A STUDY ON THE EFFECTS OF *ANDROGRAPHIS PANICULATA* (HEMPEDU BUMI) ON SERUM PROTEIN C, PROTEIN S ACTIVITY AND FASTING BLOOD GLUCOSE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

By

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ABSTRACT

A STUDY ON THE EFFECTS OF *ANDROGRAPHIS PANICULATA* (HEMPEDU BUMI) ON SERUM PROTEIN C, PROTEIN S ACTIVITY AND FASTING BLOOD GLUCOSE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS.

Background and Objectives

Patients with type 2 diabetes mellitus (DM) show enhanced activation of the blood coagulation system and decrease level of natural anticoagulant such as protein C and protein S. This is believed to contribute to the high incidence of premature atherosclerosis attributable to myocardial infarction, cardiovascular disease and peripheral vascular disease in diabetic patients. *Andrographis paniculata* (Hempedu bumi) has been well known to have blood sugar lowering properties in diabetes patients and has been used by local Malaysians as an alternative to current oral hypoglycaemic agents. With the benefit of its blood sugar lowering properties it is hoped that *Andrographis paniculata* may also increases level serum protein C and protein S in diabetic patients, thus reducing the risk of atherosclerotic disease. This study was conducted to determine the changes in fasting blood sugar levels and to assess the changes in serum protein C and protein S activity following oral administration of *Andrographis paniculata* among type 2 diabetes mellitus patients in Hospital Universiti Sains Malaysia (HUSM) Diabetic Medical Clinic.

Methodology

This is an open-labelled, randomised treatment versus control study which was conducted among type 2 diabetes mellitus patient on follow up at the Hospital Universiti Sains Malaysia (HUSM) Diabetic Medical Clinic from August 2010 till

November 2010. A total of thirty four subjects were recruited in this study. Of this, 17 were randomly given the study medication; two tablets each containing 250 mg of *Andrographis paniculata* for two weeks duration while the other 17 patients were allowed to continue with their previous medication without any alteration. Fasting blood glu**cose** (FBG), serum protein C and protein S, blood samples were taken at baseline and after two weeks intervention for both groups.

Results

A total number of 34 patients were involved in this study. The mean age was 55.2 ± 9.8 years. The baseline HbA_{1c} was 9.2% ± 2.4 % in both groups. The mean fasting blood sugar during pre intervention in the control group was 8.6 mmol/L ± 3.7 while in treatment group is 9.1 mmol/L ± 5.0 and post treatment, the mean fasting blood sugar was 8.3mmol/L ±2.9 and 9.3 mmol/L ± 4.0 respectively. The mean of protein C in the pre intervention for control group was 117.2 %± 17.3 and in the treatment group was 125.1 % ± 21.3. Post intervention mean protein C in the control group was 121.1 % ± 25.4 and in the treatment group is 125.9 % ± 18.9. The mean protein S in the pre intervention for control group was 202.4 % ± 128.0 and for treatment group is 135.4% ± 30.1 while in the post intervention mean for control group was 208.3 % ± 129.5 and in the treatment group 134.1 % ±25.3. The mean difference of fasting blood sugar, protein C and protein S between pre and post intervention for both groups were statistically not significant.

Conclusion

Our study demonstrated that generally the blood sugar control among type 2 diabetic patients is still poor with mean HbA1c of 9.2 % ± 2.4. There were no significant

changes in mean serum fasting blood glucose, protein C and protein S for pre and post - treatment of oral administration of 500 mg of *Andrographis paniculata* for two weeks duration in the treatment group when compared to control group. These findings were probably related to inadequate dose of Andrographis paniculata since there was no study of bioequivalence in this study and also inadequate study duration to produce desirable effects in this study.

ABSTRAK

KAJIAN MENGENAI KESAN HEMPEDU BUMI KE ATAS AKTIVITI PROTIN C, PROTIN S DAN PARAS GULA PUASA DI KALANGAN PESAKIT KENCING MANIS DIABETES MELLITUS JENIS KE-2.

Latar Belakang dan Objektif

Pesakit kencing manis jenis kedua menunjukkan pengaktifan system pembekuan darah dan kerendahan tahap antikoagulasi semulajadi dalam darah seperti protin C dan protein S. Ini dipercayai meningkatkan insiden aterosklerosis pramatang dan mengakibatkan penyakit kardiovascular serta penyakit salur darah periferi di kalangan pesakit kencing manis. Hempedu bumi dikenalpasti dapat menurunkan paras gula dalam darah dikalangan peakit kencing manis dan telah digunakan oleh penduduk di Malaysia sebagai alternatif kepada ubat-ubatan menurunkan paras gula dalam darah. Dengan itu, diharapkan hempedu bumi dapat meningkatkan aktiviti serum protin C dan protin S dan sekaligus mengurangkan risiko penyakit aterosklerosis. Kajian ini ingin menilai perubahan dalam paras gula dalam darah ketika berpuasa, aktiviti protin C dan protin S selepas pengambilan hempedu bumi atau "*Andrographis paniculata*" di kalangan pesakit kencing manis jenis kedua yang mendapat rawatan di Klinik Diabetik Hospital Universiti Sains Malaysia (HUSM).

Metodologi

Ini adalah kajian rawatan aktif versus control dimana pesakit serta doktor tahu akan jenis ubat yang diberi yang dijalankan di kalangan pesakit kencing manis jenis kedua. Mereka mendapat rawatan susulan di Klinik Diabetik Hospital Universiti Sains Malaysia dari Ogos 2010 hingga November 2010. Seramai 34 subjek telah diambil

untuk kajian. Seramai 17 orang subjek telah diberi *Andrographis paniculata* (Hempedu bumi) untuk tempoh dua minggu, dan seramai 17 pesakit dibenarkan mengambil ubat-ubatan diabetes yang telah sedia digunakan sebelum ini. Darah untuk paras gula semasa puasa, protein C dan protein S diambil pada permulaan dan pada penghujung kajian untuk kedua-dua kumpulan.

Keputusan

Seramai 34 orang pesakit terlibat dalam kajian ini. Purata umur untuk semua pesakit adalah 55.2 ± 9.8 tahun. Purata permulaan HbA1c untuk semua pesakit adalah 9.2 % ± 2.4 %. Purata untuk darah gula puasa sebelum intervensi dan selepas intervensi dalam kumpulan kontrol adalah 8.6 mmol/L ± 3.7 dan 8.3 mmol/L ± 2.9. Purata darah gula puasa dalam kumpulan intervensi sebelum dan selepas intervensi adalah 9.1 mmol/L ± 5.0 dan 9.3 mmol/L ± 4.0.

Purata protin C semasa sebelum intervensi untuk kumpulan kontrol adalah 117.2 % ± 17.3 dan dalam kumpulan rawatan adalah 125.1 % ± 21.3. Semasa selepas intervensi, purata protin C dalam kumpulan kontrol adalah 121.1 % ± 25.4 dan dalam kumpulan rawatan adalah 125.9% ± 18.9. Purata protein S semasa sebelum intervensi adalah 202.4 % ± 128.0 dalam kumpulan kontrol dan 135.4% ± 30.1 di dalam kumpulan intervensi. Semasa selepas intervensi, purata protein S dalam kumpulan kontrol adalah 208.3% ± 129.5 dan 134.1% ± 25.3 dalam kumpulan intervensi. Tiada perbezaan yang nyata dalam perubahan darah semasa puasa, protin C dan protin S antara sebelum dan selepas intervensi didalam kedua-dua kumpulan kontrol dan rawatan.

Kesimpulan

Kajian ini menunjukkan secara amnya kawalan gula di kalangan pesakit kencing manis jenis ke dua adalah tidak memuaskan dengan purata HbA1c sebanyak 9.2 % ± 2.4. Tiada perubahan yang nyata dalam perubahan darah gula puasa, protein C dan protein S sebelum dan selepas intervensi dua minggu menggunakan 500 mg *Andrographis paniculata.* Keputusan ini mungkin boleh disebabkan dose Hempedu bumi yang tidak cukup disebabkan tiada kajian bioequivalen ke atas ubatan ini dan mungkin jarak masa kajian selama dua minggu tidak culup untuk memberikan keputusan yang diharapkan dalan kajian ini.

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SULAILA BT. BASIAM

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
CDC	Centre for Disease Control
DM	Diabetes mellitus
FBS	Fasting blood sugar (FBS)
GDM	Gestational diabetes mellitus
HbA_{1c}	Glycosylated Haemoglobin
IDF	International Diabetes Federation
IGT	Impaired Glucose Tolerance
T1DM	Type 1 diabetes mellitus (T1DM)
T2DM	Type 2 diabetes mellitus (T2DM)
WHO	World Health Organization (WHO)

1.0 INTRODUCTION

1.1 BACKGROUND OF DIABETES MELLITUS

Diabetes mellitus (DM) was likely first described about 3500 years ago and the first case reported for diabetes was in the year of 3rd Dynasty Egyptian physician in 1555 (Gemmill, 1972). Since then a lot has been studied about nature of the disease, types of the disease and its complications.

DM has been primarily regarded as a disorder of glucose metabolism and recently has been viewed as a constellation of metabolic disturbances, including abnormalities of carbohydrate metabolism, adipose storage, lipid metabolism, and protein biochemistry. WHO criteria divided DM as Type 1 diabetes mellitus (T1DM) which is characterised by a lack of insulin production. Type 2 diabetes mellitus (T2DM) or formerly called non-insulin-dependent or adult-onset diabetes is caused by the body's ineffective use of insulin. The American Diabetes Association (ADA) Expert Committee has also introduced as in addition to type 1 and type 2 diabetes mellitus, "specific types" of diabetes are identified: gestational diabetes and secondary diabetes. Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (Metzger BE and Coustan DR, 1998).

The incidence of DM is increasing rapidly as a result of aging and an ever more obese population (Ali H. Mokdad *et al.*, 2001). Indeed, between 2000 and 2010, the number of patients with DM is expected to increase by 23 % in the United States and 46% around the world. The International Diabetes Federation (IDF) predicts by year 2025 the South

East Asia region would have an estimated diabetes prevalence of 7.5 % and IGT prevalence of 13.5 % (IDF Diabetes Atlas 2003).

In Malaysia, the number of people with diabetes is increasing with significantly high complication rates and associated diseases amongst diabetes patients. Prevalence of diabetes in Malaysian National Health Morbidity Survey 111 (Zanariah *et al*., 2006) is 14.9% compared with NHMS II in 1996 which was 8.35 %.

1.2 PATHOPHYSIOLOGY OF COMPLICATIONS OF TYPE 2 DIABETES MELLITUS

Diabetic complications can be classified broadly as microvascular or macrovascular disease. Microvascular complications include neuropathy (nerve damage), nephropathy (kidney disease) and vision disorders (e.g. retinopathy, glaucoma, cataract and corneal disease). Macrovascular complications include heart disease, stroke and peripheral vascular disease. Other complications of diabetes include infections, metabolic difficulties, impotence and autonomic neuropathy.

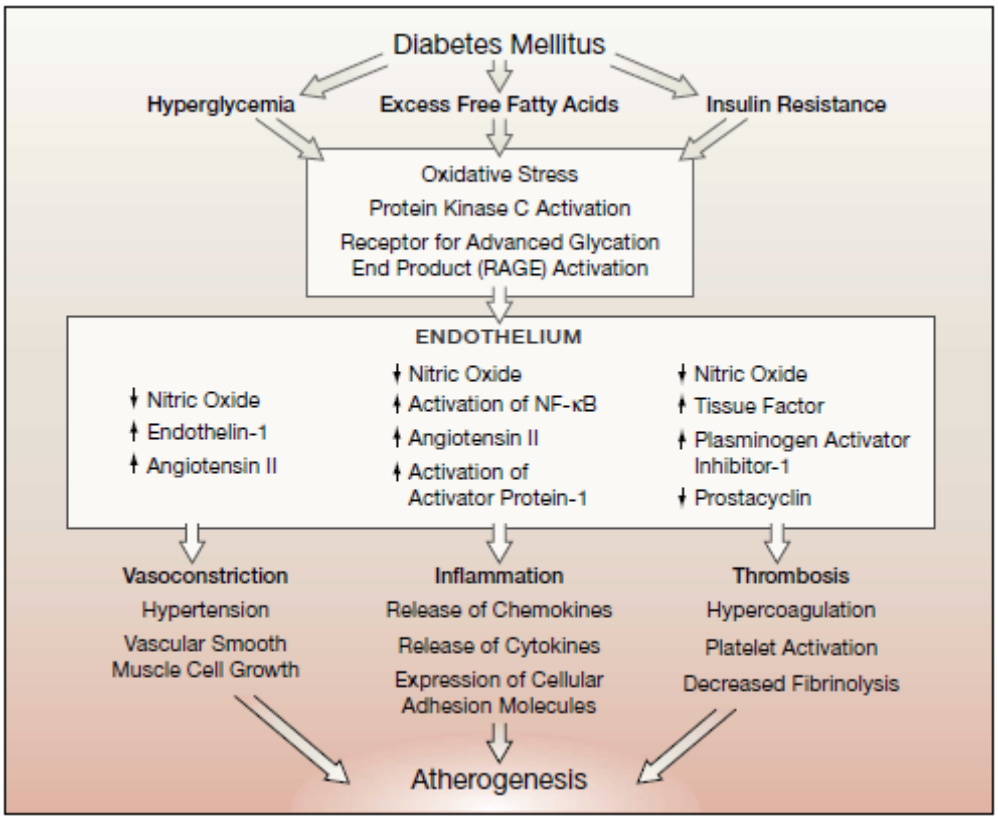
Eighty percent of patients with diabetes mellitus die a thrombotic death. Seventy five percent of these deaths are due to cardiovascular complications, and the remainder is due to cerebrovascular events and peripheral vascular complications (Carr ME., 2001).

Endothelial abnormalities, coagulation activation markers abnormalities, level of the anticoagulant protein abnormality, and increased platelet aggregation made a constellation of findings that supports the clinical observation that diabetes is a

hypercoagulable state. These changes favour the development of a hypercoagulable pro-thrombotic state, which may in turn enhance cardiovascular risk by increasing the likelihood of developing an occlusive thrombus within an artery and contributing to the development of atherosclerotic lesions (Dunn and Grant, 2005).

Endothelial dysfunction, present at disease onset, may be the risk of atherogenesis that is present throughout the course of diabetes and associated with late-stage adverse outcomes. Investigations have revealed that vascular endothelium, a crucial regulator of vascular homeostasis, participates importantly in the control of vascular tone and blood flow, coagulation and thrombosis, nutrient delivery and waste removal, inflammation, vascular smooth muscle cell growth and migration, leukocyte attraction and diapedesis (TJ.Anderson, 2003)

The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls. Atherosclerosis results from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidised lipids from LDL particles accumulate in the endothelial wall of arteries. Monocytes infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidised lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes. T-lymphocytes, in turn, induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the process is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of this lesion leads to acute vascular infarction (PJ, 2007).



- Source : Cines DB *et al.*, 1998

Figure 1.1 Endothelial Dysfunction in Diabetes Mellitus

In diabetes, hyperglycaemia, excess free fatty acid release, and insulin resistance alters adverse metabolic events within the endothelial cell. Activation of these systems impairs endothelial function, augments vasoconstriction, increases inflammation, and promotes thrombosis. Decreasing nitric oxide and increasing endothelin- 1 and angiotensin II concentrations increase vascular tone and vascular smooth muscle cell growth and migration.

Activation of the transcription factors nuclear factor kB (NF-kB) and activator protein 1 induces inflammatory gene expression, with liberation of leukocyte-attracting chemokines, increased production of inflammatory cytokines, and augmented expression of cellular adhesion molecules.

Increased production of tissue factor and plasmin activator inhibitor 1 creates a prothrombotic milieu, while decreased endothelium derived nitric oxide and prostacyclin favours platelet activation (Carr ME., 2001).

In addition to atheroma formation, there is strong evidence of increased platelet adhesion and hypercoagulability in type 2 diabetes. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation.

The platelet alterations that occur in diabetes have been extensively studied. An increased platelet aggregation has been reported in Type 1 (insulin-dependent) diabetic patients with poor metabolic control (Davis JW, 1982). Platelet hyperaggregation in diabetic patients is partly determined by an exaggerated ability of the platelets to bind both thromboxane and fibrinogen accompanied by a reduced binding capacity for prostacyclin and this can also present in diabetic patients without vascular complications and in young diabetic patients (Collier A, 1986).

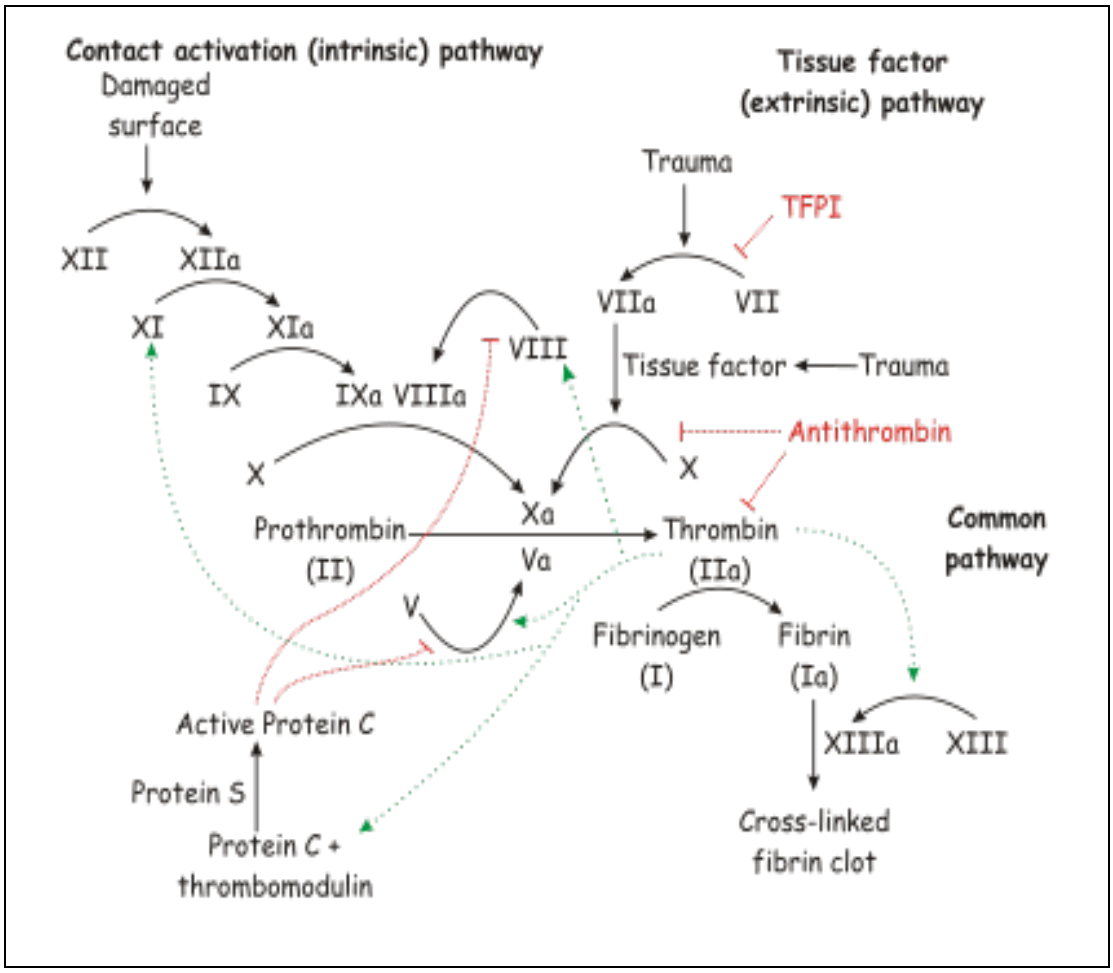
Elevated levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis in patients with diabetes (Carr M.E, 1991). Fibrinogen is a glycoprotein with a prolonged half-life. It can become hyperglycosylated circulates in an environment of containing high glucose, i.e., hyperglycemia in a poorly controlled diabetic. When such fibrinogen is clotted, the resulting fibrin structure is composed of small diameter fibers which are markedly resistant to degradation by plasmin. The higher the concentration of hyperglycosylated fibrinogen, the longer the clots take to dissolve. These results would

imply an increased resistance to fibrinolysis in poorly controlled diabetes (Carmassi, 1992).

The combination of increased coagulability and impaired fibrinolysis likely further increases the risk of vascular occlusion and cardiovascular events in type 2 diabetes (Beckman JA *et al.*, 2002).

1.3 DIABETES MELLITUS AND DISORDERS OF COAGULATION

In normal situation coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium . Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously. Proteins in the blood plasma, called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug.



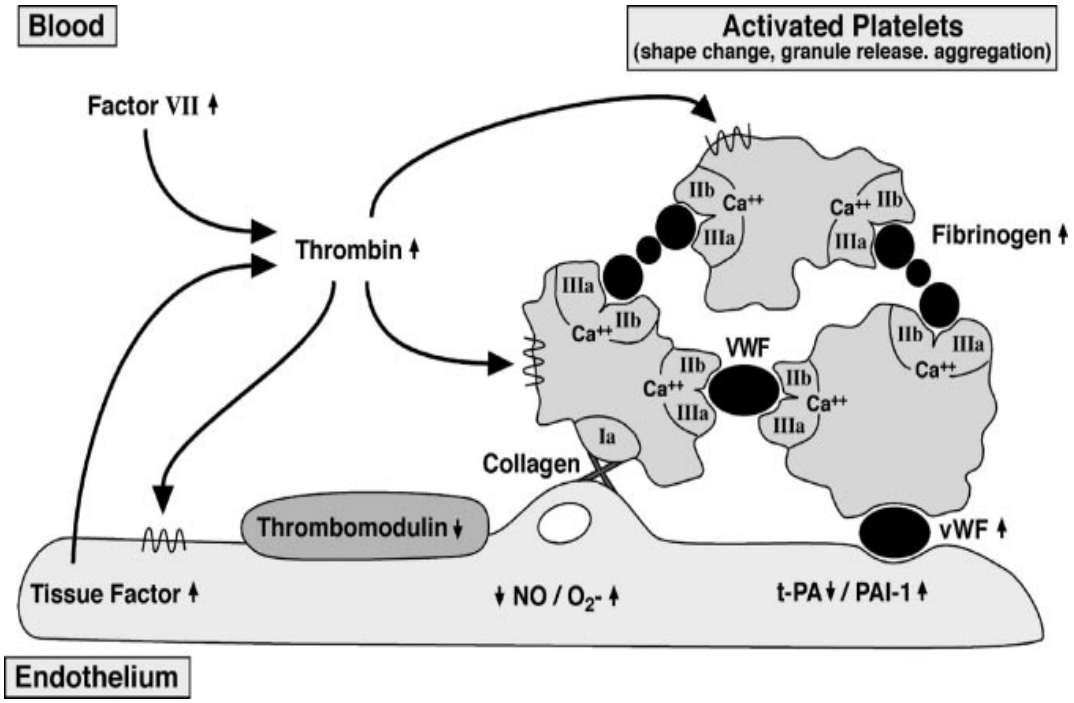
- Source : Hafer-Macko *et al.*, 2002

Figure 1.2 The coagulation cascade - secondary haemostasis

The coagulation cascade of secondary haemostasis has two pathways which lead to fibrin formation. These are known as the intrinsic pathway, and the extrinsic pathway. The primary pathway for the initiation of blood coagulation is the tissue factor pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyse the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lower case *a* appended to indicate an active form.

Following damage to the blood vessel, factor VII (FVII) leaves the circulation and comes into contact with tissue factor (TF) forming an activated complex (TF-FVIIa). This TF-FVIIa activates factor IX (FIX) and factor X (FX). And then factor VII is itself activated by thrombin, activated factor XI (FXIa), factor XII (FXII) and activated FX. The activation of FX to form activated factor X (FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).

FXa and its co-factor, activated factor V (FVa) form the prothrombinase complex, which activates prothrombin to thrombin. Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which, in turn, activates FIX), and activates and releases FVIII from being bound to Von Willebrand factor (vWF). Activated factor VIII is the co-factor of activated factor IX, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.).



- Source: Mark A. Creager *et al.*, 2003

Figure 1.3 Schematic of coagulation disorder in Diabetes compared to normal homeostasis.

Numerous epidemiological studies have concurred in recognizing fibrinogen as having an important predictive value as a marker of cardiovascular risk (Wilhelmsen L, 1984). Studies in diabetic patients also showed increase plasma level of plasminogen (Oanda OP, 1992).

Epidemiological studies also have reported that high levels of factor VII are associated with a high mortality rate for cardiovascular events it have also been reported in diabetes (Balleisen L, 1985).

In fact, in one of a study in normal subjects, a direct correlation has been reported between levels of factor VII and fasting glycaemia. This demonstrated that glycaemia

levels can directly affect the concentrations of factor VII in both diabetic patients and normal subjects (Ceriello A, 1988).

Another factor of coagulation that alters with hyperglycaemic state is factor X. The antigenic levels of factor X are increased but the activation of this factor is however reduced by the induction of hyperglycaemia, which has been observed both in diabetic patients and also in normal subjects (Ceriello A *et al.*, 1990).

Erythrocytes also play a important role in coagulation processes. When the erythrocyte membrane is impaired, ADP is released, stimulating platelet aggregation. In diabetes, the erythrocytes are damaged, resulting in reduced half-life, resulting in polycythaemia and increased volume (Jones RL, 1981). These factors may then cause blood hyperviscosity with a consequent increase in thrombotic risk.

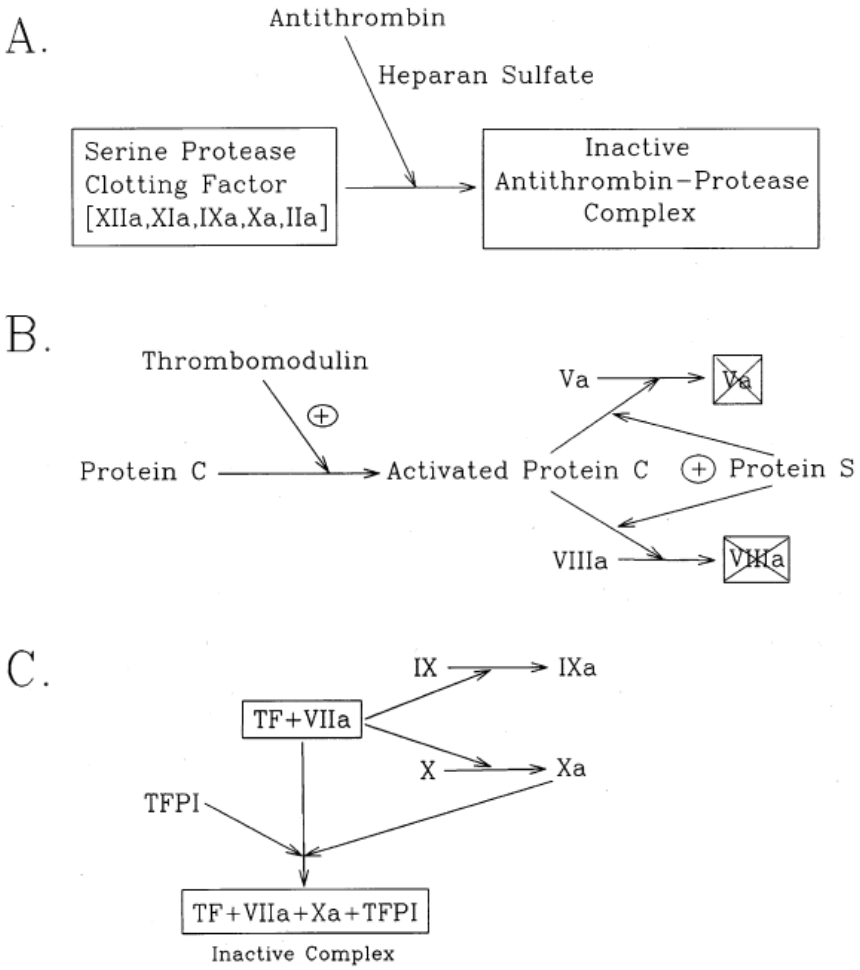
1.4 COAGULATION INHIBITORS IN DIABETES MELLITUS

Since the action of thrombin is the result of a balance between the pro-coagulant cascade and the action of the various inhibitors on it or on its production, it is reasonable to assume that these inhibitors may also play an important role in the genesis of thrombophilia state in diabetes.

There are several mechanisms that keep the coagulation cascade in check. One of the major physiological anticoagulant is protein C. It is a vitamin K-dependent serine protease enzyme produced by hepatocytes that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin binds

these proteins in such a way that it activates Protein C. The activated form, along with protein S and phospholipids as cofactors, degrades activated factor V and activated factor VIII.

If there is not enough protein C or protein S, or if either one is not functioning normally, the thrombin generation goes on largely unchecked. This can lead to excessive or inappropriate clotting that may block the flow of blood and cause thrombosis. Tests for protein C and protein S may look at their function (activity) or quantity (antigen). Functional tests for protein C and protein S are usually ordered, along with other tests for hypercoagulability, to screen for sufficiency, normality, and factor activity.



- Source: Ceriello A *et al.*, 1995

Figure 1.3 Schematic of the normal biological anticoagulant mechanisms.

Panel A **:** Antithrombin III control of the serine protease clotting factors.

Panel B **:** The proteins C and S system for control of the non-serine clotting factors Va and VIIIa.

Panel C **:** Control of factor VIIa by TFPI.

Based on the results, quantities of protein C antigen and free, or sometimes total, protein S antigen may be measured to look for decreased production due to an acquired or inherited condition and to classify the type of deficiency. Acquired protein C deficiency may be caused by large blood clots, liver disease, disseminated intravascular coagulation (DIC), infection (sepsis), and vitamin K deficiency. Treatment with warfarin or certain types of chemotherapy can also cause acquired protein C deficiency. If the shortage is due to a rare inherited genetic change, the quantity of protein C or protein S available and the degree of activity can be used to help determine whether a person is heterozygous or homozygous for the mutation.

Functional tests for protein C and protein S measure their activity and evaluate their ability to regulate and slow blood clotting. Decreased activity may be due to a decreased concentration of protein C or S or, more rarely, due to dysfunctional protein C or S.

To look for Protein C and protein S quantity, antigen tests measure the amount of the protein present. Protein S works with protein C. It is present in the blood in two forms, free or bound to another protein, but only the free form is available to combine with protein C. Protein S antigen tests measure either free protein S or total protein S.

If both the activity and the concentrations of protein C and protein S antigens are normal, it usually indicates adequate clotting regulation. Low level of protein C or protein S activity can result in excessive or inappropriate blood clotting. If the protein is dysfunctional (normal levels of protein, but it does not work correctly), the coagulation process will not be sufficiently regulated. Either situation can lead to an increased risk

of developing a clot that blocks the flow of blood in the veins, but the severity of the risk depends on the magnitude of the deficiency and/or the degree of dysfunction of the protein. The normal values of activity are 60 % to 150 % inhibition.

In diabetes, abnormalities of hemostasis have been reported in many studies over almost thirty years, but unfortunately the results have often appeared contradictory. The hemostatic alterations could lead to increased risk of vascular disease in diabetic patients. In one study, plasma coagulation factors (e.g., factor VII and thrombin) and lesion-based coagulants, tissue factor are found to be increased, and endogenous anticoagulants (e.g., thrombomodulin and protein C) are decreased (Hafer-Macko *et al.*, 2002, Ceriello A *et al.*, 1990, Ceriello A *et al.*, 1995).

In another study, they evaluated some coagulation factors (Fibrinogen, Factor II, Factor VII) and coagulation inhibitors (Protein C, Protein S), and plasminogen in fifty-four type 2 diabetic patients. They analyzed the possible relationship between coagulation factors and coagulation inhibitors and parameters for glyco-metabolic control (glycosylated haemoglobin, fructosamine) and disturbed lipid metabolism (cholesterol, triglycerides). The results showed increase of fibrinogen, correlated with the metabolic control of the disease, positive correlation between plasminogen, factor II, protein S and hypertriglycerides, decreased levels of protein C correlated neither with metabolic control of disease neither with disturbed lipid metabolism (Moccia F, 1996).

Another one most studied coagulation inhibitors is antithrombin 111(ATIII). A decrease in the biological activity of this molecule in the presence of a normal antigenic

concentration has been found in diabetic patients. In this case as well, hyperglycaemia seems capable of directly affecting the activity of the molecule, in both diabetic patients and in normal subjects (Ceriello A, 1983).

The effect of glycaemia on ATIII may have an important pro-thrombotic impact. In fact, the decreased in its biological activity results in a reduced thrombin-antithrombin complex formation with consequent hyperactivity of the thrombin and improvement in metabolic control e.g. insulin therapy, can increase the activity of the molecule (Husted SE, 1989).

{Bach, 1988 #1}{Bach, 1988 #1}

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1.5 INTRODUCTION TO TRADITIONAL MEDICINE AND DIABETES

Traditional medicine, also known as complementary medicine or alternative medicine provides the first line of primary health-care to major segments of the population throughout the world. Traditional medicine has been defined by the World Health Organization (WHO) as "health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination, to treat, diagnose and prevent illnesses or maintain well-being" (WHO, 2003).

About 25% of the drugs used in modern medicine owe their origins to plants from tropical rainforests (S.Elliot., 1986). In fact, many drugs listed as conventional medications now were also originally derived from plants; for example, salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant whereas vincristine, used to treat certain types of cancer, comes from periwinkle.

One condition for which minority populations are likely to use complementary and alternative medicine therapies is diabetes. This is particularly prevalent in many minority cultures have a long history of using herbal preparations to treat diabetes, and recent research suggests that some herbal therapies may have a role in the treatment of this complex disease (Berman, 1999).

In Malaysia, the prevalence of herbal medicines use is high (Aziz Z., 2009). Reasons for the use of herbals include that it is part of the culture and belief of some people for maintenance of health or to treat certain ailments, relatively cheaper cost of herbal products and hence

affordability to the lower income group as well as herbals are natural and that anything natural is safe (Hussin, 2001).

In another study done by the School of Pharmacy, International Medical University among patients in their outpatients clinic, this showed that a high percentage of alternative medication including herbal was used, this included of 24.6 percent among patients with chronic diseases especially in diabetes patients (35.5%) (Syed Shahzad Hasan *et al.*, 2009).

In a study carried out locally at Hospital Tuanku Jaafar, Seremban, they aimed to evaluate complementary and alternative medicine (CAM) usage among their diabetic patients. From their study, the herbal drugs (64.9%) were the most common type of CAM utilised by the patients followed by vitamins (57.9%), ginseng (12.3%), and yoga (7.9%). This study confirms an overall of a high frequency of CAM use (49.6%) among diabetic patients (Shahazad Hasan, 2011).

1.6 Andrographis paniculata ("Hempedu Bumi")

Andrographis paniculata (Acanthaceae) is a traditional medicinal plant common in south East Asia and found from India to Indo-china. Commonly called as king of bitter or kariyat, kalmegh, hempedu bumi and pokok cerita, it is an erect branch plant with green leaves and attained height of 60-70cm. The leaves and the aerial part of the plant have been used to cure various kinds of ailments.

Some important chemical compounds have been isolated from parts of the plant. The aerial part contains several diterpenoids and diterpene glycosides. Its main constituent, andrographolide, a diterpene lactone, is mainly responsible for its bitter taste. From its leaves, the active ingredients are diterpene lactones, flavone derivatives such as oroxylin and

wogonin. The major constituents are diterpene lactones (free and in glycosidic forms) including andrographolide, deoxyandrographolide, 11,12-didehydro-14-deoxyandrographolide, neoandrographolide, andrographiside, deoxyandrographiside and andropanoside.

The various routes of administration of herbal medications are typically chosen according to both the consistency of the preparation and the disease or condition under treatment. The herbs available come in several different forms: teas, syrups, oils, liquid extracts, tinctures, and dry extracts (pills or capsules). When orally consumed, andrographolide appears to accumulate in organs throughout the viscera.

Pharmacokinetics studies showed that andrographolide is quickly absorbed and extensively metabolised in rats and in human (Panossian *et al.,* 2000). 90 percent is eliminated within 48 - hour. Andrographolides are excreted fairly rapidly from the body via the urinary and gastrointestinal tract. Maximum plasma level were reach after 1.5 to 2 hours and the half life was 6.6 hours. The high pressure liquid chromatography (HPLC) is simple and rapid methods and can be use to determine concentration of active components in various extract of *Andrographis paniculata.*

1.6.1 Medicinal usage of Andrographis paniculata

Since ancient times, *Andrographis paniculata* has been used in traditional systems of medicine and some other countries for multiple clinical applications. Among the effects that have been proven by clinical trials are anti-inflammatory activities, anti-malarial activity, anti-fertility activity, hepatoprotective activity, immunological potential, respiratory system benefit, cardiovascular activity and hypoglycaemic activity.

In a study to determine the presence of antibacterial activity in the crude extracts of some of the commonly used medicinal plants in Malaysia, *Andrographis paniculata* was among five plants that were tested: It showed antibacterial activities towards *Pseudomonas aeruginosa* and being the most potent at minimum inhibitory concentration (MIC) of 2 g/disc (Zaidan *et al.*, 2005, Mishra *et al.*, 2009). In several other studies, extracts of *Andrographis paniculata* containing the active ingredients of deterpenoids were evaluated for antimalarial activity against *Plasmodium berghei,* one of the parasites that transmit malaria. The extract was found to produce considerable inhibition of parasite multiplication (Mishra *et al.*, 2009). It has also amongst the strongest activity towards *Brugia malayi* species of filaria (Zaridah *et al.*, 2001).

Andrographis paniculata is also being studied to assess the efficacy of it in the symptomatic treatment of uncomplicated upper respiratory tract infection. These studies showed significant reduction in symptom severity (Poolsup *et al.*, 2004, Gabrielian *et al.*, 2002). The prevention of the common cold with an extract of *Andrographis paniculata* was also shown in a pilot double-blind study where subjects were given a formulation of *Andrographis paniculata* and were diagnosed for the presence or absence of colds during a three-month period There was a significant decrease in the incidence of colds as compared to the placebo group who were not taking *Andrographis paniculata* formulation at the end of three months (Burgos *et al.*, 2009).

Andrographis paniculata also shows potent immunomodulatory and anti - angiogenic activities in tumour tissues. An in - vitro study (Varma *et al.*, 2009) demonstrated the capability of a compound in *Andrographis paniculata* inducing cell-cycle arrest and apoptosis in a variety of cancer cells at different concentrations. The results of a study in

Japan demonstrated that *Andrographis paniculata* also had a potent cell differentiation-inducing activity on leukaemia cells (Matsuda *et al.*, 1994).

The ability of *Andrographis paniculata* to lower fever has been demonstrated independently in several centres. Rat studies done in China have shown that andrographolide, neoandrographolide, and dehydroandrographolide can lower the fever produced by different fever-inducing agents, such as bacterial endotoxins (toxic chemicals released from bacteria), pneumococcus, haemolytic streptococcus, typhoid, paratyphoid, and the chemical 2,4-di-nitro-phenol (Madav. H.C, 1995).

In India, a study was conducted to evaluate the effect of *Andrographis paniculata* in infective hepatitis (Kapil A. *et al.*, 1993). There was marked improvement in term of appetite on the fifth day of treatment, jaundice gradually diminished and completely disappeared within 24 days, and fever subsided after 7 days on average with and improvement in liver function tests.

Other medicinal usage of *Andrographis paniculata* or its active ingredients is anti fertility where in one of the study, it resulted in cessation of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells and regressive and/or degenerative changes in the epididymis, seminal vesicle, ventral prostate and coagulating gland of tested mice (Akbarsha *et al.*, 1990, Zoha M.S. *et al.*, 1989).

As a potential antiretroviral effect, in a phase one clinical trial, showed a significant rise in the mean CD4 lymphocyte level of HIV subjects occurred after administration of 10 mg/kg andrographolide ,from a baseline of 405 cells/mm3 to 501 cells/mm3 (Carlo Calabress *et al.*, 2000).

1.6.2 Cardioprotective and homeostasis effects of *Andrographis paniculata*

To date, there are not many studies exploring the direct effect of *Andrographis paniculata* on coagulation or fibrinolytic parameters. However, in one study, the active ingredient of *Andrographis paniculata* was investigated for its suggested influence on the platelet-activating factor (PAF) which showed that andrographolide inhibits PAF-induced human blood platelet aggregation in a dose dependent manner. This result indicates that andrographolide has a mechanism of action associated with antithrombotic activity (Amroyan *et al.*, 1999).

The effect on platelet aggregation was also seen in another study where 63 patients with cardiac and cerebral vascular diseases were observed at 3 hours and/or one week after taking *Andrographis paniculata* extracts. Results showed that both 1 minute and 5 minutes platelet aggregation induced by adenosine diphosphate (ADP) were significantly inhibited. Serotonin release reaction from platelets was observed in 20 volunteers taking *Andrographis paniculata*. The observation also showed that *Andrographis paniculata* could inhibit the release of dense and alpha agranules from platelet and dilation of canalicular systems. All these findings might be due to the antiplatelet effect of *Andrographis paniculata* (Zhang *et al.*, 1994).

In another study, the three active diterpenoids from this plant, including aqueous plant extracts, were investigated for the inhibitory effect on platelet aggregation in vitro. Results indicate that andrographolide [*Andrographis paniculata* (1)] and 14-deoxy-11,12-didehydroandrographolide [*Andrographis paniculata* (3)] significantly inhibited thrombin-

induced platelet aggregation in a different concentration and time-dependent manner.. In addition, standardised aqueous extracts of *Andrographis paniculata* containing different amounts of *Andrographis paniculata* (3) inhibited thrombin-induced aggregation to different degrees. Therefore, the consumption of *Andrographis paniculata* products may help to prevent or treat some cardiovascular disorders for example, thrombosis (Thisoda *et al.*, 2006).

In experiments on dogs, the effect of *Andrographis paniculata* in alleviating the ischemia - reperfusion injury was prominent. In this study, after treatment with *Andrographis paniculata* in the ischaemia group, superoxide dismutase (SOD) in the ischemic region of myocardial tissue in the ischemia - reperfusion group was significantly decreased and calcium of ischaemic region of myocardial cell was increased. In the *Andrographis paniculata* pre-treated ischaemia - reperfusion group, on the contrary, all the above parameters were reversed. These findings indicate that *Andrographis paniculata* may improve the activity of sarcolemma adenosine triphospatase (ATPase) in alleviating the calcium and sodium overloading by decreasing the harmful effect of oxygen free radicals. Although the mechanism of action was not fully determined, it was concluded that *Andrographis paniculata* can be further be studied for the benefit of its antithrombotic activity (Guo *et al.*, 1994, Guo *et al.*, 1995).

A study conducted to determine the effect of *Andrographis paniculata* to the pharmacokinetics and pharmacodynamics of the anticoagulant warfarin in rats showed that the concomitant application of *Andrographis paniculata* and warfarin did not produce significant effects on the pharmacokinetics of warfarin, and practically no effect on its pharmacodynamics (Hovhannisyan *et al.*, 2006).

In an experimental study on the search for effective herbal drugs to reduce restenosis incidence after coronary angioplasty, *Andrographis paniculata* was used in a study on atherosclerotic stenosis and restenosis after experimental angioplasty. Preliminary results showed that *Andrographis paniculata* can significantly alleviate an atherosclerotic iliac artery. A follow-up angiography 4 weeks after angioplasty in the same patients showed the dilated iliac arteries in the control group all had severe restenosis, but in the *Andrographis paniculata* group no or only mild re-stenosis occurs. These preliminary results suggest that *Andrographis paniculata* can significantly alleviate stenosis. The above findings conclude that *Andrographis paniculata* may play an important role as an antithrombotic agent in preventing re-stenosis after coronary angioplasty (Wang and Zhao, 1993).

1.6.3 Hypoglycaemic effects of *Andrographis Paniculata*

Presently, there is a growing interest in traditional herbal remedies due to the multiple reasons associated with oral hypoglycaemic agents from the disease sufferer for the treatment of diabetes mellitus. Several species of herbal plants have been described in the scientific and popular literature as having antidiabetic activity (Valiathan M.S, 1998).

As for the situation in Malaysia, Malays believe that *Andrographis paniculata* can treat diabetes mellitus. Local Malaysian study on screening for anti hyperglycaemia activity in several local herbs showed that a few of them showed significant blood glucose lowering effecs. In one study, aqueous extract of *Andrographis paniculata* caused significant reduction in blood glucose levels (Husen *et al.*, 2004). In this study, dried raw material with dose of 50mg/kg body weight of *Andrographis paniculata* administered for 10 days has the highest

antihyperglycaemia activity among other herbs and this activity was enhanced when freeze dried extract was used.

Regarding the antihyperglycaemia mechanism of *Andrographis paniculata***,** previous studies have proposed several mechanisms. A study done to examine the effect of *Andrographis paniculata* on pancreatic β-cells showed that *Andrographis paniculata* was a very strong, dose dependent insulinotropic agent, glucose dependent and independent insulin secreting agent. This study also conclude that *Andrographis paniculata* affected membrane receptors, mostly ATP - dependent potassium channels (Wibudi *et al.*, 2008).

In another study, the extract showed appreciable alpha-glucosidase inhibitory effect in a concentration-dependent manner, and a weak alpha - amylase inhibitory activity (Subramanian *et al.*, 2008). In another animal study, oral administration of ***Andrographis Paniculata*** from aqueous extract of the leaves resulted in significant decrease in the blood glucose levels. A dose of 400 mg/kg two weeks duration lowered blood glucose level of streptozotocin- induced animals and increased activity of superoxide dismutase and catalase (Dandu and Inamdar, 2009).

Later it was found that the chloroform fraction of the plant extract (1 g/kg) significantly reduced both the blood glucose level of normoglycaemic rats and during glucose tolerance tests. These results suggested that the antidiabetic component of the *Andrographis paniculata* were present in the chloroform fraction of the extract compared to aqueous extract of *Andrographis paniculata* (Subramanian et al., 2008)**.**

In another study by Asmawi, *Andrographis paniculata* ethanolic extract was evaluated to screen the effect on insulin resistance using a combination of fat-fed diet and low dose streptozotocin. Oral administration of 1000 mg/kg extract to rats was able to cause a