td>Von well brand factor</td>
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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION:

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties. Traditional system of medicine is found to have utilities as many accounts. Due to population rise adequate supply of drug and high cost of treatment in side effect along with drug resistance has been encountered in synthetic drugs, which has lead to an elevated emphasis for the use of plants to treat human diseases. The affordability of herbals has also drawn the attraction towards their use. India is one of the oldest civilizations which is known for rich repository of medicinal plants. *Camellia sinensis* is the species of plant whose leaves and leaf buds are used to produce Chinese tea (Namita et al., 2012)

1.1.1 Types of tea:

All of which come from the leaves of *Camellia sinensis* plant. White tea is the least processed type of tea and has the highest catechin content. It is made of young tea leaves or buds steamed immediately after harvesting to inactivate polyphenols oxidase, the enzyme that destroys catechins. As a
result, white tea is richer in catechins than green tea. About three billion Kg of tea is produced and consumed every year. Green tea is mainly consumed in Japan, China and India. Of the tea produced worldwide 78% is black tea which usually consumed in western countries, 20% green tea, normally consumed in Asian countries, and 2% is Oolong tea which is produced by partial fermentation in Southern China (Nishant et al., 2012).

1.1.2 Green tea preparation

Camellia assamica plant prepared by different methods, such as daily preparation, infusions, decoctions etc. Tea also refers to the aromatic beverage prepared from the cured leaves by combination with hot or boiling water (Armoskiate et al., 2011).

1.1.2.1 Ways of preparation

Soft infusion:

In this preparation method, a tea bag was dipped in warm water having the temperature of 75-85 C for 3 to 5 minutes. This method is used by Chinese people for tea preparation.

Hard infusion:

In this brewing method, a tea bag was infused in distilled warm water having the temperature of 75-85 C for 25-30 minutes.

Ambient infusion;

This method involves dipping of tea bags in distilled water at room
temperature 25+2 C for 30 to 40 minutes

**Cold infusion:**

In this method, tea bags were infused in distilled water and maintained at room temperature for 15 minutes after that, the prepared infusion was refrigerated for an hour.

**Decoction method:**

In this recipe, tea bags or tea leaves are placed in distilled water and boiled for 3 to 5 minutes. The above mentioned five methods are predominately used in Asian cultures as in Pakistan, China, India and Bangladesh.

**Chilled green tea:**

In this type of preparation, tea bags were infused into distilled boiling water for 3 to 5 minutes and after that the infusion was refrigerated for an hour.

**Cold cocktail:**

In this alcoholic infusion, distilled water and ethanol was used with the ratio of 60 to 40%. Tea bags were infused in alcoholic solution for 15 minutes and then removed, after which the infusion was refrigerated for an hour.

**Hot Cocktail:**

Tea bags were infused in alcoholic solution for 15 minutes followed by heating up to 52 C. Hot and CCs are commonly used in western cultures and in some Asian countries like China and India.

**Pure:**

In this recipe, the tea was grounded into fine powder and blended into warm water having the temperature of 70 to 80 C for 4 to 5 minutes. The pure
obtained was filtered and analysed immediately for antioxidant activity. This type of preparation method is mostly used in Japan Sun green tea.

In this method, tea bags are infused in distilled water and put into direct sunlight for 3 to 4 hours. This brewing method is most common in United States. All Green tea samples were prepared in glass beakers of 250 ml. Infusions were prepared in distilled water and ethanol. Composition of all tea infusions include one tea bag (1.265 g of tea) in 100 ml of distilled water (Safdar, Sarfaraz, et al., 2016)

1.1.3 Classification of green tea

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<td>Dilleniidae</td>
<td>Subclass</td>
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<td>Order</td>
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<td>Theaceae – Tea family</td>
<td>Family</td>
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<tr>
<td>Camellia L – camellia</td>
<td>Genus</td>
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<tr>
<td>Camellia sinensis (L) Kuntze – tea (Nishant et al., 2012)</td>
<td>Species</td>
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1.1.4 Green tea components and benefit:

The composition of green tea is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminum; and trace amounts of lipids (linoleic and a-linolenic acids), sterols (stigma sterol), vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons). Due to the great importance of the mineral presence in tea, many studies have determined their levels in tea leaves and their infusions. Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as caffeine, theobromine, and theophyllin. In addition, there are phenolic acids such as gallic acids and characteristic amino acid such as theanine present. Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea polyphenols (GTPs) are flavonols, commonly known as catechins. Product derived from green tea are mainly extracts of green tea in liquid or powder form that vary in the proportion of
polyphenols (45-90%) and caffeine content (0.4-10%) (Chaco et al., 2010). Black tea and green tea are powerful sources of flavonoids and other poly phenolic antioxidants, which have a protective effect in coronary artery disease (CAD). Published data have documented the potential beneficial effects of tea on atherosclerosis, hypertension, serum lipids, antioxidant levels, and aging. In particular, it was documented that regular consumption of tea and green tea increases the level of antioxidants and thus reduces the risk of CAD. It was also shown that catechin contained in green tea prevents the cell proliferation of arterial wall muscle. The protective effects of flavonoids contained in green tea are not only antioxidant, anti-thrombotic, and anti-inflammatory properties but also additive to the rate of the coronary flow velocity reserve (Cheng 2005). In addition to the positive effects of tea on CAD and stroke, the beneficial effects of tea in breast colon, prostate, and pancreatic cancers have been documented. Also, tea has antimicrobial effects, and its protective effect against Alzheimer’s and Parkinson’s disease and tooth decay (jalali et al., 2008)

1.1.5 Chemical component of green tea:

The detailed analysis of green tea is relevant in the terms of preventive effect on metastasis of lung, breast cancer, prevention of inflammation, thrombosis, preventive effect on atherosclerosis and positive effect on decreasing cholesterol concentration in the blood, positive effect of its anti mutagenic and anti-carcinogenic properties, antioxidant activities established by ability to bind to free radicals and neutralize them and the effect of
decreasing the risk of renal calculi by 30%. Continuously and increasingly new articles are published in scientific literature concerning preventive or healing properties of green tea, therefore it is obvious that either scientific community, either society is interested in its properties. Whilst analyzing directly one of the main scientific databases of science, it has been calculated that in year 2007 there have been 1866 articles published about green tea, respectively 2072 articles in 2008 and 2297 articles in 2009. The amount of publications increases approximately 10% per year. Whilst evaluating the quality by the composition of green tea catechins (one of the fractions of flavonoids), such as (-)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-catechin-3-gallate, (-)-epicatechin-3-gallate, (-)-gallocatechin-3-gallate, (-)-epigallocatechin-3-gallate, theaflavin, theaflavin-3-gallate, theflavin-3,3-biggallate are indicated as major part of biologically active substances (there are up to 30% of catechins in the leaves of dry tea stock and up to 200 mg in one cup of tea). Flavonoids (and their fraction – catechins) are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism. Free radicals are the main factors responsible for the initiation of formation of cancer cells. Active oxygen forms participate in the pathogenesis of different diseases in molecular level by deformation of low-density lipoproteins (LDL), formation of secondary products (aldehydes), rapid decomposition of proteins, reduced activity of enzymes, contravention of cell membrane, DNA mutations, changes of carbohydrate receptors. This leads to the development of cancer genesis
process, sugar diabetes, establishment of cataract, renal insufficiencies, myocardial infarct, arthritic diseases, sensitivity of central nervous system, Alzheimer’s disease. The study was performed to analyze different kinds of teas of different regions existing in Lithuanian market. Evaluation of the quantity of their qualitative parameters (the amount of phenolic compounds antioxidant activity and dry residue) has been the most important subject of this study. The object of most established studies evaluating these parameters has been different kinds of teas (not exceptionally green tea) or other kinds of tea (such as roiboos or black tea). Therefore, we decided that a study concerning only different kinds of green tea would be substantial (Armoskaite et al., 2011)

1.1.6 Processing of green tea:

The assan type has a high content of polyphenols which would make green tea excessively bitter (Wilson KC., 1999). The production of green tea is characterized by an initial heating process which kills the enzyme polyphenol oxidase, which is responsible for the conversion of flavonoids in the leaf into the dark poly phenolic compounds that color black tea. The other important process is rolling in which the leaves are cut and twisted polyphenols constitute the most interesting group of green tea leaf components and in consequence green tea can be considered as an important dietary source of polyphenols, particularly flavonoids, which include catechins, are thought to be responsible for health benefits in green tea (Cabrera, Artacho et al., 2006). The final form of green tea depends on the
particular variant being produced. The rolling stage is very similar to the operation with the same name in black tea production (Thasleema., 2013).

1.1.7 Antioxidants activity:

The mechanism of oxidation reactions is strictly dependent on the chemical structure of the compound. Of interest are derivatives of plants that have high antioxidant activity, such as flavonoids, which are plant based compounds that also act as natural dyes. There are over 6000 known substances in this group (Ziyatdinova., et al. 2014). Due to the different positions of the hydroxyl group in the structure, they can be characterized by specific properties, such as pro-oxidizing and biocides. Publications in recent years mainly concern their antioxidant activity and the mechanism of oxidation and reduction.

The high antiradical activity is derived from hydroxyl groups at specific positions in the structure, such as ortho-hydroxylation on the B-ring and the C2-C3 double bond in the C-ring of the flavonoid. The high content of individual catechins, such as EGCG and EGC, affects the antioxidant potential of tea. (Tripathi R. et al., 2007) Flavonoids are mainly analysed in terms of their use for medicinal purposes. They exhibit anti-inflammatory properties; it was confirmed that they can also reduce blood pressure, strengthen the cell walls of blood vessels, and improve the immune system. They have an anti-atherosclerotic effect by inhibition of aggregation lipoproteins (LDL) responsible for transporting the so-called bad cholesterol.
Teas are imported from around the world including Vietnam, China, Japan and Thailand. Their origin often determines the values of healing. The impact of growing conditions of green tea affects its contents in terms of polyphenols. Therefore, research on these phytochemicals today is undoubtedly needed (Nunes et al., 2015). Many publications include studies of the antioxidant activity of the total content of polyphenols. Most of the polyphenols of plant origin are characterized by a very low stability. The aim of our study was to analyse the mechanism of an antioxidant mixture of flavonoids derived from green tea extract (Hodgson M.J. et al., 2010). The presented publication was created for the determination of whether multiple condensed dose levels show higher antioxidant potentials compared to isolated antioxidants (Yang et al., 2010). Several substances from the flavonoids group in one mixing may produce a synergistic effect or antagonistic oxidation reactions. (Shahidi and Wanasundara, 1999) have defined polyphenols as the powerful chain breaking antioxidants. Presented information about cancer-preventive or anticancer activities of tea. (Sadzuka et al., 1992). Describes the results regarding the use of biochemical modulators in cancer therapy. Additionally, (Sochor et al., 2013)(Belfar et al., 2015) describe the significant results of research on the positive effect of polyphenols from green tea anti-cancer prevention. This submitted manuscript presents the antioxidant poly phenol in various mixtures of oxidizing electro-oxidation induced currents in the ability to scavenge free
radicals and reduce iron ions. To evaluate the activity to deactivate free radicals, spectrophotometric methods relying on the mechanism of hydrogen atom transfer and single electron transfer were used (Williams et al., 2004)(Fisher et al., 1986). The described methods are a great tool to examine the ability to inhibit the oxidation processes of chemical compounds, for example, preventing lipid peroxidation. The applied research methods do not require special preparation of samples or specialized equipment and are low cost. The great advantage of the methods used is a high sensitivity and a very small amount of material needed for analysis. The antioxidant activity of polyphenols has been noted in several studies. The aim of the present work is to characterize the antioxidant and antiradical performance of green tea extract using electrochemical and DPPH, ABTS radical model systems, respectively (Masek et al., 2017).

1.1.7.1 Catechin:

Mechanism of the action of Catechin includes antioxidant and free radical scavenging activity which stimulate detoxification system through selective induction or modification of Phase I and Phase II metabolic enzymes. In addition, green tea may inhibit biochemical markers of tumors initiation and promotion including the rate of cell replication and thus inhibits the growth and development of neoplasm. Another potential effect due to the antioxidant activities of green tea polyphenols such as Catechin, which binds with Cd ions to form an insoluble complex - ionic salt that is used to remove Cd from biological tissues. Therefore, the aim of the present study was to
throw a light on possible prophylactic and therapeutic potential of green tea extract against the toxic effects of cadmium chloride on the testes of mammals (Sharma and Goyal, 2015).

1.1.8 Anticancer activity:

GTCs, and especially EGCG, are capable of modulating a plethora of cell signalling pathways crucial for cancer cells transformation and survival, including, but not limited to, the mitogen-activated protein kinase (MAP-kinase), the nuclear factor-kappa B (NF κB), and the insulin-like growth factor (IGF)/IGF-1 receptor pathways. With regard to the prostate-specific processes GTCs are able to effect androgen receptor (AR) down regulation and prostate-specific antigen (PSA) expression (Yang et al., 2011).

1.1.8.1 Inhibition of cell proliferation and cell cycle arrest:

GTCs exhibit ant-proliferative effects versus both androgen-sensitive and androgen-insensitive human PCa cells. The effect is mediated by cell cycle deregulation and cell death induction (Gupta S.et al., 2000). We showed that GTCs action is cancer specific, since GTCs is capable of inducing growth arrest both in SV-40 immortalized prostate epithelial cells (PNT1a) and in tumorigenic androgen-independent PCa cells (PC3), while normal human prostate epithelial cells were not significantly effected, even when EGCG was administered at higher doses (Coporali et al., 2004). The IC50 of EGCG ranges from about 40 to about 200μM, depending on the cell line type (LNCap < PNT1a < DU145 < RR PC3), as well as the length of the
experiment, ranging from 24 to 72 hours (Albrecht et al., 2008). Our results were confirmed by other authors in normal fibroblasts (Ahmed et al., 1997) (Davalli et al., 2012).

1.1.8.2 Synergistic anticancer activity of tea polyphenol and chemotherapeutic agents:

The combination of green tea catechins and anticancer drugs is a new treatment strategy that has been widely accepted by cancer researchers. Although anticancer drugs and tea polyphenols are very different in terms of structure and function, tea polyphenols can synergistically enhance the effects of anticancer drugs and make them 10–15 times more effective than mono therapy (Suganuma et al., 2011). Some studies have also reported beneficial effects of EGCG or green tea extract with anticancer drugs, such as bleomycin, cisplatin, tamoxifen, and bortezomib (Periasamy et al., 2013). We have also studied the effect of green tea extract on 5-fluorouracil (5-FU) in cancer cells and animals. Our results demonstrated that green tea catechins with anticancer agents are more effective than mono therapy (Qiao et al., 2011)

1.1.8.2.1 Combination of tea polyphenols and bleomycin:

Bleomycin is frequently used in the treatment of various cancers. However, the monotherapy strategy has often failed to produce therapeutic benefit due to multidrug-resistant cancer. Green tea polyphenols have been used as an adjuvant in bleomycin therapy. (Alshatwi et al., 2016). reported a synergistic
anticancer effect with a combination of tea polyphenols and bleomycin. Various concentrations of tea polyphenols, bleomycin, or tea polyphenols combined with bleomycin were added to cervical cancer cells (SiHa), and then the cell growth, intracellular reactive oxygen species, poly-caspase activity, early apoptosis and expression of caspase-3, caspase-8, caspase-9, Bcl-2, and p53 were observed. This study showed that tea polyphenols combined with bleomycin synergistically inhibited cervical cancer cell viability and proliferation through the induction of apoptosis. Other studies have also suggested that tea polyphenols may increase antitumor activity of bleomycin. (Chen et al., 2013)

1.1.8.2.2 Combination of tea polyphenols and cisplatin:

Cisplatin is often the first chemotherapeutic agent used to treat many forms of cancer. Unfortunately, cisplatin resistance often develops during the course of treatment. Both preclinical and clinical studies have shown that multiple mechanisms drive tumor resistance to cisplatin. The synergistic effect of cisplatin and tea polyphenols has been studied in vitro and in vivo (Mazumder et al., 2012). Tea polyphenols combined with cisplatin can decrease proliferation and induce apoptosis in breast cancer cells. Additionally, tea polyphenols plus cisplatin may minimize or slow the development of drug resistance, which may also reduce drug toxicity and improve therapeutic efficacy (Chen et al., 2013). The combination of EGCG with cisplatin has increased beneficial effects on cell cycle arrest, modulation of ROS- and apoptosis-related gene expression and potent
antioxidant activity when compared with monotherapy. EGCG may also reduce oxidative stress, inhibit proliferation, and sensitize ovarian cancer cells to cisplatin. The combination of tea polyphenols and cisplatin can synergistically inhibit the growth of various cancer cells, such as MCF-7 breast cancer cells and non-small cell lung cancer (NSCLC) A549 cells. Additionally, we found that, compared with cisplatin monotherapy, the combination of cisplatin and EGCG can significantly decrease tumor size in animal models—the data will be reported in the near future.

1.1.8.2.3 Combination of tea polyphenols and ibuprofen:

Ibuprofen is an non selective non steroidal anti inflammatory drug (NSAID), which may inhibit the growth of prostate cancer cells in both in vitro and in vivo xenograft models. The synergistic effect of EGCG and ibuprofen (EGCG+ibuprofen) treatment on DU-145 prostate cancer cells has been investigated. This study showed that EGCG + ibuprofen treatment resulted in greater growth inhibition than ibuprofen or EGCG alone. EGCG + ibuprofen treatment acts synergistically to block proliferation and promote apoptosis in DU-145 prostate cancer cells. (Kim et al., 2007)

1.1.8.2.4 Combination of tea polyphenols and tamoxifen:

Tamoxifen is an anti-estrogenic compound used for the prevention of breast cancer. Green tea is often used as a supplement in breast cancer treatment and prevention. Co-administration of green tea and tamoxifen improves experimental outcomes in breast cancer cell lines and animal models. Green
tea increased the inhibitory effect of tamoxifen on the proliferation of estrogen receptor-positive MCF-7, ZR75, and T47D human breast cancer cells in vitro (Farabegoli, et al., 2011). The combination of EGCG (75 and 100 µM) and tamoxifen (5–200 µM) significantly increased apoptosis in PC-9 cells compared to EGCG or tamoxifen alone. When MCF-7 xenograft-bearing mice were treated with both green tea and tamoxifen, their tumor sizes were significantly diminished, and more cancer cell apoptosis occurred in tumor tissue.

1.1.8.2.5 Combination of tea polyphenols and bortezomib:

Bortezomib exerts its antitumor effects by reversibly blocking the 26S proteasome (Bannerman et al., 2011). EGCG interferes with bortezomib’s anticancer activity (Golden et al., 2009). EGCG’s negative impact on bortezomib efficacy was concentration-dependent in CWR22 xenograft-bearing breast cancer mice. Only very high levels of EGCG antagonized bortezomib’s antitumor activity, while low levels of EGCG had no adverse effects in CWR22 mice (Glynn et al., 2015). This example demonstrates the negative interaction of EGCG and an anticancer drug.

1.1.8.2.6 Combination of tea polyphenols and other anticancer drugs:

Tea extract or tea polyphenols also synergistically enhance the anticancer activity of other chemotherapy drugs, such as Paclitaxel, sulindac, celecoxib, curcumin, luteolin, docetaxel, retinoids, and so on (Chen et al., 2013).
group has studied the effects of green tea extract and 5-fluorouracil treatment in SW480, BIU-87, and BGC823 human cancer cell lines; a daily dose of green tea (equivalent to <6 cups daily in humans) did not alter the cytotoxicity of 5-FU treatment in these cells.

1.1.8.3 Combination of caffeine and anticancer drugs:

Caffeine is another ingredient in tea. Caffeine can inhibit the activities of both ATM and ATR—two important protein kinases involved in DNA damage induced cell cycle arrest and apoptosis. It has been reported that caffeine increased the cisplatin-induced apoptosis in both HTB182 and CRL5985 lung cancer cells by inhibiting ATR and inducing ATM activation (Wang et al., 2015). Caffeine could enhance the antitumor effect of cisplatin; when the dosing period of caffeine was increased, the synergistic effect was increased in osteosarcoma-bearing rats (Tsuchiya et al., 2000). Significant inhibition of tumor growth and prolongation of survival time were also found in sarcoma-bearing mice (Karita et al., 2008). Caffeine significantly decreased mutagenicity of the anticancer aromatic drugs daunomycin, doxorubicin, and mitoxantrone. Caffeine decreased the anticancer drug vinblastine-induced chromosomal aberrations and mitotic index in bone marrow cells (Geriyol et al., 2015) (Cao et al., 2016).

1.1.9 Antimicrobial properties:

Green tea (Camellia sinensis L.) is an important commercial plant that is
produced in over 30 countries and has been consumed worldwide primarily as a beverage made from the processed leaf (Chung et al., 2004; Choi et al., 2006). It has a variety of secondary metabolites such as catechin, caffeine, theanine and saponin that are important for human welfare (Ogutuga and Northcote, 1970; Chung et al., 2004; Yoon et al., 2005). These compounds in green tea are well known for their broad spectrum of biological activities such as antibacterial, antioxidant, antifungal, and antitumor functions (Mabe et al., 1999; Sakanaka et al., 2000; Yang et al., 2000; Fassina et al., 2002). Tea seed saponin, natural surfactant exhibits a variety of biological activities (Sparg et al., 2004). It has been recognized that tea seed saponin account for more than 10% per dry weight of tea seed (Xingtu, 1990). It has been commercially utilized as a foam-stabilizing and emulsifying agent (Chen et al., 2010) and is extensively used in aquaculture to eliminate unwanted fish and harmful insects in prawn ponds (Chaicharoenpong and Petsom, 2009). It also has been shown to have other physiological functions such as anti-expectorant and anti-inflammatory properties (Tamura, 1956; Sagesaka et al., 1996). Control effect of saponin against insects, pests and mites has been reported (Kawai et al., 1999). In addition, It is used as a medicine for the treatment of intestinal disorders (Huang et al., 2007) and burn injuries (Huang et al., 2005). Saponin has also been reported to exert many pharmacological effects, including antihyperlipidemic (Yoshikawa et al., 2005), antiallergic (Matsuda et al., 2010), and cardio protective effects (Liao et al., 2009). Saponin as other natural products could be utilized as biological agents since it is less polluting to the environment than its
In aquaculture, diseases of microbial origin cause high mortality rates and lesions on fish, with consequent economic losses worldwide (Toranzo et al., 2005). Bacteria, mainly of Yersinia ruckeri, Pseudomonas putida, P. luteola, Aeromonas hydrophila and Listonella anguillarum (formerly Vibrio anguillarum) have been identified as the etiological agents responsible for the disease outbreaks in fish and shellfish (Toranzo et al., 2005; Austin and Austin, 2007; Radjasa et al., 2009). Moreover, these microorganisms can accumulate in the reared animal’s flesh and become a serious threat to the health of aquatic organisms (Cavallo and Stabili, 2002). Besides, the use of commercial antibiotics for disease treatment produces undesirable side effects, including toxicity to the reared organisms and release of chemical residues into the environment. These chemical residues can then pose risk to animal and human health (Cabello 2006) (H. Boran et al 2015).

daily consumption of green tea lead to kill pathogenic microorganisms like Staphylococcus aureus, Vibrio parahemolyticus, Clostridium perfringens, Bacillus cereus and Pleisomonas shigelloides. Green tea contains around 30-40% polyphenols and related compounds such as Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epigallo catechin (EGC) and Epicatechin (EC) but black tea contains only 3-7% polyphenols (Diane et al., 2007). Among these EGCG is the most luxuriant component in tea extract
and the most potent chemical tested for biological activity in terms of inhibiting the growth of pathogenic microorganisms (Archana and Abraham, 2011).

Tea leaves are polymorphic in nature and known for its antibacterial activity against many pathogenic microorganisms. It is being shown to have a wide range of antioxidant, anti-inflammatory, ant carcinogenic and ant mutagenic properties against pathogens. Antimicrobial activity of green tea and black tea extracts were studied using clinically important pathogenic microorganism and ((compared by several Investigators (Diane et al., 2007; Archana and Abraham, 2011). But no literatures are available on antibacterial activity of different green tea leaves against a wide variety of pathogenic microorganisms. Hence the present investigation made an attempt to study the antibacterial activity of different green tea leaves against selected bacterial pathogens isolated from clinical samples. (Ponnusamy Ponmurugan et al., 2016).

1.2 Literature review:

1.2.1 Hemostasis:

Hemostasis is derived from a Greek word, which means stoppage of blood flow. The process is a combination of cellular and biochemical events that function together to keep blood in the liquid state within the veins and arteries and prevent blood loss following injury through the formation of a blood clot. It consists of a complex regulated system which is dependent on
a delicate balance among several systems. The systems involved in the 
hemostatic process include the vascular system, coagulation system, 
fibrinolytic system, platelets, kinin system, serine protease inhibitors, and 
the complement system. The systems work together when the blood vessel 
endothelial lining is disrupted by mechanical trauma, physical agents, or 
chemical trauma to produce clots. The clots stop bleeding and are eventually 
dissolved through the fibrinolytic process. As a result, there is a delicate 
balance between the production and dissolution of clot during the hemostatic 
process. A disruption of this balance may precipitate thrombosis or 
hemorrhage as a result of hyper coagulation or hypo coagulation, 
respectively. Hemostasis is categorized as either a primary or secondary 
process. Primary hemostasis involves the response of the vascular system 
and platelets to vessel injury. It takes place when there are injuries to small 
vessels during which the affected vessels contract to seal off the wound and 
platelets are mobilized, aggregate, and adhere to components of the sub 
endothelium of the vasculature. Platelet adhesion requires the presence of 
various factors such as von Willebrand factor (vWF) and platelet receptors 
(IIb/IIIa and Ib/IX). Additional platelets are attracted to the site of injury by 
the release of platelet granular contents, such as adenosine diphosphate 
(ADP). The platelet plug is stabilized by interaction with fibrinogen. Thus a 
defect in platelet function or von Willebrand’s disease (vWD) may result in 
debilitating and sometimes fatal hemorrhage. Secondary hemostasis 
involves the response of the coagulation system to vessel injury. [F2] It is 
required to control bleeding from large wounds and is a continuation of the
primary hemostatic mechanisms. Whereas the outcome of primary hemostasis is the formation of the platelet plug, the outcome of secondary hemostasis is the formation of a thrombus. (Henry et al., 2002)

### 1.2.2 Primary hemostasis:

This is normally triggered by an injury to the endothelium. This produces a gap in the tissues that is immediately filled with blood from the damaged vessel(s). The two primary activations are those of the platelets and of factor VII. The blade cuts through the tissues, through the subendothelium, and then through the endothelium, producing a gap. The blade is withdrawn, and blood immediately fills the gap. The immediate response is vasoconstriction, which initially is myogenic in origin. Within the blood filling the site of trauma there are two extremely significant elements: The platelets and factor VII, and these are the initiators of hemostasis, and they do this via contact with elements in the subendothelium. These two initiators will be discussed separately, but bear in mind that their activation occurs simultaneously platelets adhesion to endothelium around the injury. This happens within 1–2s. There are numerous adhesion proteins involved here, including von Willebrand factor (this is found in the sub-endothelial space, where it was secreted by the endothelial cell in health, and also release from the Weibel–Palade bodies from damaged endothelial cells). Platelet adhesion is followed by activation of platelets within 15–20s. This is initially reversible. It results in a number of metabolic reactions which lead to Shape change Release of substances from within the platelet, most of
which, in one way or another, facilitate platelet plug formation and subsequent stabilization by coagulation.

Arachidonate release from the membrane leading to the formation of thromboxane A2. This substance has many important functions, in Promotion of the release reaction Vasoconstriction Promotion of aggregation of platelets to each other (stickiness). In this process, additional platelets are recruited to the site of injury from the circulation, mainly due to the release of ADP from activated platelet granules. In the processes of adhesion and aggregation, the platelet membrane is altered with exposure of deeper portions of the membrane. exposed membrane phospholipids are now available to take part in two important processes in the coagulation pathway, The activation of factor X (to form Xa). The formation of thrombin from the interaction of factors Xa, Va, and II.

The surface platelet receptor GPIIa-IIIbis the major platelet integrin. It forms a bridge between the cytoskeleton and the polymerized fibrin formed by the coagulation process. This receptor is important because it is the target of very many drugs being developed to decrease platelet function in many hyper coagulable states, notably in heart disease. Factor VII (as the Physiological Initiator of the Coagulation System)This too is activated by contact with a sub endothelial substance ,in this case tissue factor, a phospholipid material forming a peri vascular sheath and secreted by the endothelium. (Note that monocytes too can secrete tissue factor, and this undoubtedly plays a part in abnormal clot- ting.) In this early stage of
activation, activated VII, i.e., VIIa, now activates a small amount of IX in the circulation as well as some X. (All activated factors are designated as such by an a after the basic factor number.) IXa in turn further activates X. Xa now attaches to the sub-endothelium, especially the phospholipid of exposed tissue cell membranes, and where it binds passing Va. This complex now activates a small amount of passing prothrombin (factor II) to produce a small amount of thrombin. The initiation phase is summarized in shows factor VII making contact with the exposed collagen in the injured part of the endothelial surface. Here it becomes activated and re-enters the blood in the vicinity. Here it awaits contact with some factor IX and X passing factor VIIa activating both factor IX and X. Factor IXa further activates factor X. Xa now attaches to the sub-endothelium, especially the phospholipid of exposed tissue cell membranes, and where it binds Va. This complex now activates a small amount of passing prothrombin (factor II) to produce a small amount of thrombin. This is the end of the initiation phase. Only small amounts of activated factor are produced. There may be micro amounts of thrombin and perhaps a strand or two of fibrin present, but as yet no significant amount of thrombin have been formed. (Norman Beck., 2009)

1.2.3 Secondary hemostasis:

1.2.3.1 Initiation of coagulation:

Circulating blood reacts quickly to a disruption of the vascular endothelium.
to limit bleeding. The initial hemostatic response is triggered by tissue factor (TF; thromboplastin) expressed on sub endothelial pericytes and fibroblasts. Activated Factor VII (fVIIa), a serine protease that normally circulates in blood in low concentration, binds to TF to activate Factor X to fXa. Subsequently, fXa (also a serine protease) generates trace amounts (0.1–1 nM) of thrombin. There are two inhibitors that regulate TF-triggered procoagulant responses, thus limiting serine protease actions to the site of vascular injury. Tissue factor pathway inhibitor (TFPI) neutralizes fXa when it is in a complex with TF-fVIIa.

The other regulator of TF-trigger pro coagulant response is anti-thrombin (AT, formerly called anti-thrombin III; a serine protease inhibitor; SERPIN), which circulates at a high concentration (150 g/mL) and neutralizes the initially formed fXa and thrombin. Thus, the procoagulant triggering reaction only proceeds when TF is exposed at a high enough level to overcome inhibition by TFPI. In other words, fVIIa patrols the circulation in search of sites of vascular damage (where TF is exposed), and trace quantities of fXa and thrombin sound the alarm for any potential dangers. This activity is tightly monitored by naturally occurring inhibitors that prevent a false alarm or too extensive of a response role of coagulation in secondary hemostasis.

1.2.3.2 Propagation of Coagulation:

Platelets contribute to localized thrombus formation at the site of vascular
injury first by adherence to sub endothelial collagen-von Willebrand factor (vWF) via their glycoprotein (GP) Ib receptors. Thrombin generated by TF-fVIIa/fXa (the “extrinsic pathway”) is capable of activating adherent platelets in its vicinity via protease-activated receptors 1 and 4 (PAR1 and PAR4). Thrombin-activated platelets play a pivotal role in subsequent coagulation processes in several ways. First, platelet GPIb receptors bind to Factor XI, and they also localize Factor VIII to the site of endothelial disruption via its carrier protein vWF. Furthermore, partially activated Factor V is released from platelet granules upon platelet activation. Factors XI, VIII, and V are involved in sustaining pro coagulant responses (the intrinsic pathway) after thrombin-mediated activation. The serine protease Factor XIA mediates the activation of Factor IX to fIXa, and fVIIIa serves as a cofactor to fIXa. Factor IXa, a serine protease activates Factor X to fXa, and fVa serves as a cofactor to fXa. In the absence of fVIIIa or fIXa, as clinically observed in Hemophilia A or B, respectively, the initiation of coagulation is normal, but propagation steps are severely diminished. Patients with hemophilia develop recurrent bleeding in muscle and joints because of low-TF expression (thus, the initiation of coagulation is rapidly quenched by TFPI and AT). Using high-dose recombinant fVIIa (90–120 g/kg), recurrent bleeding can be reduced by the increased fXa production to overcome TFPI and AT (thus improved thrombin generation) in hemophilia patients with inhibitory antibodies against Factor VIII or Factor IX. Three key components (substrate, enzyme, accelerator [cofactor]) concentrated on the activated platelet surface are needed to locally generate thrombin. A
single thrombin-activated platelet exposes more than 12,000 copies of GPIIb/IIIa receptors that can concentrate fibrinogen for efficient fibrin formation. Furthermore, plasma- and platelet-derived Factor XIII are activated by thrombin to fXIIia, a trans glutaminase that rapidly cross-links fibrin monomers. Thus, localized fibrinogen and Factor XIII are final substrates that play pivotal roles in stabilizing the primary hemostatic plug. In severe fibrinogen deficiency, platelets are localized by vWF-GPIb interactions but unable to recruit fibrinogen molecules to GPIIb/IIIa receptors. Lack of fibrin formation on the platelet surface results in a dislodgement of platelet plug (Kenichi A Tanaka et al., 2009).

**Table (1.1) Coagulation factor nomenclature:**

<table>
<thead>
<tr>
<th>Fibrin subunit</th>
<th>Fibrinogen</th>
<th>I</th>
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<tbody>
<tr>
<td>Serine protease</td>
<td>Pro thrombin</td>
<td>II</td>
</tr>
<tr>
<td>Receptor/cofactor</td>
<td>Tissue factor</td>
<td>III</td>
</tr>
<tr>
<td>Cofactor</td>
<td>Labile factor</td>
<td>IV</td>
</tr>
<tr>
<td>Serine protease</td>
<td>Pro convertin</td>
<td>V</td>
</tr>
<tr>
<td>Cofactor</td>
<td>Antihemophilic factor</td>
<td>VI</td>
</tr>
<tr>
<td>Serine protease</td>
<td>Christmas factor</td>
<td>VII</td>
</tr>
<tr>
<td>Serine protease</td>
<td>Stuart power factor</td>
<td>VII</td>
</tr>
<tr>
<td>Serine protease</td>
<td>Plasma-thromboplastic antecedent</td>
<td>IX</td>
</tr>
<tr>
<td>Serine protease</td>
<td>Hageman(contact factor)</td>
<td>X</td>
</tr>
<tr>
<td>Transglutaminase</td>
<td>Fibrin–stabilizing factor</td>
<td>XI</td>
</tr>
</tbody>
</table>
### Coagulation Factors Table

<table>
<thead>
<tr>
<th>Serine protease</th>
<th>Prekallikrein (Fletcher factor)</th>
<th>XII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cofactor</td>
<td>HMWK (Fitzgerald factor)</td>
<td>XLI</td>
</tr>
</tbody>
</table>

(Victor Hoffbrand and Paul A.H.Moss., 2016)

1.2.4 **Pro thrombin time test**

Is an invitro laboratory assay for the extrinsic and common pathway of coagulation. The primary use for the PT is the monitoring of patients on oral anticoagulation therapy. The National Committee for Clinical Laboratory Standards (NCCLS) recommends that coagulation testing including prothrombin time not be performed on plasma from the first evacuation blood tube drawn from a patient, but that it be performed instead on plasma from the second or subsequent tube drawn during the same phlebotomy. This recommendation is based on the concern that tissue thromboplastin may be drawn into the first sample tube that could affect the accuracy of coagulation testing. The concerns originated at a time when the whole blood clotting time was the screening test used for disorders of the coagulation system. With more specific testing of the extrinsic and common pathway coagulation systems through the use of pro thrombin time, the small amounts of tissue thromboplastin that could be activated by a venipuncture may have less theoretical and clinical importance.

1.2.5 **Limitation of PT and APTT**

Two pathways lead to the formation of a fibrin clot, the intrinsic and
extrinsic pathway. Each pathway is initiated by a different mechanism and
both converge on a final common pathway (factors II, V, and X) leading to
thrombin generation and fibrin formation. The PT and APTT test the
integrity of the extrinsic and intrinsic pathways, respectively, while both PT
and APTT are affected by defects in the final common pathway.

The PT is the in vitro clotting time measured after addition of the PT
reagent, which contains thromboplastic (phospholipids with tissue factor)
and calcium to citrated plasma. The PT detects important deficiencies (and
rarely inhibitors) of factors II, V, VII, and X. The APTT is the in vitro
clotting time measured after addition of calcium, an intrinsic pathway
activator and the APTT reagent, which contains phospholipid (a platelet
substitute, also called ‘partial thromboplastin’ as it lacks tissue factor) to
plasma. The APTT detects bleeding disorders due to deficiencies of factors
II, V, VIII, IX, X, XI, XII and inhibitors including lupus anticoagulant and
therapeutic anticoagulants.

Prolongation of the PT and/or APTT only indicates a problem with the
quantity and/or quality of single or multiple factors within the relevant
pathways. Further specific coagulation tests are required to characterize the
actual cause. Both the PT and APTT are also subject to a number of Pro
thrombin time (PT) is an in vitro laboratory assay for the extrinsic and
common pathway of coagulation. The primary use for the PT is the
monitoring of patients on oral anticoagulation therapy.12 The National
Committee for Clinical Laboratory Standards (NCCLS) recommends that
coagulation testing including pro thrombin time not be performed on plasma from the first evacuation blood tube drawn from a patient, but that it be performed instead on plasma from the second or a subsequent tube drawn during the same phlebotomy. This recommendation is based on the concern that tissue thromboplastic may be drawn into the first sample tube that could affect the accuracy of coagulation testing. The concerns originated at a time when the whole blood clotting time was the screening test used for disorders of the coagulation system. With more specific testing of the extrinsic and common pathway coagulation systems through the use of pro thrombin time, the small amounts of tissue thromboplastin that could be activated by a venipuncture may have less the critical and clinical importance (Y L Chee., 2014).

1.2.6 Green tea and Hemostasis:

According to the available published reports, tea could be beneficial to one’s health, such as decrease of the incidence of hyper-lipidemia, atherosclerosis and anti-oxidant (Mannu GS, Zaman MJ, Gupta A, et al; 2013). Black tea and green tea are potent sources of flavonoids and other polyphenolic antioxidants, which have a protective outcome in coronary artery disease (CAD) (Elattar et al., 2000; Hakim IA et al 2003; Jun Pang, Zheng Zhang et al 2014). It was also shown that catechin contained in green tea prevent the cell proliferation of arterial wall muscle. (Benzie IF, Stvain JJ, T omlinson B., 1999). The protective effects of flavonoids enclosed in green tea are not only antioxidant, anti-thrombotic, and anti-
inflammatory properties, but also additive to the rate of the coronary flow velocity reserve (Cheng TO, 2005).

Even though drinking tea is one of the most popular Sudanese behavior but green tea is still infrequently used for daily consumption by Sudanese population in spite of their beneficial effect. since there is a published data on its beneficial effects on fibrinogen level this study was conducted to verify whether green tea well affect the coagulation profile or not.

1.2.7 Previous studies:

- A study done by F.Jalali MD et al (2008), which study the effect of Green tea on serum lipids Antioxidants and Coagulation Tests in stable Coronary Artery Disease in Iran reported that after regular consumptive of 4g/d green tea for one month there was a small significant decrease reduction in pro thrombin time level.

- Another study done by Hussam MA et al (2017) which study the effect of green tea consumption on coagulation profile and fibrinogen level reported that after regular consumptive of two cup of green tea for one month there was a small significant decrease in pro thrombin time level.

1.2.8 Rationale:

Although green tea is more frequently available in Sudan it is not commonly used for daily consumption as a drink although sometimes it is consumed by females to reduce their weight, there are no documented data on its beneficial effects on coagulation parameters and its antioxidant Effect at the national level. We, therefore, conducted this prospective, interventional study into the effect of green tea on Pro thrombin time of appernately healthy Sudanese volunteers.
1.2.9. Objectives:

1.2.9.1 General objective:

To study the effect of green tea intakes on Pro thrombin time level among healthy Sudanese volunteers.

1.2.9.2 Specific objectives:

1- To determine the mean of Pro thrombin time among healthy Sudanese volunteers pre and post consuming two cup of green tea per day for one month using Stago semi-automated coagulometer analyzer.

2- To correlate the effect of green tea consumption on Pro thrombin time to the participants gender and age.
Chapter Two

2. Materials and Methods:

2.1 Study design:
This study was a prospective, interventional (before- after)

2.2 Study area and duration:
The study was conducted at Khartoum state in the period from March to May 2018.

2.3 Study population and sample size:
The study was conducted on 40 # apparently healthy volunteers to study the effect of green tea on pro thrombin time (PT); the volunteers consumed two cups of green tea per day for one month. PT time was estimated for each participant before and after the consumption of green tea for the one month study period

2.4 Study variables:
Dependent variable:
Pro thrombin Time Test

Independent variables:
Age
Gender
Green tea
2.5 Sample collection:

Three (ml) of venous blood was collected in 3.2% tri-sodium citrate containing container from each participant before and after the consumption of green tea.

2.6 Reagent:

Calcified thromboplastin (Biomed)

2.7 Instruments:

Optic semi automated coagulometer

2.8 Preparation of platelet poor plasma (PPP):

We are prepared PPP by centrifugation at 2000 g for 15 minutes (approximately 4000 rev / min in a standard bench). Then we count plate let by chamber which gave count below 10/ml, we stored the sample as batch at 30 c for several weeks, the stored sample then mixed after thawing and centrifuged before testing.

2.9 Determination of Prothrombin time:

Determination of prothrombin time (PT) was analyzed by a coagulometer. The clotting time of plasma was analyzed in the presence of an optimal concentration of tissue extract (thromboplastin), in presence of calcium chloride (CaCl2). The endpoint of clot was determined by semi-automated coagulometer (Stago, Japan) (Appendix 1).
2.10 Data collection:

Patient’s data collected directly from participants

2.11 Data analysis:

The data were analyzed using SPSS software version 16; the paired t-test was applied to analyze the changes in the pro thrombin time level. A P-value less than 0.05 was considered as significant.

2.12 Ethical consideration:

Informed consent was taken from all subjects before sample collection
Chapter Three

Results:
This study was done in Khartoum state at Sudan university of since and technology in the period from March to May 2018 to evaluate the effect of green tea consumption on prothrombin time test. 40 healthy Sudanese volunteers aged between 20-45 years were enrolled to participate in this study. Twenty one of them were females and nineteen of them were males. The studied subjects were consume a two cubs of green tea per day for one month. Then pro thrombin level was measured from pre and post samples collected from them.
Figure (3.1) distribution of study group according to age group
Figure (3.2) distribution of study group according to gender
Table (3.1) Summary of Mean of PT before and after green tea consumption:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (±SD)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Green Tea</td>
<td>Post -Green Tea</td>
<td>p-value</td>
</tr>
<tr>
<td>PT before and after green tea consumption</td>
<td>14.33 (±0.96)</td>
<td>14.06 (±0.91)</td>
</tr>
</tbody>
</table>

- Significant at <0.05

The statistical analysis of the results showed that there is a significant difference (p.value= 0.000) among participant’s PT between before 14.06 (±0.91) and after 14.33 (±0.96) green tea consumption samples (Table 3.1).

Table 3.2 PT according to the Gender:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gender</th>
<th>Mean (±SD)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post -green tea consumption PT</td>
<td>Female</td>
<td>13.690 (± 0.85)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>14.468 (±0.81)</td>
<td></td>
</tr>
<tr>
<td>Pre-green tea consumption PT</td>
<td>Female</td>
<td>13.8 (±0.72)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>14.85 (±0.90)</td>
<td></td>
</tr>
</tbody>
</table>

- Significant at <0.05

When we compared the PT in both males and females the mean of pre green tea consumption samples among females was 13.8 (±0.72) and among
males was 14.85 (±0.90) , Whereas the mean of PT in post green tea consumption samples collected from females was 13.7 (±0.85) while it was 14.5 (±0.81) in samples collected from males . The result showed that there is a significant difference between males and females in the pre and post green tea consumption samples (P value= 0.005 and 0.000 respectively).

Table (4) PT level according to age group:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age Group</th>
<th>Mean (±SD)</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post -green tea consumption PT</td>
<td>20-32 years</td>
<td>13.8 (±0.90)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>33-45 years</td>
<td>14.3 (±0.88)</td>
<td></td>
</tr>
<tr>
<td>Pre-green tea consumption PT</td>
<td>20-32 years</td>
<td>14.0 (±0.80)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>33-45 years</td>
<td>14.6 (±1.0)</td>
<td></td>
</tr>
</tbody>
</table>

- Significant at <0.05

When we compared the Pro thrombin time level in the different aged group, the mean of PT for Pre-green tea consumption samples among group one (20-32 years) was 14.0 (±0.80) and among group two (33-45 years) was 14.6 (±1.0) , while the mean of pro thrombin time of post-green tea consumption samples among group one 13.8 (±0.90) and it was 14.3 (±0.88) 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 0.09 and 0.06 respectively).
Chapter Four

Discussion, Conclusion, and Recommendations

4.1 Discussion:

Pro thrombin time test is important test that used to evaluate the extrinsic and common pathway of coagulation and it is used to the monitoring of patients on oral anticoagulation therapy. This study was carried out to evaluate the effect of green tea consumption (two cups per day) on pro thrombin time level in apparently 40 healthy Sudanese people aged between 20-45 years.

In this study we found that the mean of pro thrombin time level in the post samples were significantly lower than the mean of pre-samples, our results agrees with findings of study done by F jalali et al (2008) reported that after regular consumptive of 4 g/d green tea for one month there was a small significant decrease in pro thrombin time level.

Another study done by Hussam et al (2017) which agreed with our study and found that after regular consumption of two cup of green tea for one month there was a small significant decrease in pro thrombin time.

No statistically significant difference when we compared mean of pro thrombin time level between males and females.

No statistically significant difference when we compared mean of pro thrombin time level between aged group. Another study done by Kannan et al (2014) which study the effect of green tea on pharmacodynamics of
warfarin and showed that after regular consumptive of green tea for one month there was a small significant reduction in the mean of pro thrombin time level after green tea consumption and patients who used green tea with warfarin were reduced in pro thrombin time level more than who used warfarin alone.

4.2 Conclusion:

The study concludes that consumption of two cup of green tea per day for 30 days significantly reduce the level of pro thrombin time level among healthy Sudanese volunteers.

4.3 Recommendation:

Further study should be conducted to evaluate the effect of green tea consumption on prothrombin time level among subjects with known higher prothrombin time level.

Another study should have done with suitable sample size, different numbers of cups per day and with different duration of consumption for more obvious findings.
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REQUIRED MATERIALS NOT PROVIDED:

General Laboratory Equipment and instrumentations

PROCEDURE:

Manual method

1. Patients samples should be tested in parallel with pooled fresh normal plasma (FNP) and suitable controls.
2. Bring the reagent vial to room temperature (20-30°C). Mix the contents of the vial to homogenise the suspension completely.
3. Aspirate from the reagent vial enough reagents for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred.)
4. To a test tube 75 mm test tube add 0.1 ml of Plasma (PP) and place the tube in a water bath for 3 to 5 minutes at 37°C.
5. To the tube add 0.2 ml of BioMed-Liquifast plasma (pre-warmed at 37°C for at least 10 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
6. Gently slide the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel clot formation begins. Record the time in seconds.
7. Repeat steps from 4 to 6 for a duplicate test on the same sample.

CALCULATION:

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

Or as a ratio:

\[ R = \frac{\text{Mean of patient plasma PT in seconds}}{\text{Mean of patient plasma PT in seconds}} \]

Or as an International Normalised Ratio (INR), INR = (R) \text{MNPT for the reagent.}

It is recommended by the WHO that MNPT should be established for each lot of PT reagents by each laboratory, since PT result are dependent on the combination of reagent lot, instrument and technique followed at each laboratory.

Usually plasma from at least 20 normal healthy individuals should be used to establish the MNPT. The average of such PT results in seconds = MNPT.