MODULATORY EFFECT OF METFORMIN AND ARTEMETHER-LUMEFANTRINE ON BLOOD COAGULATION IN DIABETIC MICE CO-INFECTED WITH MALARIA

BY

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19010102022

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY,

COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY

SEPTEMBER 2022

DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

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……..

DATE

CERTIFICATION

This is to certify that the content of this project entitled '**MODULATORY EFFECT OF METFORMIN AND ARTEMETHER-LUMEFANTRINE ON BLOOD COAGULATION IN DIABETIC MICE CO- INFECTED WITH MALARIA'** was prepared and submitted by **BABALOLA IYANUOLUWA ISAAC** in partial fulfilment of the requirement for the degree of **BACHELOR OF SCIENCE IN BIOCHEMISTRY.**

The original research work was carried out by him under my supervision and is hereby accepted.

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DEDICATION

I would like to dedicate this report to Almighty God and also dedicate this to my Father & Mother (Mr. O.J and Mrs. O.F. Babalola) for their continuous financial and moral support which has kept me going all through the period of my undergraduate studies.

ACKNOWLEDGMENTS

My utmost gratitude goes to the Almighty GOD, who in his infinite mercies inspired the conception of this project write-up and also made it possible to be a great success. My special thanks also goes to Dr. (Mrs.) Ayodele O.O. and Dr. (Mrs.) Oyerinde O.R. who supervised my project and also put me through with my project write-up.

I also would like to appreciate MOUNTAIN TOP UNIVERSITY for giving me the opportunity to pursue a B.Sc degree in Biochemistry. I sincerely wish to express my sincere gratitude to the entire staff of the department of staff of Biochemistry, most especially the HOD, Dr. Kayode O.T.; without your efforts thus far, the knowledge I had before embarking on this training will be nothing to write home about. May the almighty God continue to bless you all, all the days of your lives.

I am especially greatly indebted to my parents (Mr. O.J and Mrs. O.F Babalola) for their love, encouragement, and investment in me. I sincerely wish to appreciate everyone that has influenced me positively and assisted me into becoming the young man I am today. To my colleagues Adeleke Wisdom, Olaiya Opemipo and Judith Richards who were there through the early mornings and late nights working in the lab, thank you.

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ABSTRACT

Blood coagulation is a process describing conversion of soluble materials within the blood into an insoluble gel that plugs injury/break site within blood vessels; this process requires regulation. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, defectiveness of insulin hormone has been found to have an increased risk of coagulation and can lead to cardiovascular disorders. Malaria is a disease caused by plasmodium parasite, when the parasite enters the bloodstream; proteins are secreted into the host's red blood cells, which stick to other blood cells making them adherent to blood vessel walls reducing detection and destruction of infected blood cells by the immune system which potentially causes blood clots. Effect of anti-malaria drugs on blood coagulation has not been properly investigated, as diabetics have been found to have possible co-infection with malaria, this study aims to provide information on the modulation of blood coagulation by metformin and artemether-lumefantrine in diabetic mice co-infected with malaria.

Fifty albino mice (BALB/C strain) were divided into 8 groups for this study; group I served as the normal control, group II served as diabetes negative control, group III served as malaria only, group IV served as diabetes co-infected with malaria group, group V served as malaria treated with 6.86 mg/body weight of artemether-lumefantrine, group VI served as diabetes treated with 200 mg/body weight of metformin, group VII served as diabetes co-infected malaria and treated with 200 mg/body weight of metformin and group VIII served as diabetes co-infected with malaria and treated with 200 mg/body weight of metformin and 6.86 mg/body weight of artemether-lumefantrine.

The results showed artemether-lumefantrine having an anti-coagulant effect on prothrombin time of extrinsic factors in malaria positive treatment group, metformin having a pro-coagulant effect on intrinsic factors in diabetes positive control group, and artemether-lumefantrine elevating the fibrinogen concentration in malaria positive control group. These findings suggest that while metformin is the first-line drug with an anti-coagulant effect on diabetes, artemether-lumefantrine not only could cure plasmodium falciparum but also possesses an anticoagulant effect.

Keywords: Artemether-Lumefantrine, Blood coagulation, Diabetes, Malaria, Metformin.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Malaria is a disease condition caused by parasites of the genus *Plasmodium*, which is transferred to humans by a bite of an infected *Anopheles* female mosquito. Malaria infection is regarded as one of the primary causes of mortality world-wide; it was recorded in 2017 to have affected an estimated 219 million people globally causing 435,000 deaths (WHO, 2017). Globally the mortality rate is seen to range between 0.3–2.2% and in cases of critical cases of malaria in tropical climate areas from 11–30% (White *et al.* 2014). Often regarded as a prevalent disease in Africa and some countries located in Asia, while in the urbanised parts of world malaria exists as imported from endemic areas (White *et al*. 2014).

When the parasite enters the bloodstream, it secretes proteins into the red blood cells of the host which are visible on its outer surface. These proteins stick to other blood cells making them adherent and to blood vessel walls reducing the likelihood of infected blood cells being detected & destroyed by the immune system while also being circulated and passed through the pancreas and potentially causing blood clots (Heledd Davies *et al.* 2020).

Diabetes mellitus is defined as a group of metabolic abnormalities characterized by chronic hyperglycemia occurring as a consequence of defects in action of insulin,its secretion, or both (Kharroubi & Darwish, 2015). Metabolic irregularities in carbohydrates, lipids, and protein levels occur as a result of insulin's importance as an anabolic hormone, which could either be caused by reduction of insulin levels normally required to achieve the required response and/or resistance of insulin to target tissues; adipose tissues etc. (Craig *et al*. 2009). Extent of the damage is majorly dependent on and diabetes type and duration.

Diabetic patients can sometimes be asymptomatic; particularly individuals which have been diagnosed with type 2 diabetes during the early periods of the disease, others with observable elevated blood sugar or hyperglycemia, and especially in children with complete insulin production insufficiency could suffer from weight loss, blurred vision, polyuria, polyphagia etc. Mis-managed diabetes may lead to stupor & coma situations and if not properly treated eventual death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome (Galtier; 2010). Compromised fibrinolysis and increased rate of coagulation have been inferred in the

pathological process of CVD in type 2 diabetes and insulin resilience syndromes (Sobel, 2002).

Blood coagulation can be described as a process which requires regulation; it involves conversion of circulating substances within the blood into an insoluble gel that plugs leakages in blood vessels to minimize blood loss (Furie *et al*. 2005). The process involves coagulation factors, calcium, and phospholipids. The coagulation factors (proteins) are synthesised by the liver and, ionized calcium (Ca^{++}) is present in the blood and from intracellular sources. A change in the equilibrium between clot formation and coagulation inhibition, favouring either pro or anticoagulation, can lead to potentially fatal bleeding (Ayodele *et al*. 2019).

Coagulation is known to be initiated by either of two distinct pathways, regardless of which pathway starts coagulation, completion of the process follows a general pathway. The common pathway involves activation of factors: I, II, V, X and XIII. Both extrinsic and intrinsic pathways are needed for systematic hemostasis and positive feedback loops exist between the two pathways. Deficiencies or abnormalities in any one factor can slow the overall process, increasing haemorrhaging possibilities.

The coagulation system has been progressively recognized to play a key role in malaria (Francischetti *et al*. 2008). Obstruction of small vessels and adhesion of parasitized red blood cells to endothelial cells are essential proceedings in the pathogenesis of severe malaria, also with endothelial cell activation and activation of the coagulation cascade which are proposed to be included in this process (Angchaisuksiri *et al*. 2014). Numerous works have shown alteration in levels of coagulation parameters in malaria patients (Moxon *et al*. 2009).

Diabetes has been described as a procoagulant state (Carmassi *et al*. 1992); metabolic defects interrupt these physiological mechanisms, causing a prothrombotic state categorised by platelet hypersensitivity, coagulation factor disorders, and hypofibrinolysis (Lemkes *et al*. 2010). Conversion of fibrinogen to fibrin is the last critical step in extrinsic and intrinsic coagulation pathways, and higher circulating fibrinogen levels are observed in T1DM and T2DM patients, resulting in a more compacted clot structure along with increased resistance to fibrinolysis (Neergaard-Petersen *et al*. 2014).

Metformin, is majorly described as the first line anti-diabetes drug, with its capability of lowering coagulation factor and platelet activity and also preventing the development of fibrinolysis-resistant clots (Verdoia *et al*. 2018). The attenuation of coagulation properties was described by an improvement of insulin sensitivity and a standardization of endothelial

function for metformin treatment (Markowicz-Piasecka *et al*. 2019). A study currently discovered that metformin was able to directly lower the expression and activity of Tissue factor in patients diagnosed with chronic hyperglycemia and abysmally controlled glucose, which was mediated by reduction of endothelial inflammation (Witkowski *et al*. 2020).

Published reports have stated that metformin decreases cardiovascular events and mortality in diabetic patients (Inzucchi *et al*. 2007), this finding is also consistent with results from a study which suggests that metformin may have great use as an anti-thrombotic agent (Xin *et al*. 2016).

1.2 Problem statement

Diabetes mellitus is a morbid condition (with a global population of about 463 million people and 1.5 million deaths reported in 2019; a global population of over 750 million people with diabetes has been projected for 2045 (WHO, 2021). Nigeria is one of the most affected countries in Africa with 2.7 million people living with diabetes (Uloko *et al*., 2018), which affects blood coagulation.

Malaria is perhaps regarded as the world's most devastating human parasitic infection (Udeme & Omotayo, 2012) and Africa bears the greatest burden of this disease. Nigeria is one of the countries most affected by malaria and accounts for 25 percent of world-wide malaria cases (Obianime & Aprioku, 2009) and diabetic patients could also be infected with malaria.

Several medications are available for the treatment of these two disease conditions. Artemether is a drug substance used for treating uncomplicated malaria caused by *P .falciparum* parasite, some research has also stated that it can also be used to treat complicated malaria (Esu *et al*. 2019). Metformin is regarded as first-line therapy for diabetic patients due to its hypoglycemic activity (Nasri & Rafieian-Kopaei 2014). As blood coagulation has been associated with both disease conditions, it is, therefore, important to investigate the mechanistic effect of metformin and artemether-lumefantrine on blood coagulation in diabetic mice co-infected with malaria.

1.3 Aim and Objectives

The study aims to investigate the modulatory effect of metformin and artemether-lumefantrine on blood coagulation in diabetic mice co-infected with malaria parasite by achieving specific objectives which are to:

i. Determine the effects of metformin and artemether-lumefantrine administration on the packed cell volume (PCV) of the experimental mice.
ii. Determine the effects of metformin, and artemether-lumefantrine on activated partial thromboplastin time (APPT) and prothrombin time (PT) of diabetic mice co-infected with malaria.
iii. Determine the effects of metformin and artemether-lumefantrine on fibrinogen concentration of diabetic mice co-infected with malaria.

1.4 Scope of the study

This study entails whether metformin and artemether-lumefantrine have effects on blood parameters of diabetic mice co-infected with the malaria parasite. The blood coagulation and packed cell volume are parameters and factors that will be used for study determination.

1.5 Significance

Adding to its metabolic characteristics, metformin treatment has been connected with reduction in coagulation proteins (Grant, 2003), it has also been reported to improve impaired fibrinolytic activity (Hamilton *et al*. 2007). Artemether is pre-scribed as the first line of anti-malaria treatment because of its efficacy in rapid reduction of parasitemia, acceleration of recovery, and is believed to decrease the likelihood of drug resistance development (White, 2008). However there is insufficient knowledge about its effect on blood coagulation.

This study will provide information on the modulatory effects of metformin, artemether-lumefantrine on blood coagulation parameters, packed cell volume in diabetic mice co-infected with malaria parasite.

CHAPTER TWO
LITERATURE REVIEW

2.1 General overview of Diabetes

Diabetes mellitus is a metabolic abnormality categorized by hyperglycaemia and also associated with high mortality and morbidity (Daneman, 2001). This condition results as an effect of interrupted metabolism of carbohydrates, proteins, and fats, due to insufficient or inept activity of insulin (American Diabetes Association, 2009). Diabetes is observed as a public health challenge, with a worldwide population of about 463 million people and about 1.5 million deaths reported in 2019; a global population of over 750 million people with diabetes has been estimated for 2045 (WHO, 2021). Nigeria is considered to be one of the most affected countries with the disease in Africa with about 2.7 million people living with diabetes (Uloko *et al*., 2018**).**

Although the categorization of diabetes is important and has suggestions for the management techniques of the disease, this is considered to be quite a difficult task as many patients do not purely fall under a distinct class especially younger adults (Rosenbloom *et al*. 2009) and a research has stated that a re-assessment may be required on an estimated 10% of those initially classified (Cakan *et al*. 2012). The mainstream classification of diabetes as put forward by the American Diabetes Association (ADA) in 1997 as type 1, type 2, other types, and gestational diabetes mellitus (GDM) is still the most commonly accepted categorisation and adopted by ADA (American Diabetes Association, 2014).

2.1.1 Type 1 diabetes

Type 1 diabetes is mainly characterised by deficiency of insulin consequential from pancreatic beta cells destruction, and may be related with acidosis or ketosis in children and adolescents (Albert & Zimmet, 2004). Also incorporated to the importance of genetic susceptibility in type 1 diabetes, numerous environmental factors are considered to be involved in the causality of the disease (Canivell & Gomis, 2014).

Type 1 diabetes most often develops unexpectedly and can produce clinical symptoms such as extreme fatigue, polyuria, polyphagia, polydipsia, recurrent infections, slow-healing wounds etc. (Aguiree *et al*. 2013).

Children with this metabolic abnormality present more severe symptoms in comparison to adults. Patients identified with autoimmune type 1 diabetes have been discovered to be susceptible to other autoimmune disorders such as Addison's disease, vitiligo, autoimmune hepatitis, Graves' disease (American Diabetes Association, 2014).

2.1.2 Type 2 diabetes

Based on a report issued by the International Diabetes Federation (IDF) in 2013 the world wide occurrence of diabetes in adults (20-79 years old) was 8.3% (382 million people), the majority ranging between the ages 40 and 59 years (Aguiree *et al*. 2013). In addition, the number is predicted to escalate above 592 million by 2035 with an approximated 10.1% global prevalence. With an estimate of 175 million cases still undiagnosed, the total number of individuals currently suffering from diabetes surpasses half a billion. The peak frequency of diabetes is observed in the Middle East and North Africa regions (10.9%), although, the Western Pacific region recorded the highest number of adults diagnosed with diabetes (138.2 million) it also comprises of countries with the highest prevalence (Aguiree *et al*. 2013). contains

Insulin resistance in patients diagnosed with type 2 diabetes increases the need for insulin in insulin-target tissues. With the inclusion of insulin resistance, the increase in demand for insulin could not be met by the pancreatic β cells due to deficiencies in the function of these cells (Halban *et al*. 2014).

In addition to diabetes, insulin resistance exhibits many clinical symptoms such that include systemic inflammation, critical hypertension, obesity, non-alcoholic fatty liver disease, etc. (Rosenbloom *et al*. 2009), sporadic moments of critical dehydration and the occurrence of ketoacidosis in some paediatric patients with diabetes type 2(American Diabetes Association. 2001) had led to the mis-categorization of diabetes type 2 to type 1.

2.1.3 Gestational diabetes

Hyperglycemia during pregnancy either in the form of type 2 diabetes diagnosed either before or during pregnancy or in the existence of gestational diabetes has an elevated risk of unpropitious maternal, fetal and neonatal outcome. Mothers diagnosed with gestational diabetes and infants born to such mothers have increased risk of developing diabetes later in

life. Hyperglycemia in pregnancy is responsible for the increased tendency to have macrosomia (birth weight ≥ 4.5 kg), large for gestational age births, preterm births and caesarean delivery due to large babies (HAPO Study Cooperative Research Group, 2008). Risk elements for gestational diabetes include family history of diabetes, maternal age, obesity, personal history of gestational diabetes, polycystic ovary syndrome, inactive lifestyle, and exposure to toxic factors (Galtier, 2010).

2.2 General overview of Malaria

Malaria disease is caused by unicellular micro-organisms of the *Plasmodium* group (WHO. 2014). The main method for transmission is through the bite of an infected Anopheles mosquitoe. The mosquito bite introduces the parasites from the mosquito's saliva into the bloodstream of the host (Caraballo & King 2014). The parasites mobilize to the liver where they develop and multiply. There are five known species of *Plasmodium* can impair and be spread by humans. Mortalities are majorly caused by *P. falciparum,* although *P. vivax, P. ovale,* and *P. malariae* normally cause a milder form of malaria. Malaria is classically diagnosed by microscopic reading of blood using blood films, or with antigen-based rapid diagnostic tests.

Occasional doses of the combination medication sulfadoxine/ pyrimethamine are prescribed in infants and after the first trimester of pregnancy in locations with high prevalence of malaria (WHO, 2014). As of 2020, there is one vaccine that has been shown to reduce the occurrence of malaria to about 40% in children in Africa (WHO, 2016). In areas where the disease is common, it is recommended that malaria be confirmed before treatment is started due to concerns of increasing drug resistance.

Major prevalence of the disease occurs in tropical and subtropical regions; this comprises much of Asia, Latin America and sub-Saharan Africa locations (WHO, 2014). In 2020, a study assessed there were about 241 million cases of malaria globally resulting in approximately up to 627,000 deaths. An estimated total of 95% of cases and deaths were prevalent in sub-Saharan Africa. Rates of the disease had decreased from 2010 to 2014 but progressed from 2015 to 2020 (WHO. 2021).

2.3 The Blood Coagulation Process

Blood coagulation is a process that involves conversion of circulating substances within the blood into an insoluble gel. The resulting gel seals leakages in blood vessels to reduce loss of blood (Favaloro & Lippi 2020). The process requires coagulation factors, calcium, and phospholipids. The coagulation factors (proteins) are synthesised by the liver, ionized calcium (Ca^{++}) is present in the blood and from intracellular sources, phospholipids are important elements of cellular and platelet membranes. They contribute a surface upon which the chemical reactions of coagulation can occur.

Coagulation can be initiated by either of two distinctive pathways:

Through the intrinsic pathway which is initiated by actions that occur within the lumen of blood vessels. Elements required for the extrinsic pathway are located within the vascular system which include clotting factors, Ca^{++}, platelet surface etc.

The other route to coagulation is through the extrinsic pathway. It involves the Tissue Factor (tissue thromboplastin), an element that does not ordinarily circulate in the blood vessel. Release of the tissue factor occurs in the case of a rupture in the vessel wall (Kasthuri *et al*. 2010).

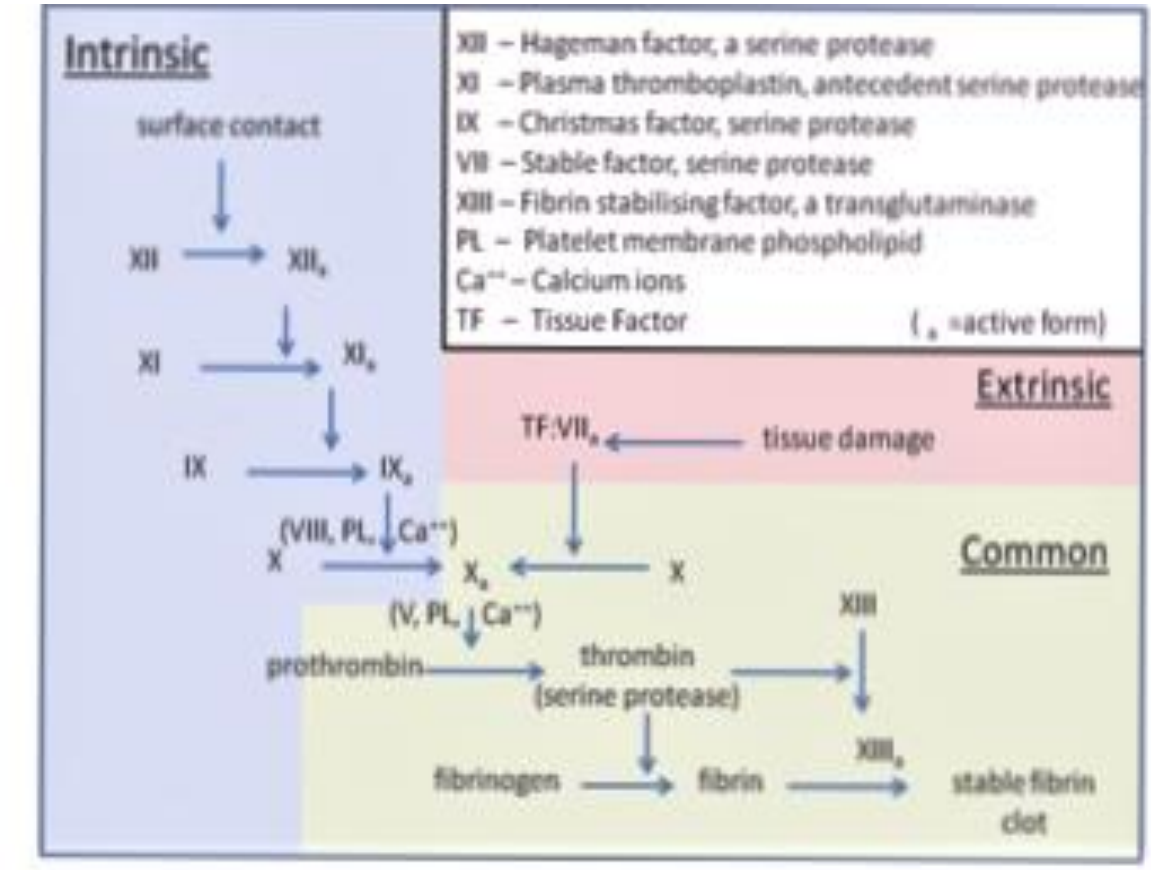

Figure 2. 3: Pathways of blood coagulation; intrinsic, extrinsic, and common pathway

The extrinsic coagulation or Tissue Factor (TF) pathway is initialized with activation of tissue factor/Factor VIIa (TF/FVIIa) complexes and plays an essential role in thrombus generation (Kasthuri *et al*. 2010). The intrinsic coagulation pathway entails successive activations of factors XI, XI and XII. Conversion of fibrinogen to fibrin is the last critical step in extrinsic and intrinsic coagulation pathways as shown in figure 2.1 (Neergaard-Petersen *et al*. 2014). Defects or irregularities in any one factor can hinder the general process, increasing the likelihood of haemorrhaging.

Coagulation factors and their common names include:

Factor I – fibrinogen, Factor II – prothrombin Factor III – tissue thromboplastin (tissue factor), Factor IV – ionized calcium (Ca^{++}), Factor V – labile factor or proaccelerin, Factor VI – unassigned, Factor VII – stable factor or proconvertin, Factor VIII – antihemophilic factor, Factor IX – plasma thromboplastin component, Christmas factor, Factor X – Stuart-Prower factor, Factor XI – plasma thromboplastin antecedent, Factor XII – Hageman factor, Factor XIII – fibrin-stabilizing factor (Michelson & Alan, 2006).

Breaks in atherosclerotic plaques exposes a prothrombotic element that comes into contact with platelets and coagulation factors. Primarily, platelets adhere to the location of the break become moderately stimulated. Factor VII conjoins to exposed tissue factor (TF) and produces a complex, which consequently stimulates Factors IX and X, resulting in Factor V activation. Activated Factors V and X adhere to prothrombin to synthesise partial amounts of thrombin, significantly enough to preserve platelet activation but deficient to support thrombus development. Platelet activation by thrombin and exposed collagen causes total and complete stimulation along with degranulation, in succession with Factor V release and activation by both thrombin and Factor Xa. Thrombin also initiates Factor VIII and this can develop into an active complex along with Factor IXa, which further enhances the coagulation procedure. Accordingly, an adequate amount of thrombin is produced for the conversion of soluble fibrinogen into a system of fibrin fibres. More stability of the clot is attained by thrombin-activated factor XIII which conjoins adjoining fibrin fibres and integrates other proteins such as α2-antiplasmin and fibronectin into the complex, as a result this increases the clot stability and resistance to lysis (Ariens *et al*. 2002). A resistance mechanism against thrombosis occurs as a result of synthetic anti- coagulants, such as protein C, which inhibits factors V and VII, and tissue factor pathway inhibitor that as a result blocks Tissue factor-VII coagulation initiation (Ajjan & Ariens 2009).

2.4 Blood coagulation disorders

Blood coagulation disorder is a disease that prevents blood from clotting normally, substances in the blood is transformed from liquid to solid during the clotting event, known as coagulation. In the scenario of an injury, the blood typically initiates clotting to prevent massive blood loss. Certain diseases might cause blood to clot improperly, resulting in excessive or persistent bleeding (Kahn *et al*. 2018). It can lead to irregular bleeding either external or internal. Some diseases cause a significant increase in the volume of blood that leaves the body for example when a person has a cut on the skin. Other ailments can initiate bleeding beneath the skin or in vital organs such as the brain. Some of the best known coagulation disorders include are Von Willebrand disease, Hemophilia other examples include deep vein thrombosis, clotting factor deficiencies and hypercoagulable states (Kahn *et al*. 2018).

2.5 Blood coagulation in diabetes

Diabetes has been described as a procoagulant state (Lemkes *et al*. 2010), micro and macro vascular difficulties in diabetes are connected with increased platelet activation, irregular function of the vascular endothelium and altered coagulation system. Following are the abnormalities associated with the state of coagulation & fibrinolytic system and its laboratory parameters in diabetes mellitus.

2.5.1 Increased levels of coagulation factors

Some research works have reported defects of Factors XII, XI, VIII, Kallikrein and von Willebrand of the intrinsic pathway linked with hyperinsulinemia and hyperglycemia (van der Toorn *et al*. 2019) and elevation factors of extrinsic pathway i.e. tissue factor and factor VII are elevated in patients with diabetes mellitus (Boden, Rao, 2007). Diabetic vascular difficulties have been found to be associated with increased factor V activity. Similarly, an increase in the activity of factor VII has also been reported in patients diagnosed with diabetic nephropathy (Erem *et al*. 2005). In diabetes, plasma fibrinogen levels have been found to be both the most consistently irregular clotting factor and also the best researched. Increased plasma fibrinogen levels have been regularly diagnosed in patients with diabetes mellitus (Carr, 2001).

2.5.2 Glycosylated fibrinogen

It has been reported that fibrinogen becomes hyper glycosylated in the presence of hyperglycemia (Pieters *et al*. 2007). When fibrinogen is stimulated in this state, the subsequent fibrin structure is comprised of fibers small in diameter (Carr, 2001). These small diameter fibers are specifically impervious against the degradative action of plasmin. Plasmin is less effective against this type of hyper glycosylated fibrinogen.

The degree of hyperglycosylation is directly proportional to the dissolution of the clot (Weisel, 2007); when fibrinogen is converted to fibrin by the action of thrombin, fibrinopeptide A (FPA) is released, with the resultant increase in its level during clotting. Measurement of FPA in diabetes shows conflicting results; elevated (Roshan *et al*. 2000) and normal (Bae *et al*. 2003) FPA levels have been observed during different studies in patients with diabetes.

2.5.3 Coagulation studies in diabetes mellitus

Conflicting results of prothrombin time (PT) and activated partial thromboplastin time (APTT) test have been observed in patients diagnosed with diabetes mellitus. Shorter PT and aPTT indicate the presence of activated coagulation factors *in vivo* (Pomero *et al*. 2015). Conversely, normal PT in patients with type 2 diabetes mellitus has also been observed (Erem *et al*. 2005).

2.5.4 Coagulation Activity in Malaria

A number of research studies with humans and animals have indicated that there is undeniably increased coagulation action in malaria. Majorly found to occur in uncomplicated (mild) cases (Grobusch & Kremsner 2005) and although considered not to be very clinically significant, it can be established as an equilibrium condition according to *in vivo* coagulation tests (Angchaisuksiri, 2014). Previous research works have confirmed that the extent of coagulation derangement was often connected to the severity of the disease (Ifeanyichukwu & Esan 2014).

Additionally, in other studies, it corresponds with parasitemia levels (Rana & Tanveer 2004). The fact that apparent Disseminated Intravascular Coagulation (DIC e.g., bleeding) is absent in patients diagnosed with severe malaria does not exempt an active role for the coagulation cascade in malaria pathogenesis.

2.5.5 Fibrin and Malaria

The actuality that fibrin has been displayed in some instances (Looareesuwan *et al*. 2009) but inconsistently found (Clark *et al*. 2003) or undetectable (Rakotoarivelo *et al*. 2012) in others, could be clarified by the fact coagulation initiation is asissted by compensatory fibrinolysis (Dempfle, 2004). Some other mechanisms may also be included, such as increased levels of neutrophil elastase (which degrade fibrin-stabilization factor XIII) which is located in the plasma of patients diagnosed with malaria (Hemmer *et al*. 2006).

A final integral feature associated to the coagulation cascade in the concept of malaria pathogenesis is physiologic alterations in the plasma level of coagulation factors and inhibitors that are apparent in children (Parmar *et al*. 2006) and also during the course of pregnancy (Franchini, 2006). In certain populations, which are have an increased tendency for developing severe malaria (Greenwood *et al*. 2005), there is reduced fibrinolytic activity,

which results in a hypercoagulable condition. While these changes are physiologic (Franchini, 2006), they may however influence a procoagulant tonus in pregnant women and children that may give rise to a severe disease (Von Seidlein *et al*. 2012).

2.6 Metformin

Metformin is an oral anti-hyperglycemic agent described to diabetic patients for its glucose lowering effect (Ferrannini, 2014) and also its ability to improve insulin sensitivity (Kaul *et al*. 2015).

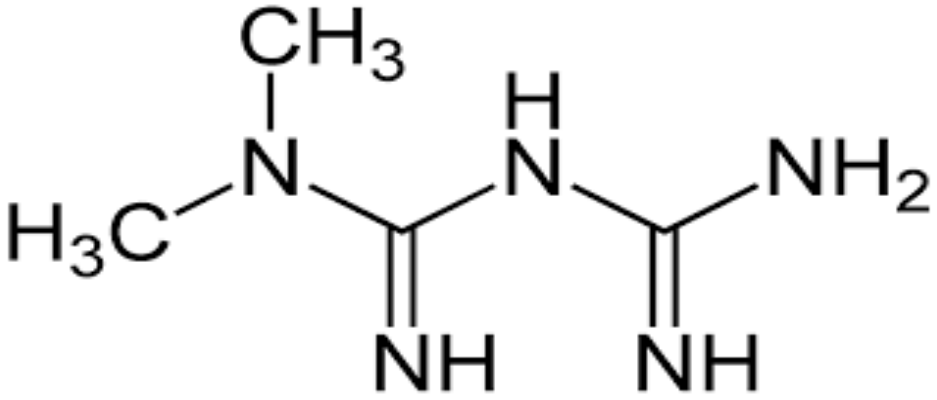

Figure 2. 4: Molecular structure of metformin (Rizvi *et al*. 2015)

2.7 Artemether-Lumefantrine drug

Artemisinin-based combination therapies (ACTs) are referred to as the preferred treatment of falciparum malaria world-wide, because they effeciently reduce parasitaemia, accelerate recovery, and lower the possibility of developing drug resistance (White, 2008). Of the obtainable ACTs, artesunate-amodiaquine (AA) and artemether-lumefantrine (AL) appear to be the most generally used (Sowunmi *et al*. 2007).

2.8 Infection and Circulating Blood Glucose Level

The role of infection in the regulation of blood glucose is complex. The exact effect depends on the infectious agent, the extent of the infection, the immune status as well as the immune response to the infectious agent. Although the existence of some contaminant agents such as the Plasmodia are separately linked with elevated glucose level through insulin resistance (Acquah *et al*. 2014).

2.9 Principles of Assay

2.9.1 Packed Cell Volume

Principle: This is a measurement of the proportion of blood that is made up of cells.

On the tail, a standard incision is performed. Blood is collected with a capillary tube and sealed. Capillary tube is centrifuged with a hematocrit centrifuge. The hematocrit is then read using a hematocrit reader. This measures percentage of red blood cells present in total blood volume (Bull *et al*. 2000).

2.9.2 Prothrombin Time

Prothrombin Time is an indicator of the extrinsic blood coagulation mechanism. Deficiencies of prothrombin and co factors (V, VII and X) give rise to a prolonged clotting time. Activation time is proportional to concentration of individual clotting factors involved in the coagulation cascade. This assists in estimating cause & extent of haemorrhagic disorder.

2.9.3 Activated Partial Thromboplastin Time

Activated Partial Thromboplastin Time is an appropriate method for detecting coagulation abnormalities in the intrinsic and common pathway; it is sensitive to deficiencies or abnormalities of co-factors (II, VIII, IX, X, XI, and XII) and fibrinogen degradation products. Prolongation of aPTT is generally related to decreasing of one or more intrinsic co-factors, by functional deficits (liver disease), and the presence of inhibitors (Levi & Sivapalaratnam 2019).

2.9.4 Fibrinogen Test

Thrombin converts soluble fibrinogen into insoluble fibrin, which when cross-linked forms a fibrin clot as the last step in the coagulation cascade. High fibrinogen levels are associated with atherosclerotic cardiovascular diseases, also in response to physiological stimuli such as tissue inflammation or injury. Reduced fibrinogen levels are prevalent in liver disease, malnourishment, disseminated intravascular coagulation etc.

CHAPTER THREE
METHODOLOGY

3.1 Materials and Chemicals

The reagents used for the study were all analytical grades.

Volumetric flask, weighing balance, dropper, test tubes, test tube racks, beaker, measuring cylinder, spatula, pH meter, ice bath, water bath, hand gloves, distilled water, chloroform, dessicator, sodium citrate, glucose, glucometer, syringe, aluminium foil, sodium chloride, methanol, glass slide, pin, microscope, Giemsa stain, micropipettes and tips, Eppendorf bottles, Hematocrit centrifuge (Jintan zhengji instruments co. ltd, China), capillary tubes, plasticine, dissecting kit and tray.

Artemether-Lumefantrine and Metformin were obtained from Fidson pharmaceuticals, Nigeria. STZ was obtained from ApexBio technologies (USA), reagent kits for activated partial thromboplastin time (aPTT), and Fibrinogen was obtained from Fortress Diagnostics Limited (United Kingdom). Prothrombin time (PT) was purchased from Agappe Diagnostics (India).

3.2 Study Design: The study was carried out *in vivo* using mice model

3.2.1 Experimental Animals

The Animal Facility at Mountain Top University, Ogun, Nigeria provided fifty four male BALB/C mice for the study. The mice were kept in cages at Mountain Top University's Animal Facility, where they were fed a regular rat diet and had unlimited access to water. They were acclimatized for two weeks.

3.2.2 Grouping of Experimental Animals

Experimental animals were randomly allocated into 8 groups as described below:

Group 1: Normal control group

Group 2: Diabetes only group

Group 3: Malaria only group

Group 4: Malaria and Diabetes only group

Group 5: Malaria treated with Artemether-Lumefantrine (6.86 mg/kg).

Group 6: Diabetes treated with metformin drug (200 mg/kg).

Group 7: Diabetes and Malaria treated with metformin drug (200 mg/kg).

Group 8: Diabetes and Malaria treated with metformin and Artemether-Lumefantrine (200/6.86 mg/kg respectively).

3.3 Fasting Blood Glucose

The mice were subjected to overnight fasting. By the following day (at most 12 hours after animals had last eaten), their blood sugar level was checked by collection of blood from the tail vein which was placed on a glucose strip and read using a glucometer (Accu-chek).

3.4 Malaria Parasite

Malaria Parasite (*Plasmodium berghei* NK65) was obtained from Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria.

3.5 Diabetes induction

Mice selected for diabetes induction had food removed from their cages prior to STZ treatment the next day. Blood glucose level was checked as a baseline, by blood collection from the tail and measured using a glucometer. Diabetes was induced by intraperitoneal injection of streptozocin (STZ; 40 mg/kg body weight) in sodium citrate buffer (pH 4.5) for 5 consecutive days. Hyperglycemia was established at blood glucose level ≥150 mg/dL (Furman, 2021).

3.6 Inoculation of mice

Infected mouse was bled by cardiac puncture with heparinised syringe. The blood was diluted immediately with normal saline to provide the desired dose per injection. On the 14^{th} day of the experimental setup, all groups to be infected were injected with 0.2 mL of malaria parasite inoculum intraperitoneally using an insulin syringe of 1.0 mL gauge needle.

3.7 Preparation of Thin Blood Films

Blood was taken from the tail every four days. A drop of blood was placed on the edge of a clean microscopic slide, spread across to a length of about 5cm, and left to dry. The cells were then fixed with absolute methanol and stained with prepared Giemsa stain solution. It was stained for about 15 minutes, rinsed with running tap water, and allowed to dry.

3.8 Determination of Percentage (%) Parasitaemia

The method estimates the percentage of red blood cells (RBC) infected with malaria parasites. The prepared thin blood films were carefully viewed under the microscope using a high magnification with oil immersion for intracellular stages of the *Plasmodium berghei* to estimate the number of non-parasitized and parasitized RBCs. At least a total of 800 cells were counted from the blood film to calculate the percentage parasitaemia.

$$\text{PARASITAEMIA (\%)} = \frac{\text{Number of parasitized RBC} \times 100}{\text{Total number of RBC}}$$

3.9 Determination of Packed Cell Volume (PCV)

Small volume of blood was collected from the tip of the animal tail (tail tip amputation) into a heparinised capillary tube. The capillary tube was spun for ten minutes using haematocrit centrifuge to separate the blood into plasma and packed cell. The percentage of packed cells was determined using a haematocrit reader.

3.10 Preparation of Blood Plasma

The collected blood sample was transferred into centrifugation tubes containing 3.2% sodium citrate which was used for blood coagulation assays (9:1). The plasma was separated. Plasma from the same group was pooled together and then kept at 4^{o}C in the refrigerator until use.

The blood samples were centrifuged at 2500 revolutions per minute (RPM) for 10 minutes using Thermo Scientific Centrifuge (Heraeus Megafuge 8). The plasma samples were aspirated using a Microflux pipette into dry, clean sample bottles and were stored frozen at (-4^{0} C) overnight. The homogenates obtained were centrifuged at 3000 revolutions per minute (RPM) for a period of 10 minutes to obtain the supernatants which were then gently collected into sample bottles, stored frozen (4^{0} C) overnight before being used for the various assays.

3.11 Assay Methods

3.11.1 Prothrombin Time

The prothrombin time of mice was determined following the manufacturer's instruction in kit purchased from Agappe Diagnostics (India).

Procedure prothrombin time reagent was reconstituted and pre-warmed at 37^{o}C for four minutes. Plasma of the mice (0.5 mL) was pipetted into a test tube and incubated at 37^{o}C for three minutes. 100 µL of pre-warmed prothrombin time reagent was forcibly added, while the timer was started. The time of clot formation was recorded in seconds.

3.11.2 Activated Partial Thromboplastin Time (aPPT)

The assay was done using assay kit purchased from Fortress diagnostics (United Kingdom) according to manufacturer's method.

Procedure: $CaCl_2$ was pre-incubated at 37^{o}C for ten minutes. 100 µL of plasma sample was pipetted into a test tube & placed in a water bath. 100 µL of aPPT reagent was added to the sample, gently mixed & incubated at 37^{o}C for 5 minutes. 100 µL of pre-incubated $CaCl_2$ was forcibly added while the stopwatch was started simultaneously. The test tube was gently tilted back and forth till a clot was formed. The timer was stopped, and time was recorded in seconds.

3.11.3 Fibrinogen test

The assay was done using assay kit purchased from Fortress diagnostics (United Kingdom) according to the manufacturer's instructions.

Procedure: A serial dilution of 1:10 was made of plasma sample to Imidazole buffer and placed in a test tube. The test tube containing the plasma sample and Imidazole buffer was incubated in a water bath at 37^{o}C for 3 minutes. Then,100 µL of the diluted and pre-warmed sample was pipetted into a test tube. 50 µl of thrombin reagent was added to the pre-warmed solution while the stopwatch was simultaneously timed. Clotting time was recorded in seconds.

3.12 Waste Disposal

The rat carcasses were buried at the designated burial site, while sample bottles containing unused blood and other biological samples were incinerated.

3.13 Statistical Analysis

Data obtained from the study was statistically analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple comparisons (post-hoc) using GraphPad prism 9.2.0. Results were expressed as a mean ± standard error of mean (SEM). P values less than 0.05 ($p < 0.05$) were considered statistically significant.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Effect of metformin and artemether-lumefantrine on body weight of diabetic mice co-infected with malaria

Presented in figure 4.1 is the effect of metformin and artemether-lumefantrine on body weights of diabetic mice co-infected with malaria. The peak values were located in group 5 (Malaria + AL) and 6 (Diabetes + MTF) on days 17 and 21 respectively. Also on Day 21, group 7 (Malaria + Diabetes + MTF) recorded the lowest value. Group 1 (Normal Control) in contrast with other groups showed no difference in weight, excluding group 5 (Malaria + AL) which received treatment on days 17 and 21 (p= 0.039 and 0.017 correspondingly). In addition, the results demonstrate that the weight of group 2 (Diabetes only) was quite low compared to group 6 (Diabetes + MTF). The weights of Group 3 (Malaria only) and Group 7 (Malaria + Diabetes + MTF) showed a significant difference in their weights (p = 0.038) on Day 17.

Groups 5 (Malaria + AL) & Group 4 (Malaria + Diabetes) exhibited a statistically significant weight increase (p= 0.041) on Day 17. Group 5 (Malaria + MTF) and Group 7 (Malaria + Diabetes + MTF) showed a statistical significant lowered weights on days 17 (p = 0.006) and 21. Group 6 (Diabetes + MTF) in contrast to Group 7 (Malaria + Diabetes + MTF) displayed a significant increment in weight on days 17 & 21 (p = 0.026 and 0.009 respectively). Weight of mice in group 8 (Malaria + Diabetes + MTF + AL) was significantly reduced compared to that of group 6 (Diabetes + MTF) on day 21.

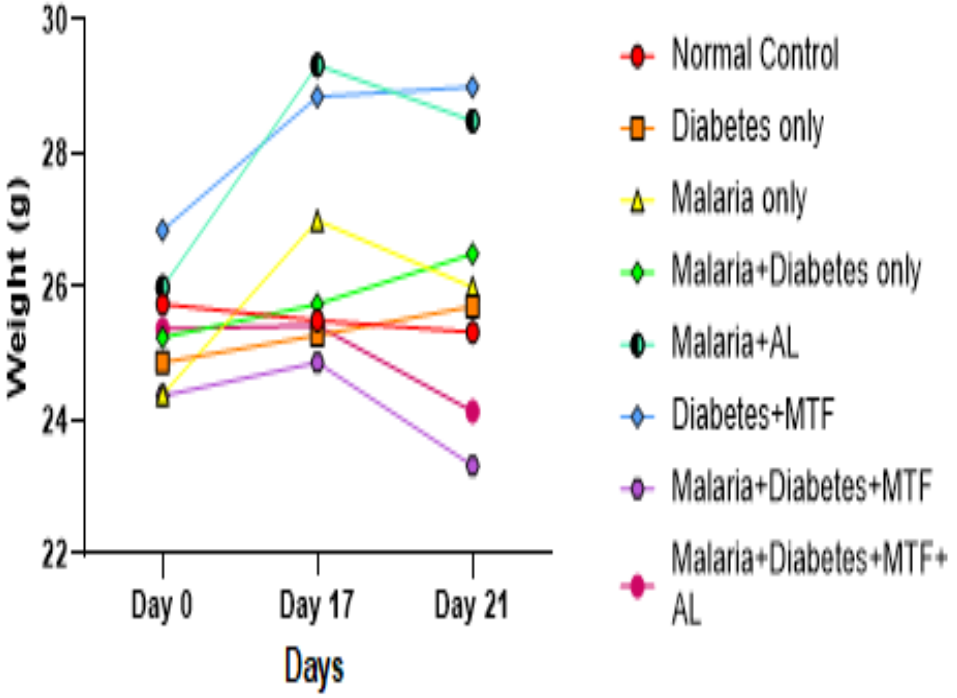

Figure 4.1: Body weights of experimental mice in grams on day 0, day 17 and day 21

Data are represented as n = 8. A/L = Artemether-Lumefantrine, MTF = Metformin.

4.2 Effect of metformin and artemether-lumefantrine on blood glucose level of diabetic mice co-infected with malaria

Presented in figure 4.2 is the effect of metformin and artemether-lumefantrine on blood glucose levels of diabetic mice co-infected with malaria. Group 8 (Malaria + Diabetes + MTF + AL) exhibited the peak mean value while group 3 (Malaria only) showed the least value on day 21. Group 1 (Normal Control) in comparison to all other groups showed no significant difference in fasting blood glucose levels. Groups 2 (Diabetes only) and 6 (Diabetes + MTF) displayed a significant increase in fasting blood glucose levels on day 0 and 14 ($p = 0.002$ and 0.005 correspondingly). Groups 2 (Diabetes only) and 8 (Malaria + Diabetes + MTF + AL) showed a significant difference in fasting blood glucose levels on days 14 & 21 ($p = 0.011$ and 0.022 respectively).

Group 4 (Malaria + Diabetes only) showed considerably higher fasting blood glucose levels compared to group 3 (Malaria only) on Day 21 ($p = 0.014$). Fasting blood glucose of group 5 (Malaria + AL) in contrast to that of group 3 (Malaria only) showed a significant increment in its levels ($p = 0.020$) on day 21. Fasting blood glucose of group 8 (Malaria + Diabetes + MTF + AL) when compared to group 3 (Malaria only) showed exceptional significance on day 21 ($p = 0.017$). On day 0 ($p = 0.005$), fasting blood glucose values in group 4 and 6 was significantly increased on day 14 ($p = 0.027$). Group 4 (Malaria + Diabetes only) in comparison to group 8 (Malaria + Diabetes + MTF + AL) had a significantly increased fasting blood glucose from day 0 to 14 ($p = 0.001$ and 0.018).

On day 21, group 5 (Malaria + AL) and group 8 (Malaria + Diabetes + MTF + AL) showed a significant reduction in fasting blood glucose levels ($p = 0.015$). Group 6 (Diabetes + MTF) and Group 7 (Malaria + Diabetes + MTF) displayed a significant increase in fasting blood glucose levels from day 0 ($p = 0.027$) to day 14 ($p = 0.043$). Group 6 mice (Diabetes + MTF) in comparison to group 8 (Malaria + Diabetes + MTF + AL) had a significant reduction in fasting blood glucose from day 0 ($p = 0.002$) through day 21 ($p = 0.025$).

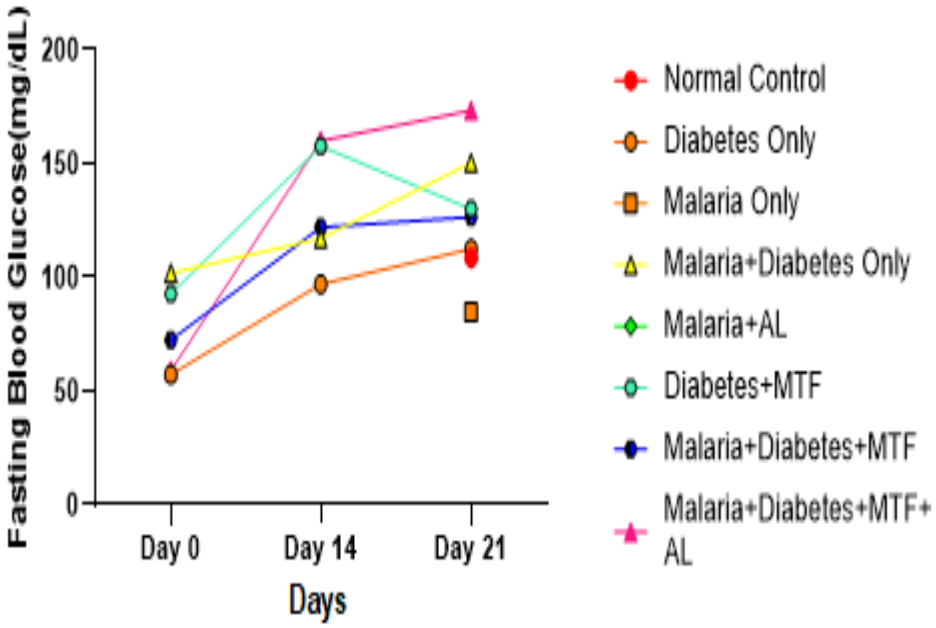

Figure 4.2: Fasting blood glucose (mg/dL) of experimental mice on days 0, 14 and 21 Data are represented as n = 8. A/L = Artemether – Lumefantrine, MTF = Metformin.

4.3 Effect of metformin and artemether-lumefantrine on parasitaemia count of diabetic mice co-infected with malaria

Presented in figure 4.3 is the effect of metformin and artemether-lumefantrine on parasitaemia count of diabetic mice co-infected with malaria. Group 7 (Malaria + Diabetes + MTF) was found to have the peak parasitaemia count reading when compared to other groups on Day 21/D7 PI/72H PT, while group 8 (Malaria + Diabetes + MTF + AL) was found to have the least reading on the same day. Results showed there was no observable difference in parasitaemia count between group 3 (Malaria only) and all other groups, excluding group 7 (Malaria + Diabetes + MTF) which was observed to have a considerably higher parasitaemia count on Day 21/D7 PI/72H PT ($p = 0.046$). Group 8 mice (Malaria + Diabetes + MTF + AL) were observed to have significantly lower parasites than group 3 (Malaria only) between Day 20/D6 PI/48H PT and Day 21/D7 PI/72H PT (p = 0.014 and 0.017 correspondingly). Group 5 (Malaria + AL) mice were found to have a significantly reduced parasitaemia count than group 4 alone (Malaria + Diabetes only) on Day 21/D7 PI/72H PT ($p = 0.017$). Similar parasitaemia counts were observed for group 8 (Malaria + Diabetes + MTF + AL) in comparison to group 4 (Malaria + Diabetes only) on Day 20/D6 PI/48H PT and Day 21/D7 PI/72H PT (p = 0.007 and 0.002 respectively).

Group 7 (Malaria + Diabetes + MTF) was found to have a significantly higher parasitaemia count than group 5 (Malaria + AL) on Day 21/D7 PI/72H PT (p = 0.020). On day 17/D3 PI and day 21/D7 PI/72H PT groups 8 (Malaria + Diabetes + MTF + AL) and group 5 (Malaria + AL) were observed to have significantly lower parasitaemia counts (p = 0.036 and 0.034 correspondingly). Group 8 (Malaria + Diabetes + MTF + AL) also when compared to group 7 (Malaria + Diabetes + MTF) was discovered to have a reduced parasitaemia count on day 19/D5 PI/24H PT, day 20/D6 PI/48H PT and day 21/D7 PI/72H PT (p = 0.028, 0.010 and 0.002 respectively).

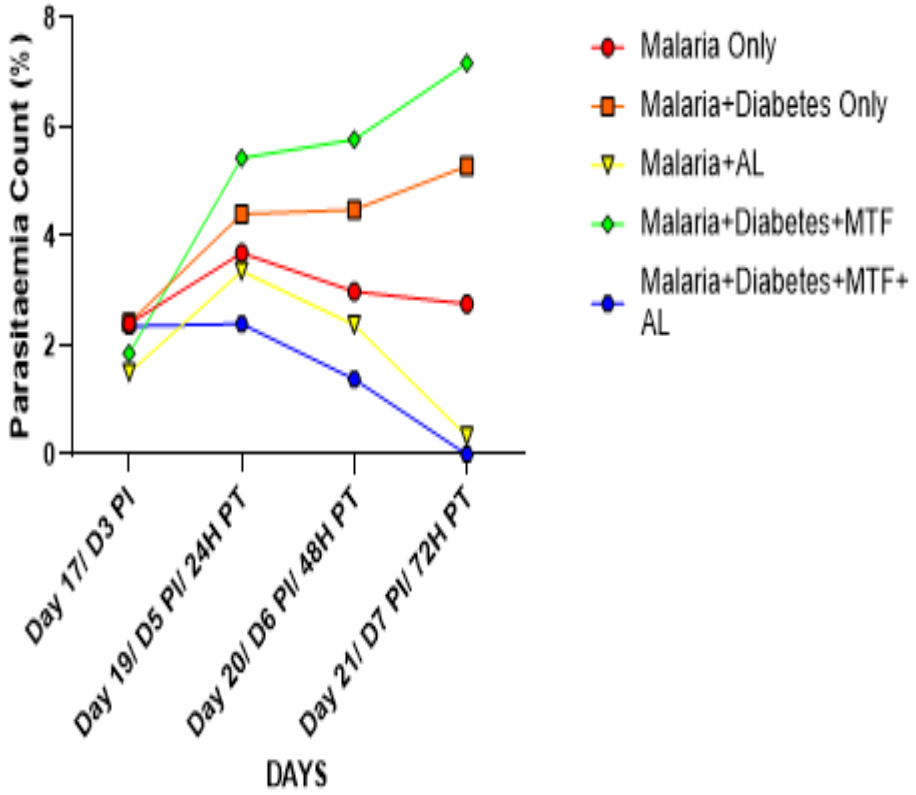

Figure 4.3: Parasitaemia count (%) of Experimental mice on Day 3 PI, day 5 PI/24H PT, day 6 PI/48H PT, day 7 PI/72H PT

Data are represented as n = 5.

D3: Day 3, D5: Day 5, D6: Day 6, D7: Day 7, H: Hour, PI: Post Induction, PT: Post Treatment, A/L = Artemether –Lumefantrine, MTF = Metformin.

4.4 Effect of metformin and artemether-lumefantrine on packed cell volume of diabetic mice co-infected with malaria

Presented in figure 4.4 is the effect of metformin and artemether-lumefantrine on packed cell volume of diabetic mice co-infected with malaria. Group 3 (Malaria only) was observed to have the highest packed cell volume reading from day 0 to day 18, while group 7 (Malaria + Diabetes + MTF) was seen to have the lowest readings between those days. While from days 18 to 21, group 6 (Diabetes + MTF) was seen to have the peak packed cell volume readings and group 4 (Malaria + Diabetes only) had the lowest readings on the same days.

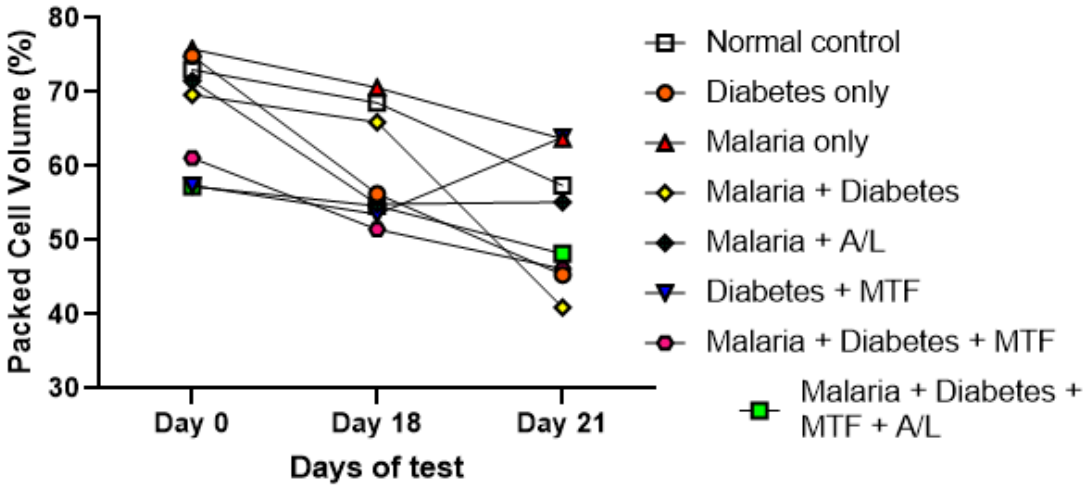

Figure 4.4: Graphical illustration of packed cell volume of experimental mice on day 0, 18 and 21

Data are represented as n = 8.

DB = Diabetes, Mal = Malaria, A/L = Artemether – Lumefantrine, MTF = Metformin.

4.5 Effect of metformin and artemether lumenfantrin on prothrombin time in diabetic mice co-infected with malaria

Presented in figure 4.5 is the effect of metformin and artemether lumenfantrin on prothrombin time in diabetic mice co-infected with malaria. From the results it was observed that group 5 (Malaria + AL) had the longest clotting time, while group 1 (Normal Control) had the shortest time. Group 3 (Malaria only) was observed to have a significantly lower clotting time compared to group 5 (Malaria + AL) (p = 0.0097)

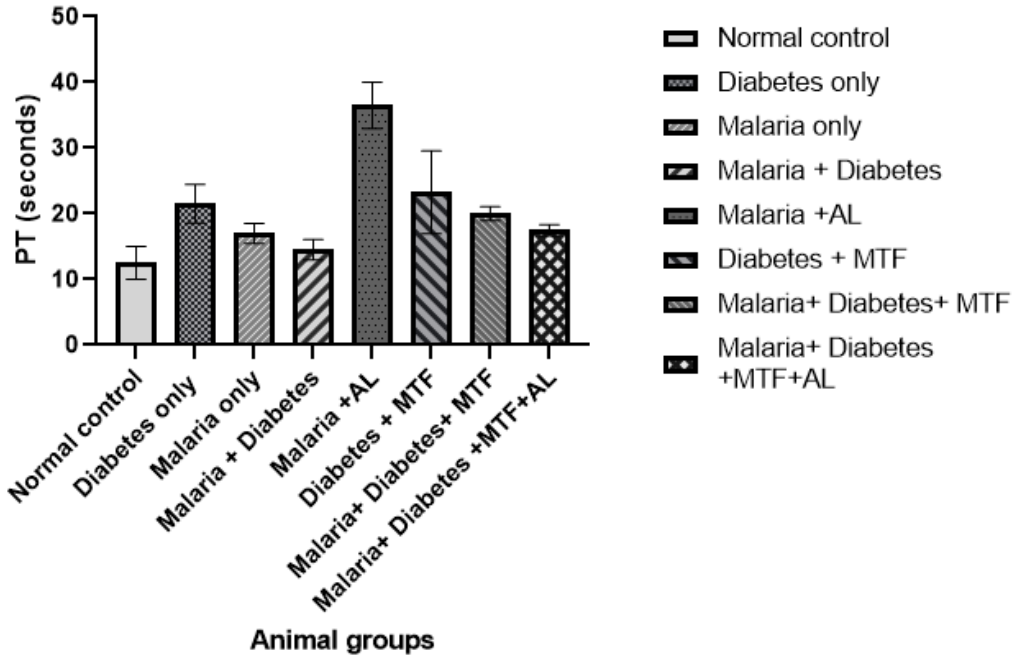

Figure 4.5: Prothrombin time of control and test groups of experimental mice

Data are presented as Mean ± SEM; n=8

AL: Artemether-Lumefantrine, DB: Diabetes, Mal: Malaria, MTF: Metformin

4.6 Effect of metformin and artemether lumenfantrin on activated Partial Thromboplastin Time (aPPT) in diabetic mice co-infected with malaria

Presented in figure 4.6 is the effect of metformin and artemether-lumefantrine on activated partial thromboplastin time in diabetic mice co-infected with malaria. Group 4 (Malaria + Diabetes) and 5 (Malaria + AL) were found to have the shortest clotting time compared to all other single and co-infected groups, while group 1 (Normal control) was found to have the longest clotting time of all the groups.

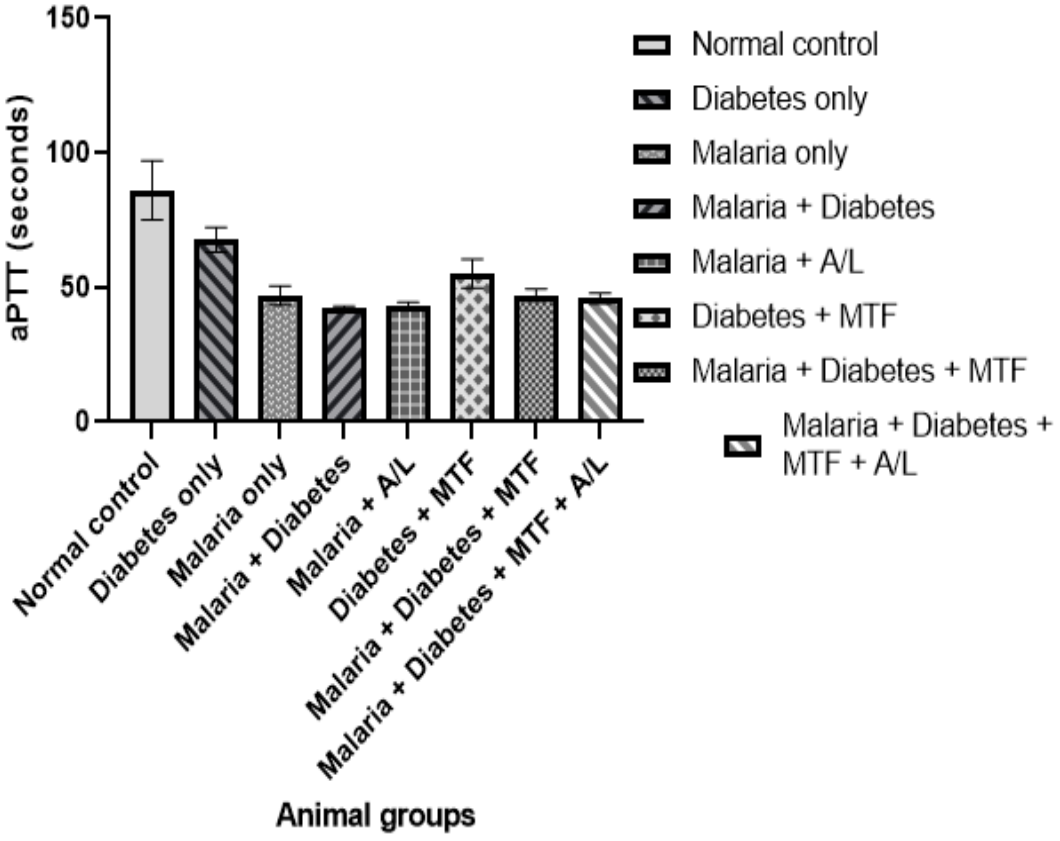

Figure 4.6: Activated Partial Thromboplastin Time (aPTT) of control and test groups of experimental mice.

Data are presented as Mean ± SEM; n=8

AL: Artemether-Lumefantrine, DB: Diabetes, Mal : Malaria , MTF: Metformin

4.7 Effect of metformin and artemether-lumefantrine on plasma Fibrinogen time in diabetic mice co-infected with malaria

Presented in figure 4.7 is the effect of metformin and artemether lumenfantrin on fibrinogen time in diabetic mice co-infected with malaria. Group 8 (Malaria + Diabetes + MTF + AL) was found to have the highest clotting time of all single and co-infected groups followed by group 4 (Malaria + Diabetes) while the shortest clotting time was found to be in group 1 (Normal control).

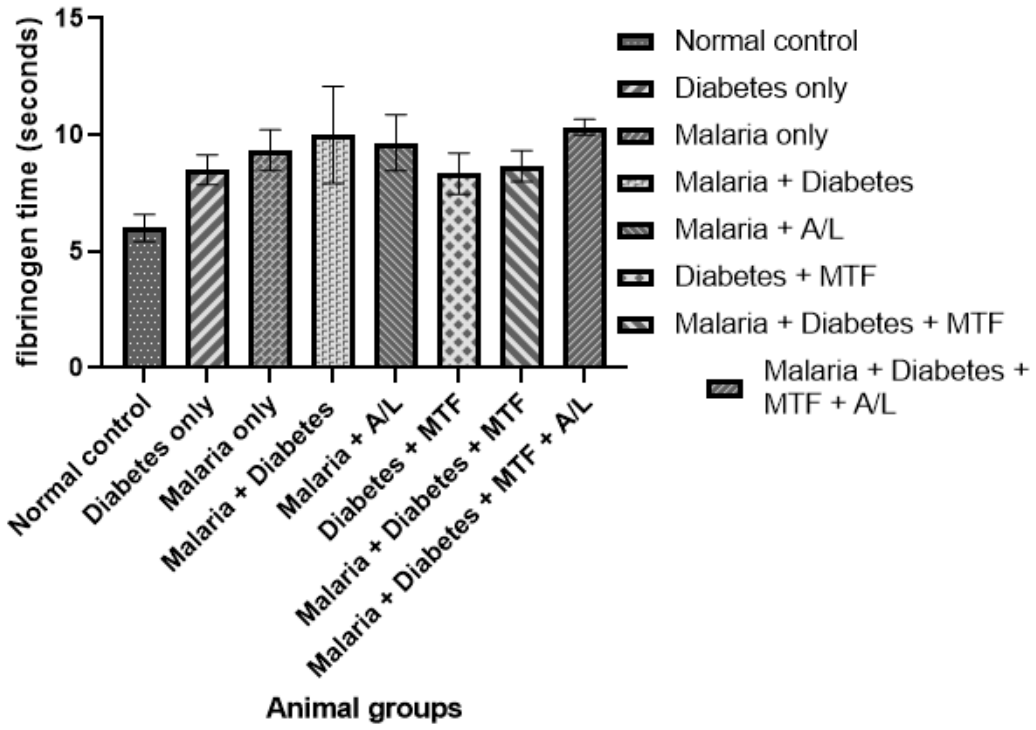

Figure 4.7: Fibrinogen time of control and test groups of experimental mice

Data are presented as Mean ± SEM; n=8

A/L: Artemether-Lumefantrine, DB: Diabetes, Mal : Malaria , MTF: Metformin

4.8 Discussion

This experiment studied the modulation of blood coagulation by metformin and artemether-lumefantrine in diabetic mice co-infected with malaria. Hyperglycemia is a metabolic state which occurs as a consequence of defective insulin action, secretion of insulin or possibly both (Craig *et al*. 2009). Malaria is a world-wide disease principally occurring in the tropical areas caused by protozoan parasites (genus *plasmodium*), the parasites are transmitted to humans by an infected female anopheles mosquito (Uloko *et al*. 2018).

Diabetes and malaria infection have consequences on the host weight as shown in the result of groups with singular infection and conditions of both diabetes and malaria infection. Additional probable causes for the reduction in body weight include the injection of streptozotocin which has been found to produce common diabetic symptoms including irregular food and water intake actions (Xue *et al*. 2017).

This study findings show that co-infection of diabetes and malaria has influence on the blood glucose and parasitaemia count of infected groups. Diabetic individuals are prone to be susceptible to other disease infections such as malaria, according to a similar study conducted by Ch'ng *et al*. 2021; a higher growth of malaria parasites was observed in diabetic patients. It can also be hypothesised that diabetes can aggravate the co-morbidity and complications of malaria. This was similarly reported in a study which reported that individuals infected with malaria parasites had an increased likelihood to develop severe malaria if also accompanied with diabetes (Wyss *et al*. 2017). It was also observed during the study that as fasting blood glucose increased the parasitaemia count similarly increased in comparison to the singular infected groups. The exact reason for the increase in malaria infection is uncertain; a probable explanation would be the increment in blood glucose which could be because of compromised defence against liver and/or blood stage parasites from extended persistence, contributing to the host developing insulin resistance. The results obtained from this study are in co-relation with Ch'ng *et al*. 2021 which surmised that a probable basis for the association between diabetes and severe malaria is that increased blood glucose levels stimulates parasite growth, which in turn leads to hyperparasitemia and severe malaria.

Metformin is a synthetic chemical substance which possess hypoglycemic activity (Nasri & Rafieian-Kopaei, 2014), it is regarded as the first-line drug in type 2 diabetes (T2D) treatment, it has also been evidenced to improve insulin sensitivity in type 1 diabetics (Kaul *et al*. 2015), this drug has also been found to have an anti-malaria effect; with its ability to reduce prevalence of malaria parasites (Jones & Ward 2002). During this study it was observed that the group treated with metformin showed a significant increase in its parasiteamia level which corresponds with the conclusion by Jones & Ward (2002).

According to the findings of this study, the group co-infected with diabetes and malaria while treated with metformin drug had an increased clotting in prothrombin time compared to the co-infected group that received no treatment, this clearly demonstrates the inhibition of extrinsic coagulation factors by metformin, which is supported in a study by Verdoia *et al*. 2021, which stated that metformin was able to reduce the coagulation factor activity. It was also observed during the study that the group induced with malaria and treated with arthemeter-lumefantrine had an increased time for clotting in prothrombin time compared to the malaria infected group which received no treatment, Francischetti *et al*. 2008 in a study stated that there is a definite increase in coagulation activity in malaria patients, occurring in uncomplicated cases and is sometimes regarded as not clinically significant (Grobusch & Kremsner 2005) as a result the effect of anti-malaria drugs on coagulation has not been properly looked into, this study was able to prove that artemether-lumefantrine has an anti-coagulant effect on malaria conditions and also an ability to inhibit extrinsic coagulation factors.

Findings of this study also observed the diabetic group treated with metformin had an increased time for clotting in activated partial thromboplastin time compared to the group that received no treatment and all other single and co-infected groups, this result goes against that of Xin *et al*. 2016 which stated that metformin was observed to have no significant effect on activated partial thromboplastin time in their study, the results are able to prove that metformin not only has an anti-coagulant effect but also an ability to inhibit intrinsic coagulation factors.

The final vital step in the extrinsic and intrinsic coagulation pathways is the conversion of fibrinogen to fibrin and higher circulating fibrinogen levels have been observed in diabetic patients, resulting in a more compacted clot structure along with increased resistance to fibrinolysis (Neergaard-Petersen *et al*. 2014). This study supports that of Francischetti *et al*. 2008 which describes malaria condition as a pro-coagulant state and some studies have

theorized the irregularity of the presence of fibrin (Clark *et al*. 2003) can be explained by the fact that coagulation initiation is supplemented by compensatory fibrinolysis (Dempfle, 2004). The group co-infected with both malaria and diabetes while receiving metformin treatment was found to have a lower fibrinogen time compared to the malaria diabetes co-infected group that received no treatment, this result is also in disparity with that of Xin *et al*. 2016 which stated that metformin appeared to have no significant influence on fibrinogen in their work.

Artemether-lumefantrine drugs are regarded as the recommended first-line treatment of falciparum malaria globally, due to their ability to their ability to rapidly decrease parasitemia, hasten recovery and reduce chances of drug resistance development (White, 2008).

A study by Clemens *et al*. 1994 stated that in uncomplicated malaria, the coagulation cascade is seen to be activated, however, the underlying mechanisms, and its wider pathophysiological role, remain uncertain. In a study on severe malaria both factor XII and its substrate, prekallikrein, were found to have been reduced significantly, insinuating activation of the intrinsic system. Activation of the intrinsic coagulation pathway is regularly associated with activation of the fibrinolytic system. Earlier studies in severe malaria have shown consistently elevated concentrations of fibrin degradation products, usually with increased levels of fibrinogen, suggesting increased fibrinogen turnover (Vreeken & Cremer-Goote, 1978).

Hyperglycemia and insulin resistance exert synergistic effects on the TF pathway in diabetic patients, leading to an increment in pro-coagulatory activity and FVIIa consumption (Boden & Rao, 2007), which corresponds with the results in this study as observed by the junction in clotting times of groups . The intrinsic coagulation pathway involves progressive activations of FXII, FXI and FIX, and recent studies have reported disfunctions of intrinsic coagulation associated with hyperglycemia and hyperinsulinemia in DM (van der Toorn *et al*. 2019), this is coincidental with results in this study as shown by the significantly reduced clotting time in diabetic infected groups indicating stimulation of intrinsic coagulation factors.

In addition, low-grade inflammation in diabetes has been found to directly stimulate hepatocytes to synthesize more fibrinogen (Yamaguchi *et al*. 2000). Modified quality of fibrinogen in diabetes patients has recently been demonstrated: an increased glucose level has been found to amplify glycation of fibrinogen and disrupts the fibrinolysis process, and these

alterations could be attenuated by tight glucose control (Pieters *et al*. 2007). Thrombin is derived from prothrombin and is an essential factor which converts fibrinogen into fibrin. In diabetic patients, increased thrombin levels lead to greater fibrin generation and clot density, thereby contributing to the pro-thrombotic state (Park *et al*. 2018). These earlier findings are also similar with results of this study stating that there was a significant increase in fibrinogen levels in groups diagnosed with diabetes as shown by the increment in fibrinogen clotting time.

CHAPTER FIVE

CONCLUSION AND RECCOMENDATION

5.1 Conclusion

The findings of this study suggest that while metformin is the first line drug with an anti-coagulant effect on diabetes, artemether-lumefantrine not only could cure *plasmodium falciparum* but also possesses an anti-coagulant effect by inhibiting factors of the extrinsic coagulation pathway via prolonged prothrombin time as observed in *Plasmodium*-infected rats treated with artemether-lumefantrine in this study.

5.2 Recommendation

Further study should be carried out to elucidate the possible mechanism for the prolonged effect of Artemether-lumefantrine on prothrombin time and the effect of metformin on fibrinolysis

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