EFFECTS OF DIABETES AND *Plasmodium berghei* INFECTION ON SOME IMMUNE AND BIOCHEMICAL PARAMETERS IN BALB/c MICE

BY

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DECLARATION

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

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ADELEKE, WISDOM ADEOLA

……..

DATE

CERTIFICATION

This is to certify that the content of this project entitled '**Effects of Diabetes and *Plasmodium berghei* Infection on Some Immune and Biochemical Parameters in BALB/c Mice'** was prepared and submitted by **ADELEKE, WISDOM ADEOLA** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOCHEMISTRY.**

The original project work was carried out by her under my supervision and is hereby accepted.

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This project is dedicated to God Almighty, to my parents Pastor Sunday Adeleke and Mrs. Joke Adeleke, and My brother, Joseph Adeleke and to everyone that contributed to the success of this project.

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ABSTRACT

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. Malaria is a life-threatening disease caused by protozoan parasites of the *Plasmodium* species that are transmitted by infected mosquitoes. Diabetes and Malaria are prevalent diseases in Nigeria. Malaria has been documented to be more common in diabetes, therefore it is essential to investigate the effects of the co-morbid conditions on immune and biochemical parameters.

Fifty- four male BALB/C mice were used in this study. The mice were grouped into eight groups, Group 1 (Normal control), Group 2 (Malaria only), Group 3 (Diabetes only), Group 4 (Diabetes + Malaria), Group 5 (Malaria + AL), Group 6 (Diabetes + MTF), Group 7 (Malaria + Diabetes + MTF) and Group 8 (Malaria + Diabetes + MTF + AL). Diabetes was induced using Streptozotocin (40 mg/kg) for 5 consecutive days while malaria was established by inoculating each mice with 0.2 mL of *Plasmodium berghei*. 200 mg/kg body weight of Metformin and 1.14/ 6.86 mg/kg body weight of Artemether Lumefantrine were used for treatment of diabetes and malaria respectively. This study showed a significant decrease in creatinine concentration of infected groups with treatment when compared to negative control groups. The results reveal that there was no significance in the Urea Concentration in the Control Groups (Diabetes Only, Malaria Only, Malaria + Diabetes Only) when compared across all other groups. The result of serum levels of TNF –α conducted in experimental mice revealed that there is no significant increase in the median concentration of TNF –α across all the groups when compared. The result of serum levels of interleukin-6 conducted in experimental mice revealed that there was a significant increase in IL-6 Concentration ($p=0.046$) in group 4 (Malaria + Diabetes Only) when compared with the Normal Control. The findings of this study provides evidence for increased risk for *P. berghei* infection in Diabetes and also showed that Metformin does not have a prophylactic effect on malaria treatment.

Keywords: Diabetes, Malaria, Tumor Necrosis Factor-α, Interleukin- 6, Creatinine, Urea.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Hyperglycemia (high blood glucose level) caused by errors in insulin production or irregularities in action can be a symptom of diabetes mellitus (DM), a set of metabolic diseases (Alhaider, 2011). Patients with high blood sugar generally have polyuria (frequent urine), as well as polydipsia and polyphagia (increased thirst and hunger) (Suresh, 2016). The homeostasis of the circulatory system is impacted by diabetes (Ayodele *et al*., 2020). More than 80% of the estimated 382 million individuals with diabetes who resided in low- and middle-income countries in 2013 (LMIC). Nigeria has the highest incidence of diabetes in sub-Saharan Africa, with an estimated 3.9 million cases (or an extrapolated prevalence of 4.99%) among adults aged 20 to 79. According to studies done in Nigeria, the prevalence of diabetes among adults ranged from a low of 0.8% among those living in rural highlands to over 7% in urban Lagos, with an average of 2.2% overall (Dahiru *et al*., 2016). According to estimates, 65.1 million individuals in India had diabetes in 2013, placing it in second place among the top 10 nations with the highest prevalence of the disease.

Additionally, diabetes increases the risk of developing heart disease, kidney disease, pneumonia, bacteremia, and tuberculosis (TB), all of which are morbidities (Dahiru *et al*., 2016). In affluent nations, diabetes mellitus is a significant contributor to cardiovascular morbidity and mortality, with atherothrombosis being the primary cause of death for those with the condition (Stratmann and Tschoepe, 2008).

Despite the crippling consequences of diabetes, the endemic nature of the disease in tropical areas enhanced the likelihood of cohabitation with other common infections like malaria. A potentially fatal parasite illness called malaria is spread by female Anopheles mosquitoes (Akande and Musa, 2005). *An. gambiae sensu stricto*, *An. funestus*, and *An. arabiensis* are the three species that are most common in Africa (Nkumama *et al*., 2017). The disease of malaria is really serious. Malaria, which affects 190–330 million people worldwide and is endemic in 109 countries, is the most common mosquito-borne disease, according to Akinneye and Afolabi (2014). It results in the death of roughly 1 million people each year.

In Nigeria, where malaria is endemic, it increases morbidity and death. The prevalence of malaria in Nigeria demonstrated seasonal fluctuation across a number of different geopolitical coverage areas (Ogundeyi *et al*., 2015). For instance, Ogundeyi *et al*. (2015) showed that the prevalence of malaria was 32.7% in the South-South, 36.6% in the South-West, 30.7% in the South-East, 58.8% in the North Central, 55.3% in the North East, and 33.6% in the North East (North West). Every year, malaria normally affects all age groups and around 50% of the population (Ogundeyi *et al*., 2015). Malaria disrupts metabolic and cellular function most likely as a result of liver damage brought on by *P. falciparum*'s exoerythrocytic form, which lives in the liver (Onyesom *et al*., 2012). Malaria is caused by infection with protozoan parasites of the *Plasmodium* species. *Plasmodium falciparum* is widespread in Africa while *P. vivax*, *P. ovale*, and *P. malariae* infections are less common and geographically restricted.

Malaria and diabetes mellitus are both prevalent and serious killer illnesses in poor countries (Ikekpeazu *et al*., 2010). However, (Acquah *et al*., 2014) pointed out that diabetes mellitus and malaria continue to affect millions of people worldwide, particularly in developing countries, and as such, diabetes mellitus and malaria can be considered as a global phenomenon, implying that developing nations separately or synergistically fight against diabetes mellitus and malaria. Due to this tendency and the endemic nature of malaria in sub-Saharan Africa, it is necessary to understand how diabetes mellitus and malaria interact in order to prevent any potential compounding health impacts (Acquah *et al*., 2014).

In developing countries like Nigeria, where both malaria and diabetes mellitus are common, it is necessary to evaluate the biochemical profile of diabetic patients who have malaria parasitemia in order to determine whether renal organ malaria infection may contribute to the pathophysiology of diabetes mellitus (Ikekpeazu *et al*., 2010). A rise in the incidence of diabetes mellitus might increase the number of people at risk of contracting malaria (Danquah *et al*., 2010).

1.2 Statement of the Problem

In 2014, 422 million individuals worldwide have diabetes. Since 1980, the prevalence of type-2 diabetes (T2DM) has increased by nearly doubling, from 4.7% to 8.5% (Sanjay, 2017).

Diabetes, especially when it is not well managed, includes innate immune system dysfunction and causes persistent low-grade inflammation. Numerous studies have shown that circulating

levels of inflammatory markers, such as C-reactive protein (CRP), sialic acid, and interleukin-6 (IL-6), are independent predictors of the future development of diabetes in people with impaired fasting blood glucose (Mirza *et al*., 2011). Furthermore, prior studies have linked obesity and insulin resistance to high levels of circulating acute phase proteins (IL-6), tumor necrosis factor-α (TNF- α), and other inflammatory mediators. Furthermore, several investigations have shown that people with clinically diagnosed diabetes have higher levels of IL-6 and TNF- α (Mirza *et al*., 2011). The rate of rise in type 2 diabetes mellitus is highest in Sub-Saharan Africa; adaption to Western lifestyles and genetic predispositions may quicken this trend. In urban Ghana, type 2 diabetes mellitus prevalence was 6.3% ten years ago (Danquah *et al*., 2010).

Globally, type 2 diabetes is the primary cause of albuminuria, reduced kidney function, and the need for renal replacement treatment, imposing a significant strain on health-care systems. According to current international guidelines, patients with type 2 diabetes who develop albuminuria (e.g., urinary albumin-to-creatinine ratio [UACR] >3 mmol/mol), a decrease in estimated glomerular filtration rate (eGFR) to less than 60 mL/min per 173 m2, or both, sustained over at least 3 months, are considered to have chronic kidney disease. Even when kidney histology has not been properly evaluated by biopsy, chronic kidney disease found in the setting of type 2 diabetes is typically referred to as diabetic nephropathy or diabetic kidney disease (Muskiet *et al*., 2018).

Similar startling figures can be seen with malaria. According to the WHO, there were 207 million cases of malaria worldwide in 2012, resulting in 6,270,000 fatalities. African countries contributed 80% of these cases followed by South East Asia Region (SEAR) (13%) (WHO, 2013). India contributes 61% of cases and 41% deaths due to malaria in SEAR (Sanjay, 2017).

Plasmodium falciparum malaria is still a fatal illness whose pathophysiology has been well studied from several perspectives. Despite being demonstrated to be transient, naturally acquired antibody responses to important parasite antigens have been found to play essential roles in the development of anti-malarial immunity (White *et al*., 2014; Partey *et al*., 2018). When generated in an uncontrolled way, pro-inflammatory cytokines such TNF- α, IFN- γ, IL-6, and IL-1 have been linked to the pathophysiology of cerebral malaria and are associated with disease severity and mortality (Kinra and Duttan, 2013).

In tropical regions, glomerular disorders were first firmly linked to malaria, a parasite illness (Elsheikha and Sheashaa, 2007). Infection brought on by severe malaria can affect the interstitial area, tubules, and glomeruli. Erythrocyte abnormalities are the main cause of kidney damage in malaria. The involvement of the kidneys occurs often in *P. falciparum* and *P. malariae* infections, although *P. vivax* infections have also been documented. Malaria kidney problems are mostly brought on by immunological response and hemodynamic dysfunction (Silva *et al*., 2017).

Numerous studies from Africa have shown that malaria is more prevalent in people with diabetes (Sanjay *et al*., 2017), hence it is crucial to look at how the co-morbid illnesses affect immunological and metabolic markers.

1.3 Aim and Objectives of the Study

This study uses a mouse model to assess changes in a few immunological and biochemical markers in both single and combined infections with diabetes and malaria.

The specific objectives are to:

(i) determine the effects of diabetes and rodent malaria co-infection on parasitaemia and fasting blood glucose levels.

(ii) determine the effects of the single and co-infections on creatinine and urea levels.

(iii) to evaluate changes in two cytokines in single and co- infections of diabetes and *Plasmodium berghei.*

(iv) determine the effects of antimalarial and/or metformin treatments on the host parameters

1.4 Scope of the Study

This study investigates the impact of diabetes and *Plasmodium berghei* co-infection on several immunological and biochemical markers in BALB/c mice. Tumor Necrosis Factor- α (TNF- α) and Interleukin- 6 are two cytokines that must be tested for as immunological parameters (IL-6). The Creatinine and Urea assays are the relevant biochemical parameters. The results of these tests will be used to assess how these illnesses affect the control, treated, and untreated groups.

1.5 Significance of the Study

The co-morbid conditions' effects on biochemical variables like creatinine and urea concentrations will be discussed in this study. Additionally, it will detail the alterations in two cytokines in both single and combined infections with Plasmodium berghei and diabetes.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview of Diabetes

Atypically high blood sugar levels and the excretion of extra glucose in the urine are two symptoms of diabetes (Balogun *et al*., 2012). Hyperglycemia, or a high blood glucose level, is a sign of diabetes mellitus (DM), a group of metabolic illnesses caused by problems with insulin secretion or irregularities in action (Alhaider, 2011). Patients with high blood sugar generally have polyuria (frequent urine), as well as polydipsia and polyphagia (increased thirst and hunger) (Suresh, 2016). The homeostasis of the circulatory system is impacted by diabetes (Ayodele *et al*., 2020). In the poor world, malaria and diabetes are both more prevalent and present serious public health issues (Kalra *et al*., 2017).

2.2 Prevalence of Diabetes

The prevalence of diabetes is rising quickly on a global scale. According to the International Diabetes Federation's (IDF) Diabetes Atlas, there were 382 million (or 8.3% of the adult world population) diabetics worldwide as of the end of 2013, with 80% of them residing in low- and middle-income nations. It is predicted that this number will increase to 592 million in about 25 years (by 2035). Sub-Saharan Africa is currently predicted to have 20 million diabetics, of which around 62% are undiagnosed. By 2035, the region is expected to have 41.4 million diabetics, an increase of 109.1%. Nigeria has the greatest prevalence of diabetes in sub-Saharan Africa, with an estimated 3.9 million cases (or an extrapolated prevalence of 4.99%) of the adult population aged 20 to 79 years old. One study showed a traditional community with a very low prevalence of diabetes as well as highly specific communities. The prevalence of diabetes ranged from 0.8% to 11% and involved both urban and rural populations. The sixth edition of the International Diabetes Federation (IDF) Diabetes Atlas, which was already mentioned, reveals that Nigeria is the top country in Africa in terms of the number of people with diabetes, with 3.9 million having the disease and 105,091 deaths from diabetes in 2013, with an estimated annual increase of 125,000 between 2010 and 2030 (Dahiru *et al*., 2016).

2.3 Classification of Diabetes

2.3.1 Type 1 Diabetes

This kind of diabetes is brought on by the pancreatic beta cells being destroyed by the immune system. The rate of annihilation varies quite a little, being quick in some people and gradual in

others (WHO, 1999). Insulin is not produced by the body. This kind of diabetes may also be referred to as juvenile diabetes, insulin-dependent diabetes, or early-onset diabetes (Suresh, 2016). Typically, young people and children are the ones who are diagnosed. Type 1 diabetes affects up to 10% of patients with diabetes.

2.3.2 Type 2 Diabetes

This is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest (WHO, 1999). Insulin resistance sometimes referred to as insufficient insulin production or insulin resistance is a condition in which the body's cells do not respond to insulin (Suresh, 2016). Type 2 diabetes affects up to 95% of patients with diabetes. Diabetes with obesity and diabetes without obesity are the two subsets of T2DM. Due to changes in cell receptors, obese T2DM patients frequently acquire resistance to endogenous insulin; this is linked to the distribution of abdominal fat. Along with inadequate insulin synthesis and release, non-obese T2DM also exhibits some insulin resistance at the post receptor levels. Since obesity and aberration of metabolic variables are crucial to the development of TIDM, significant dietary and lifestyle changes in emerging countries are evidences that these nations have the greatest prevalence of diabetes (Kirti *et al*., 2013).

2.3.3 Gestational Diabetes

Some women experience this sort of development while pregnant. After pregnancy, gestational diabetes often disappears. Despite the fact that this kind of diabetes cures at the end of the gestation, it is possible for problems to arise that may be permanent. For instance, gestational diabetes is a possible teratogen and significantly raises the risk of death in both the mother and the fetus. Additionally, the development of diabetic nephropathy in GDM can result in pre-eclampsia, which is linked to a number of anomalies in the development of the fetus, including intrauterine growth retardation (lUGR), preterm labor, and stillbirth (Kirti *et al*., 2013).

2.4 Symptoms of Diabetes Mellitus

Both type 1 diabetes, which develops rapidly into severe hyperglycaemia, as well as type 2 diabetes, which has very high levels of hyperglycaemia, frequently experiences the traditional symptoms of diabetes, such as polyuria, polydypsia, and polyphagia. Only type 1 diabetes or type 2 diabetes that has gone undiagnosed for a long time causes severe weight loss. Other

typical symptoms of undiagnosed diabetes include unexplained weight loss, exhaustion, restlessness, and physical discomfort. Mild symptoms or those that appear gradually may also go missed (Ramachandran, 2014).

2.5 General Overview of Malaria

A potentially fatal parasite illness spread by mosquitoes is malaria (Akande and Musa, 2005) Infection with *Plasmodium* species protozoan parasites is what causes malaria. While *P. vivax, P. ovale,* and *P. malariae* infections are less prevalent and more localized, *Plasmodium falciparum* is pervasive throughout Africa. Anopheles mosquitoes transmit the parasites; the three species that are most common in Africa are *An. gambiae sensu stricto*, *An. funestus*, and *An. arabiensis* (Nkumama *et al*., 2017).

2.5.1 Epidemiology of Malaria

The illness was previously ubiquitous throughout the world, but by the middle of the 20th century, it had been completely eradicated from many temperate nations. Currently, malaria poses a threat to 40% of the world's poorest nations. The majority of the world's tropical and subtropical areas (Akande and Musa, 2005). In 2020, there were a projected 241 million cases of malaria globally and 627 000 malaria-related deaths, according to the World Health Organization's (WHO) Malaria Report 2021. This amounts to 69 000 more fatalities and approximately 14 million more cases in 2020 than in 2019 (WHO, 2021). In India, infections with *Plasmodium falciparum* or *Plasmodium vivax* are the main vectors for malaria transmission. According to estimates, India and 15 sub-Saharan African nations would bear 80% of the global malaria burden in 2018 (Rita *et al*., 2020). There are 37 known species of Anopheles mosquito in Nigeria. *P. falciparum* is the primary cause of malaria. Only a tiny portion of the southern half of the country experiences year-round transmission, which happens throughout the whole nation. Malaria epidemic risk is about 3%, but endemic malaria risk is 97%. In Nigeria, where malaria is a serious problem and a leading cause of illness and death (Akande and Musa, 2005).

2.5.2 Pathogenesis of Malaria

Due to the primary causative parasite, *Plasmodium falciparum*, approximately 3.3 billion people (or half of the world's population) are at risk of contracting malaria. Malaria is one of the most significant health issues in the world, with over 200 million cases annually, 90% of which occur in Africa, and 655 000 deaths from the disease in 2010 (Mawson, 2013). The many host and

parasite variables that contribute to disease are referred to as pathogenesis (Miller *et al*., 2015). Each person's response to an infection is determined by these variables, which are frequently ill-defined (Miller *et al*., 2002). When a person is bitten by an infected female Anopheles mosquito, *Plasmodium spp*. (species) parasites in the form of sporozoites are injected into the circulation, starting the malarial infection process. In the following 7–10 days, the sporozoites proliferate asexually as they make their way to the liver. There are no symptoms at this time. The parasites now appear from the liver cells in vesicles called merozoites and go via the heart to the lungs' capillaries (Mawson, 2013).

2.6 Cytokines

White blood cells and other cells release cytokines, which are regulatory proteins or glycoproteins, in response to a variety of stimuli (Mandala *et al*., 2017). Despite having more particular names based on the types of cells that make them and the tasks they carry out, cytokines are often referred to as "cytokines". Lymphokines, for example, are produced by lymphocytes, whereas monocyte and macrophages produce monokines. At infection sites, lymphokines like interferon gamma (IFN- γ) and interleukin 4 (IL-4) recruit and activate immune cells like macrophages and other lymphocytes while also stimulating B cells to make antibodies (Khan, 2008). Contrarily, inflammatory functions are played by monokines such tumor necrosis factor alpha (TNF- α), IL-1, IL-6, and IL-8, which also chemotactically draw neutrophils (Akdis *et al*., 2011). The bulk of cytokines, however, may now be generated by a variety of distinct cell types, casting doubt on the seeming exclusivity of the terms "lymphokine" and "monokine."

When categorized according to how they affect inflammation, cytokines can be divided into pro-inflammatory and anti-inflammatory categories. Pro-inflammatory cytokines include IL-1, TNF- α, IFN- γ, IL-12, and IL-18, while anti-inflammatory cytokines include IL-4, IL-10, IL-13, and transforming growth factor beta (TGF- β).

2.6.1 Role of Tumor Necrosis Factor - α and Interleukin- 6 in Diabetes

An adipocytokine called tumour necrosis factor alpha (TNF- α) is implicated in systemic inflammation and activates the acute phase response (Moller, 2000). The majority of cells that release TNF- α are macrophages, although there are many other types of cells as well, including adipocytes (Jatla *et al*., 2012). TNF- α reduces the action of insulin and affects how quickly

glucose is metabolized (Zou and Shao, 2008). Type 2 diabetes mellitus may develop and proceed more slowly as a result of disturbances in TNF- α metabolism since these disturbances have been linked to metabolic diseases including obesity and insulin resistance. It is unclear how TNF- α affects the course and development of the illness, though.

A multifunctional cytokine called interleukin 6 (IL-6) has been linked to the etiology of type 2 diabetes (T2D). An independent predictor of T2D, the increased circulating amount of IL-6 is thought to play a role in the emergence of inflammation, insulin resistance, and beta-cell dysfunction. On the other hand, mounting data points to the anti-inflammatory and enhanced glucose metabolism of IL-6. The pleiotropic properties of IL-6 may be explained by the cytokine's intricate signal transduction process. Classic signaling and trans-signaling are two different signaling routes that IL-6 uses to exert its effects. Despite the fact that both signaling pathways activate the same receptor subunit, their biological outcomes are very different (Akbari and Zadeh, 2018). While deteriorating glucose control is positively and linearly related with high levels of IL-6 and leptin, diabetes overall is substantially connected with raised levels of IL-6, leptin, CRP, and TNF- α (Mirza *et al*., 2012).

2.6.2 Role of Tumor Necrosis Factor - α and Interleukin- 6 in Malaria

Malaria immunity is both humoral and cellular in nature and includes a number of pathways. According to Langhorne *et al*. (2008), *P. falciparum*-induced antibodies play a part in both the pathogenesis and protection against malaria. Different lymphocyte subsets are also involved (Langhorne *et al*., 2008). Despite being demonstrated to be transient, naturally acquired antibody responses to important parasite antigens have been found to play essential roles in the development of anti-malarial immunity (White *et al*., 2014; Partey *et al*., 2018). Pro-inflammatory cytokines are known to have a role in clearing the initial parasitemia in the early stages of *P. falciparum* infection. They are generated by a variety of cells, including lymphocytes, monocytes, macrophages, fibroblasts, neutrophils, endothelial cells, and mast cells. When generated in an uncontrolled way, pro-inflammatory cytokines such TNF- α, IFN- γ, IL-6, and IL-1 have been linked to the pathophysiology of cerebral malaria and are associated with disease severity and mortality (Kinra and Duttan, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Drugs, Assay Kits, Test strips and other chemicals and apparatus

Artemether Lumefantrine (1.14/ 6.86 mg/kg), STZ (40mg/kg), Metformin (200 mg/kg), Volumetric flask, Beakers, Stirring rod, Whatman's No. 1 filter paper, sodium chloride, picric acid, tri- sodium citrate, citric acid, Sodium Hydroxide, Methanol, measuring cylinder, UV-Visible Spectrophotometer, cuvette, potassium chloride, formalin, measuring cylinder, UV-Visible Spectrophotometer Conical flask, capillary tubes, microscopic slides, weighing balances, analytical balances, dissecting tools, distilled water, test tubes and test tube racks, dissecting kit, and pins Giemsa Stain, a pH meter, a mortar and pestle, and droppers.

We bought ELISA kits for TNF- and IL-6 from Elabscience Biotechnology in the USA. We bought urea and creatinine assay kits from Randox Laboratories Ltd. in the UK. Test strips and a glucometer from Accu-check Active strip Compact Plus were used.

3.2 Study Design

In vivo animal research was used in the study. 54 healthy male mice (26g ± 4g) were procured from the Mountain Top University's animal holding facility in Ogun State, Nigeria. The rodents were housed in cages at the Animal Facility of Mountain Top University, where they were given mouse pellets and had unrestricted access to water. Two weeks were spent acclimatizing them.

At the beginning of the trial, baseline measures of weight, fasting blood glucose (FBG), and packed cell volume (PCV) were collected. Streptozotocin (STZ) (40 mg/kg) was administered to mice in numerous, modest doses intraperitoneally over the course of 5 days to cause diabetes (Brian, 2021). By monitoring the diabetic mice's fasting blood glucose on Day 14, hyperglycemia was confirmed, and metformin therapy started the same day. Using normal saline as a vehicle for induction, the malaria parasite was administered to the malaria groups. To track the levels of parasitemia in mice, thin smears were made on microscope slides on Day 3 PI, Day 5 PI/24 hours PT, Day 6 PI/48 hours PT, and Day 7 PI/72 hours PT. Additionally, Artemether Lumefantrine (1.14/ 6.86 mg/kg) was administered orally to the malaria therapy groups twice daily for three days (Otuchere *et al*., 2012).

On days 17 and 21, weights and packed cell volume measurements were once more obtained consecutively, and on days 14 and 21, fasting blood glucose readings were once more done sequentially. After the animals were sacrificed, blood samples and important organs were taken for further examinations on the bench. Procedures for handling animals were compliant with the Institutional Animal Ethics Committee's guidelines for the care and use of laboratory animals (IAEC).

3.2.1 Grouping of Experimental Animals

Group 1: Normal Control

Group 2: Diabetes Only

Group 3: Malaria Only

Group 4: Malaria + Diabetes Only

Group 5: Malaria + AL

Group 6: Diabetes + MTF

Group 7: Malaria + Diabetes + MTF

Group 8: Malaria + Diabetes + MTF + AL

AL: Artemether Lumefantrine, MTF: Metformin.

3.3 Determination of Weights

The mice were weighed on day 0, day 17 and day 21 using an electronic sensitive weighing scale.

3.4 Induction of Diabetes

By withholding food and water from their cages, the mice were forced to fast for 12 hours the next day. To get a final concentration of 4 mg/mL, 4 mg of STZ was measured and dissolved in 50 mm of sodium citrate buffer (pH 4.5). Streptozotocin (STZ) was given to mice in numerous, modest doses (40 mg/kg, intraperitoneally) over the course of five days, and on the sixth day of the experiment, sucrose water was created in place of the normal water (Brian, 2021). On Day 14, hyperglycemia was identified, and the mice's Fasting Blood Glucose levels were checked to confirm it.

3.4.1 Determination of Fasting blood glucose Levels

By withholding food and water from their cages, the mice were forced to fast for 12 hours the next day. The tips of the mouse tails were cut, and blood was taken and placed on a glucose test strip that was then inserted into a glucometer. On Days 0, 14, and 21, the Fasting Blood Glucose were measured, accordingly. Diabetic mice were those with fasting blood glucose levels ≥190 mg/dL.

3.5 Malaria Parasite Inoculation

The Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria, collected malaria parasites (*Plasmodium berghei* NK65). A heparinized syringe was used to pierce the heart of an infected mouse to get blood. To deliver the desired dosage per injection, the blood was instantly diluted with ordinary saline. After the onset of hyperglycemia on day 14, all P. berghei infection groups received an intraperitoneal injection of 0.2 mL of the malaria parasite inoculum using an insulin syringe with a 0.1 mL gauge needle.

3.5.1 Preparation of Thin Blood Films

Blood was drawn from infected mice on day 17 (day 3 post infection) by rubbing the tip. Near its frosted end, the tip was slightly sliced, and a little drop of blood was deposited on a labeled, well cleaned slide. The drop was allowed to spread along the contact line of the two slides and allowed to dry before another slide was brought up to it at a 45° angle. After that, the cells were fixed with 100% methanol and traditionally stained for 5 minutes using easily available Giemsa stain solution (Ranjo Medix Laboratories LTD).

3.5.2 Percentage Parasitemia Count

The technique calculates the proportion of red blood cells (RBC) harboring malaria parasites. To determine the number of non-parasitized and parasitized RBCs, the generated thin blood films were carefully examined under a microscope at a high magnification of ×100 with oil immersion for intracellular stages of the *Plasmodium berghei*. To determine the proportion of parasitaemia, the blood film had at least 1000 cells that had to be counted.

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

3.6 Drug Dosage Determination and Administration

In order to determine the drug dosage in mg/kg, the manufacturers' standard dose was used. The STZ dose was 40 mg/kg. Metformin (Glucophage, Nigeria) had a dosage of 200 mg/kg, whereas Artemether Lumefantrine (Fidson, Nigeria) had a dosage of 1.14/6.86 mg/kg.

The dosage of the drugs was 1 mg/0.5 mL. For induction, STZ was dissolved in 50 mM citrate buffer (pH 4.5). Metformin was supplied in a total dose of 326.025 mg in a vehicle of 161 mL of normal saline. Daily Metformin medication began on day 14, the day when hyperglycemia was detected. Artemether Lumefantrine (AL) was delivered in a total amount of 15 mg over 42 mL of sterile saline. Treatment for AL began on day 18 (day 4 post infection).

3.7 Collection and Preservation of Blood and Tissue Samples

Ocular punctures were used to collect whole blood from the experimental animals into simple test tubes. The labels on the simple tubes were accurate. After centrifuging the blood samples at 1000 g for 10 min., the serum was extracted, and it was kept at -20 °C until it was needed for cytokine and biochemical tests.

3.8 Sacrifice of Experimental Animals

On Day 21, all experimental mice were put to death. Prior to cervical dislocation, anesthesia was administered to the mice.

3.9 Biochemical Parameters

Creatinine and urea tests were performed as biochemical parameters in this investigation.

3.9.1 Determination of Creatinine Levels

Using a Randox test kit for the quantitative detection of creatinine in serum taken from mouse blood, a creatinine test was conducted.

REACTION:

GLDH

NH_3 + α-oxoglutarate + NADPH + H^+ ⟶ L- Gluatamate + $NADP^+$ + H_20

PROCEDURE

The serum sample or Standard was pipetted into test tubes with labels in the amount of 0.1 mL. The tubes' contents were then mixed well with 1 mL of R1A reagent before being incubated for 5 minutes at room temperature. The solutions in the plain tubes received an addition of 0.2 mL of R1B reagent. The mixtures were properly combined before being placed in the incubator at 37°C for one minute. A UV-Spectrophotometer was used to measure the initial absorbance (A1), and a timer was set for three minutes to collect the final absorbance readings (A2).

CREATININE CALCULATION USING SERUM SAMPLES

$$\frac{A_{sample}}{A_{standard}} \times \text{Standard concentration (µmol/L)}$$

3.9.2 Concentration levels of Urea in samples

The Urease Berthelot Method was used for the Urea Test to determine the amount of urea in mouse serum quantitatively in vitro. United Kingdom-based Randox Laboratories Ltd. sold the urea test kit.

PRINCIPLE

When urease is present, serum urea hydrolyzes to produce ammonia. Berthelot's reaction is then used to measure the ammonia photometrically.

Urease

Urea + H_2O ⟶ $2NH_3$ + CO_2

NH3 + hypochlorite + phenol ⟶ Indophenol (blue compound)

PROCEDURE

100 mL of Reagent 1 were added after pipetting 10 mL of the sample into simple tubes with labels. Another tube with a label on it received 10 L of the standard, which was measured out.

Next, 100 L of Reagent 1 were added to the mixture. 100 mL of Reagent 1 were added to 10 mL of distilled water, which served as the blank. The mixes were carefully combined before being put in the incubator to incubate at 37°C for 15 minutes. The blank, standard, and samples each received 2.50 mL of Reagent 2. The mixes were gently combined before being placed in the incubator for 15 minutes at 37°C.

In a UV-Spectrophotometer, the absorbance of the samples (Asample) and standard (Astandard) were measured against the blank at a wavelength of 546 nm. These measurements were taken and written down.

UREA CALCULATION USING SERUM

$$\text{Urea Concentration} = \frac{A_{sample}}{A_{standard}} \times \text{Standard concentration (mmol/L)}$$

3.10 Immune parameters

Tumor Necrosis Factor-α and Interleukin-6 were the cytokines assayed for in this study using enzyme-linked immunosorbent assay (ELISA).

3.10.1 TNF-α Assay Procedure

Tumor Necrosis Factor-α ELISA kit was gotten from Elabscience Biotechnology Inc, USA. All procedures were carried out according to the guidelines stated in the manual.

PROCEDURE

The samples were diluted 1: 8 before being combined. Additionally, the supplied standard was diluted with 1 mL of diluent and centrifuged at 10,000g for 1 minute before being let to stand for roughly 10 minutes. Using 500 L of diluent each dilution, the 1000 pg/mL standard solution was serially diluted to 7.8 pg/mL. Each well was then filled with 100 L of a standard, a blank, or a sample, sealed, and incubated for 90 minutes at 37°C. The concentrated biotinylated detected antibody was centrifuged at 800g for roughly one minute during incubation. 5940 mL of antibody diluent were mixed with 60 mL of concentrated antibody (Ab). The ELISA plate was removed after incubation, and the liquid was decanted from each well. Each well was then filled

with 100 L of biotinylated detected antibody, capped with fresh sealer, and incubated for an additional hour at 37°C. The wash buffer was made by mixing 15 mL of concentrated wash buffer with 360 mL of distilled water during the incubation period. The solution was removed from the well after incubation. Each well received 350 l of wash buffer, which was added, steeped for one minute, and then decanted. Three times were spent washing in this manner. Following washing, 100 L of the conjugate of horseradish peroxidase (HRP) was pipetted into each well. A fresh sealer was applied to the plate, which was then incubated at 37°C for 30 minutes.After 30 minutes of incubation, the solution in the plate's wells was decanted and washed five more times with 350 L of wash buffer. After washing, 90 L of substrate reagent was applied. A fresh sealer was applied to the plate, which was then incubated for 20 minutes at 37°C. After an incubation period of 20 minutes, a color shift was noticed. A stop solution of 50 L was added to each well. A microplate reader tuned to a wavelength of 450 nm was used to read the optical density (OD) of each sample in each well at once. Curve expert 1.4 was used to produce the concentration and the standard curve.

3.10.2 IL-6 Assay Procedure

Interleukin- 6 kit was gotten from Elabscience Biotechnology Inc, USA. All procedures were carried out according to the guidelines stated in the manual.

PROCEDURE

The samples were created by dilution in a 1:8 ratio. 25 µL of samples and 175 µL of diluent were pipetted into an Eppendorf tube. The standard was centrifuged for one minute at 10,000 g. The standard was mixed with 1 mL of diluent and let to stand for 10 minutes. 1 mL of diluent was mixed with the supplied standard before being allowed to stand for 10 minutes. Then, it was serially diluted into Eppendorf tubes from 1000 pg/mL to 7.5 pg/mL. Each well in the ELISA plate received 100 L of the standard, blank, and sample. After being sealed, the plate was incubated at 37°C for 90 minutes. The samples were made by diluting the original substance 1:8. A pipet was used to transfer 25 mL of samples and 175 mL of diluent into an Eppendorf tube. The standard was centrifuged at 10,000 g for one minute. One milliliter of diluent was added to the standard, and it was let to stand for 10 minutes. The given standard was combined with 1 mL of diluent and let to stand for 10 minutes. Then, it was serially diluted from 1000 pg/mL to 7.5 pg/mL into Eppendorf tubes. 100 L each of the standard, blank, and sample were placed in each

well of the ELISA plate. The plate was sealed, and it was incubated for 90 minutes at 37 °C. The plate received a fresh sealer, which was then incubated for 30 minutes at 37°C. The solution in the wells was decanted and given five additional washes with 350 L of wash buffer after 30 minutes of incubation. 90 µL of substrate reagent were added after washing. The plate was coated with fresh sealer and then incubated for 20 minutes at 37°C. A color change was seen after 20 minutes of incubation. A stop solution of 50 µL was added to each well. The optical density (OD) of each sample in each well was measured using a microplate reader calibrated to a wavelength of 450 nm. Using Curve expert 1.4, the concentration and the standard curve were produced.

3.11 Statistical Analysis

On GraphPad Prism 9.2.0, the study's data were calculated, and descriptive statistics for frequencies, means, median values, and standard deviation were run. Using the Mann-Whitney U test of comparisons and one-way Analysis of Variance (ANOVA), significant differences between the groups were discovered. Statistical significance was defined as a P value less than 0.05 ($p < 0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Weight of Mice (g)

The highest value was discovered in group 5 (Malaria+AL) and 6 (Diabetes+ MTF) on days 17 and 21, respectively, as illustrated in Figure 4.1. On day 21, the group 7 (Malaria+Diabetes+MTF) reading was the lowest. When the normal control group was compared to all other groups, the results show that there was no difference in weight, with the exception of the malaria only group that received AL treatment on days 17 and 21 ($p=0.039$ and 0.017, respectively). Additionally, the results showed that the weight of the diabetes only group was much lower than that of the diabetes group receiving MTF treatment. On Day 17, the weights of the malaria only group and the malaria plus diabetes group receiving metformin medication differed significantly ($p=0.038$).

On day 17, both the malaria group receiving AL therapy and the malaria group who also had diabetes experienced a statistically significant weight gain ($p=0.041$). On days 17 ($p=0.006$) and 21, there was a statistically significant difference in weight between group 5 (malaria+AL) and group 7 (malaria+diabetes+MTF). On day 21, a comparable result was seen between groups 5 and 8 (Malaria + Diabetes + MTF + AL) ($p=0.038$). On days 17 ($p=0.026$) and 21 ($p=0.009$), there was a statistically significant increase in weight in Group 6 (Diabetes + MTF) compared to group 7 (Malaria + Diabetes + MTF). On day 21, the weight of group 8 (Malaria + Diabetes + MTF + AL) was significantly lower than that of group 6 ($p=0.014$).

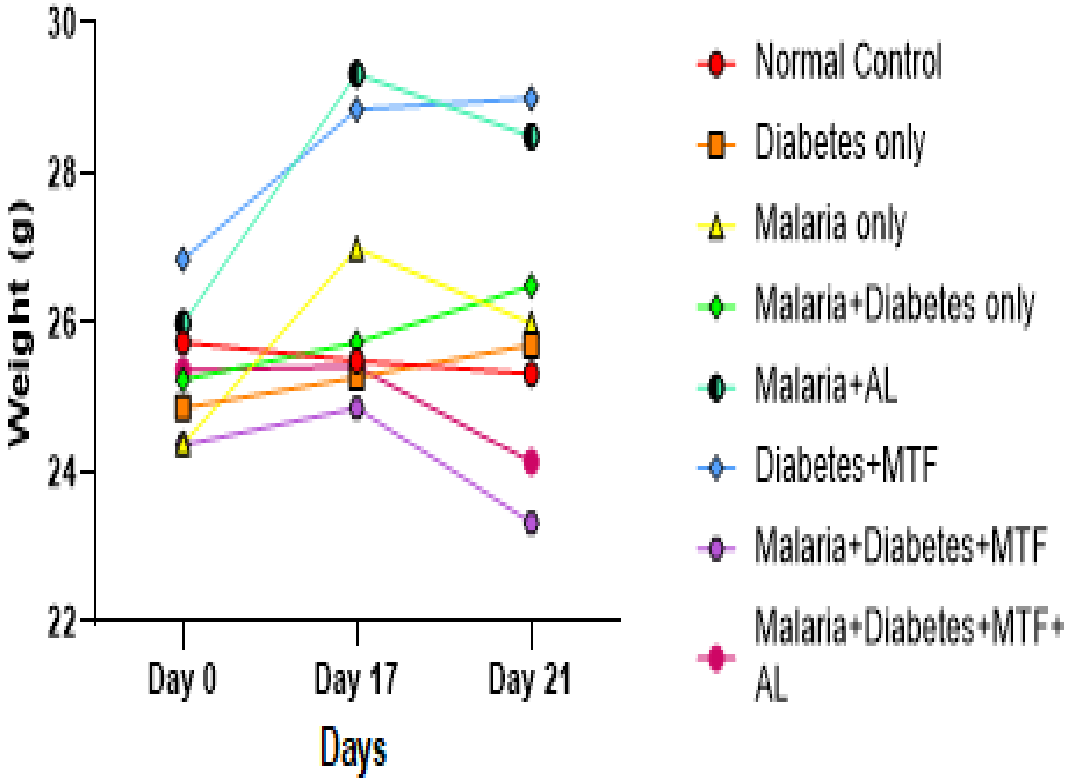

FIGURE 4.1: Graphical illustration of the weight of experimental mice in grams on day 0, day 17 and day 21

Values are presented as mean, n=8

MTF: Metformin; AL: Artemether Lumefantrine

4.2 Fasting Blood Glucose (mg/dL)

Figure 4.2 illustrates that on day 21, group 8 (Malaria+ Diabetes +MTF+AL) had the greatest mean value, whereas group 2 (Diabetes Only) had the lowest value. The findings show that when the normal control group's fasting blood glucose was compared to that of all other groups, there was no statistically significant difference. On days 0 (p=0.002) and 14 (p=0.005), the fasting blood glucose of the mice in group 2 (Diabetes Only) and group 6 (Diabetes +MTF) significantly increased. On days 14 (p=0.011) and 21, there was a significant difference in fasting blood glucose between group 2 (Diabetes Only) and group 8 (Malaria+ Diabetes +MTF+AL) (0.022).

On day 21, mice in group 4 (Malaria+ Diabetes only) had substantially higher fasting blood glucose levels than mice in group 3 (Malaria only) (p=0.014). On day 21, the fasting blood glucose of group 5 (malaria Plus AL) was significantly higher than that of group 3 (malaria only) (p=0.020). When compared to group 3, the mice in group 8 fasting blood glucose was extremely significant on day 21 (p=0.017) (Malaria only). When compared to Day 0, the mice's fasting blood glucose in groups 4 (Malaria + Diabetes Only) and 6 (Diabetes +MTF) was considerably higher on day 14 (p=0.005) (0.027).When compared to group 8 (Malaria + Diabetes + MTF+ AL), mice in group 4 (Malaria+ Diabetes only) had a significantly higher fasting blood glucose from day 0 (p=0.001) to day 14 (p=0.018).

On day 21, the mice in groups 5 (malaria + AL) and 8 (malaria + diabetes + MTF+ AL) had significantly lower fasting blood glucose levels (p=0.015). The fasting blood glucose of mice in groups 6 (Diabetes + MTF) and 7 (Malaria + Diabetes + MTF) significantly increased from day 0 (0.027) to day 14 (p=0.043). When compared to group 8 (Malaria + Diabetes + MTF+ AL), mice in group 6 (diabetes + MTF) had a significantly lower fasting blood glucose from day 0 (p=0.002) through day 21 (p=0.025).

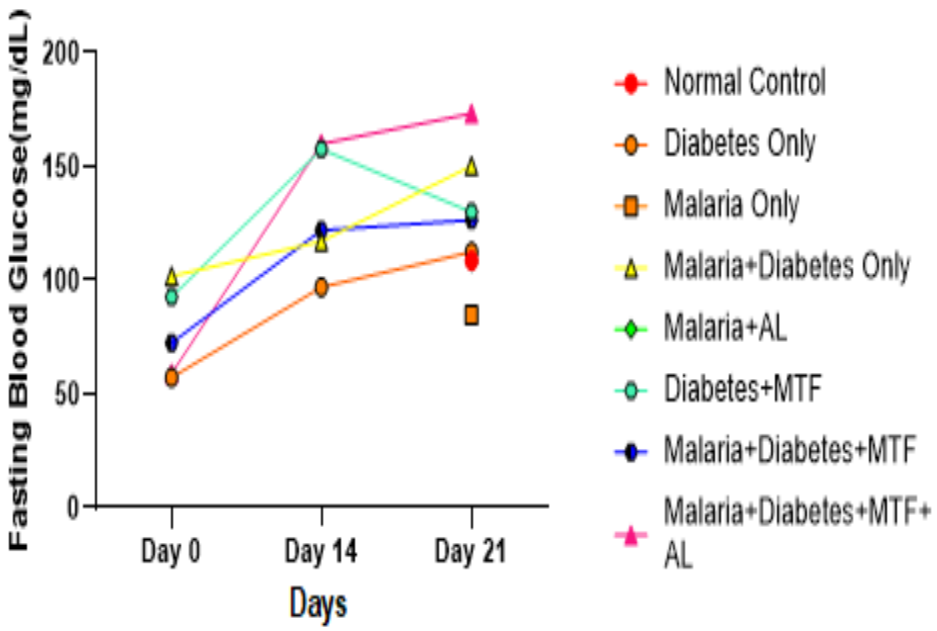

FIGURE 4.2: Graphical illustration of the fasting blood glucose test (mg/dL) on experimental mice on day 0, day 14 and day 21

Values are presented as mean, n=4

MTF: Metformin; AL: Artemether Lumefantrine

4.3 Percentage Parasitemia Count

According to figure 4.3, group 7 had the highest parasitemia count reading on Day 21/D7 PI/72Hr PT, while group 8 had the lowest reading on the same day. With the exception of group 7 (Malaria + Diabetes +MTF), which had a substantially higher parasitemia count (p=0.046) on Day 7 PI/72Hr PT, the results show that there was no discernible difference in parasitemia count between the malaria only group and any of the other groups. Between day 6 PI/48Hr PT and day 7 PI/72Hr PT, mice in group 8 (Malaria + Diabetes + MTF+ AL) had significantly fewer parasites than mice in group 3 (p=0.014 and p=0.017, respectively) (Malaria Only). On Day 7 PI/72Hr PT, the mice in group 5 (malaria + AL) had significantly lower parasitemia counts than those in group 4 (malaria + diabetes alone; p=0.017). When group 8 (Malaria + Diabetes + MTF+ AL) was compared to group 4 (Malaria + Diabetes Only), the results between Day 6 PI/48Hr PT and Day 7 PI/72Hr PT were similar (p=0.007 and p=0.002, respectively).

On Day 7 PI/72Hr PT, mice in group 7 (malaria + diabetes + MTF) had significantly more parasitemia than mice in group 5 (malaria + AL; p=0.020). On Days 3 PI (0.0036) and Day 7 PI/72Hr PT, respectively, the mice in Groups 8 (Malaria + Diabetes + MTF+ AL) and Group 5 (Malaria + AL) had significantly lower parasitemia counts (0.034). When compared to group 7 (Malaria + Diabetes +MTF), mice in group 8 (Malaria + Diabetes + MTF+ AL) had significantly lower parasitemia counts on days five, six, and seven (p=0.028, 0.010, and 0.002, respectively).

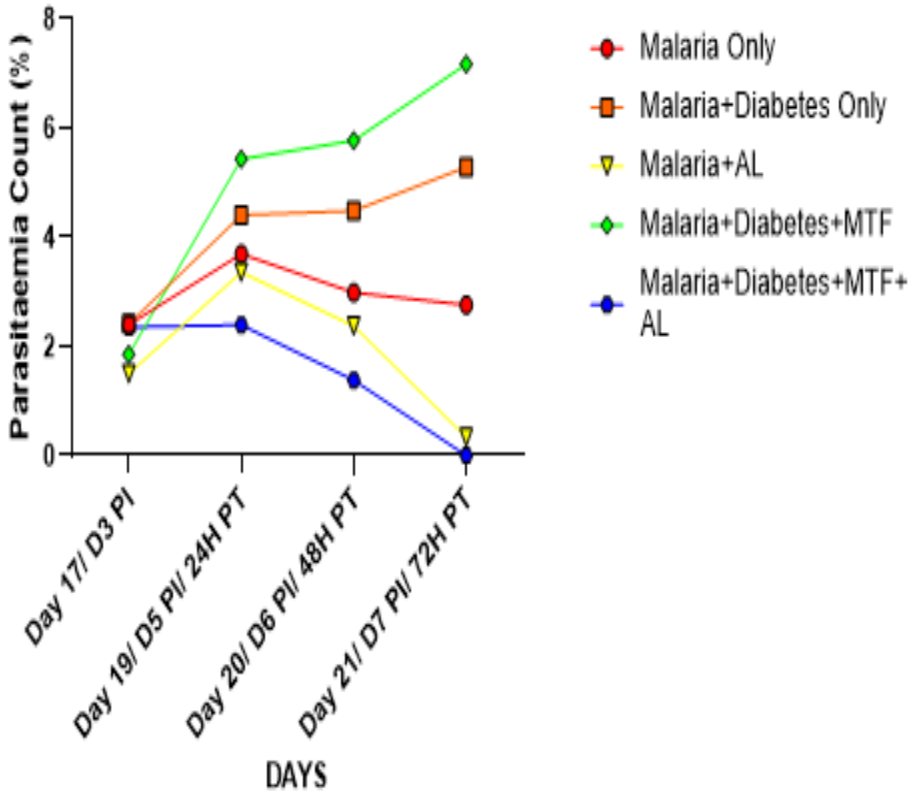

FIGURE 4.3: Graphical illustration of the parasitemia count (%) on day 3 PI, day 5 PI/24Hr PT, day 6 PI/ 48Hr PT and day 7 PI/ 72Hr PT.

Values are presented as mean, n=4

D3: Day 3; D5: Day 5; D6: Day 6; Hr: Hour PI: Post Induction; PT: Post Treatment

4.4 Creatinine Assay Interpretations in µmol/L

According to figure 4.4, group 8 (malaria + diabetes + MTF + AL) had the highest mean creatinine concentration, while group 5 (malaria + AL) had the lowest. The findings show that when the creatinine concentration in the normal control was compared to all other groups, there was no significant difference. In comparison to group 8 (Malaria + Diabetes + MTF+ AL), group 5 (Malaria + AL) had a considerably lower creatinine concentration ($p=0.021$). Additionally, group 8 (Malaria + Diabetes + MTF + AL) had significantly higher levels of creatinine than group 7 (Malaria + Diabetes +MTF) ($p=0.043$).

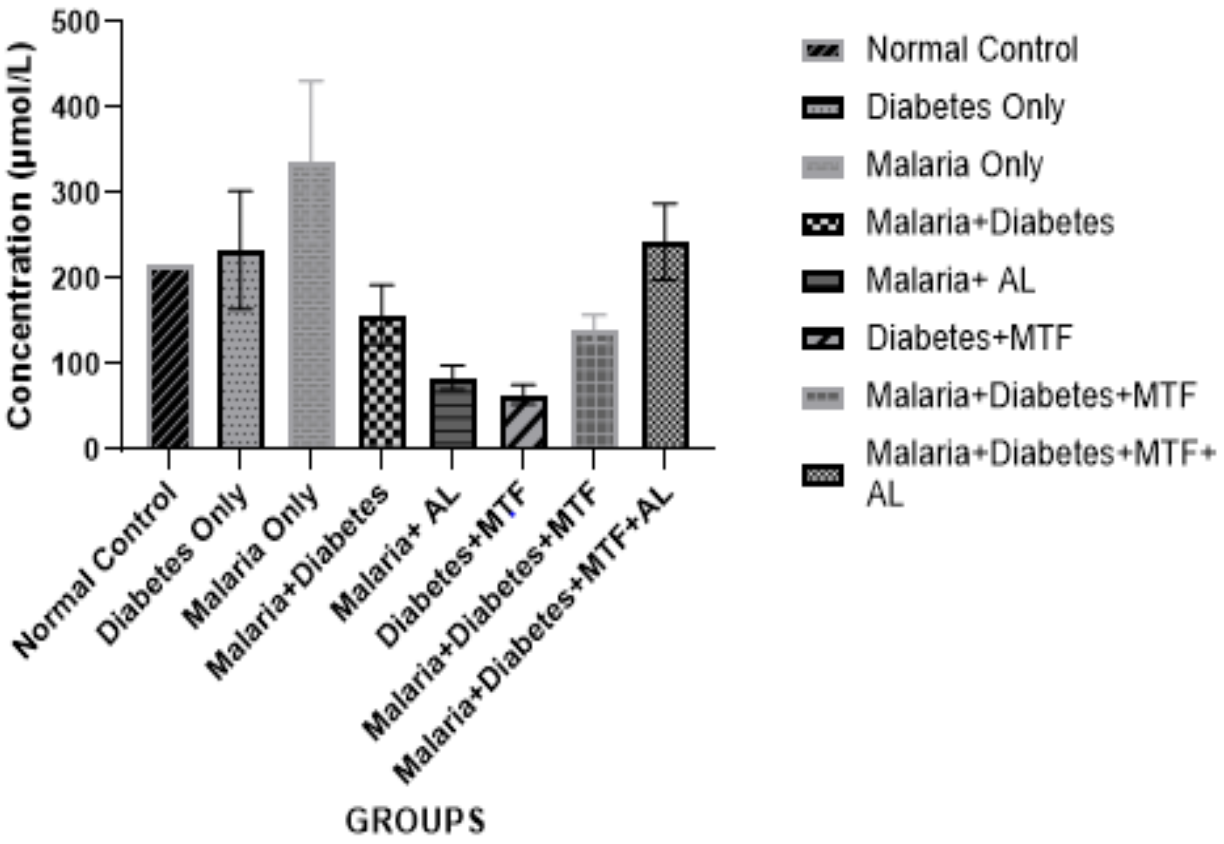

FIGURE 4.4: Creatinine concentration in control groups and test groups of experimental mice

Values are presented as mean ± SEM, std. deviation, n=4

MTF: Metformin; AL: Artemether Lumefantrine

4.5 Urea Assay Interpretations in Mmol/L

According to figure 4.5, group 6 (Diabetes +MTF) had the lowest mean value whereas the normal control group had the highest.

The findings show that when the urea concentration in the control Groups (Diabetes Only, Malaria Only, and Diabetes + Malaria Only) was compared to the other groups, there was no statistically significant difference. In compared to group 6 (Diabetes +MTF), the urea concentration considerably increased ($p=0.021$) in group 7 (Malaria + Diabetes +MTF).

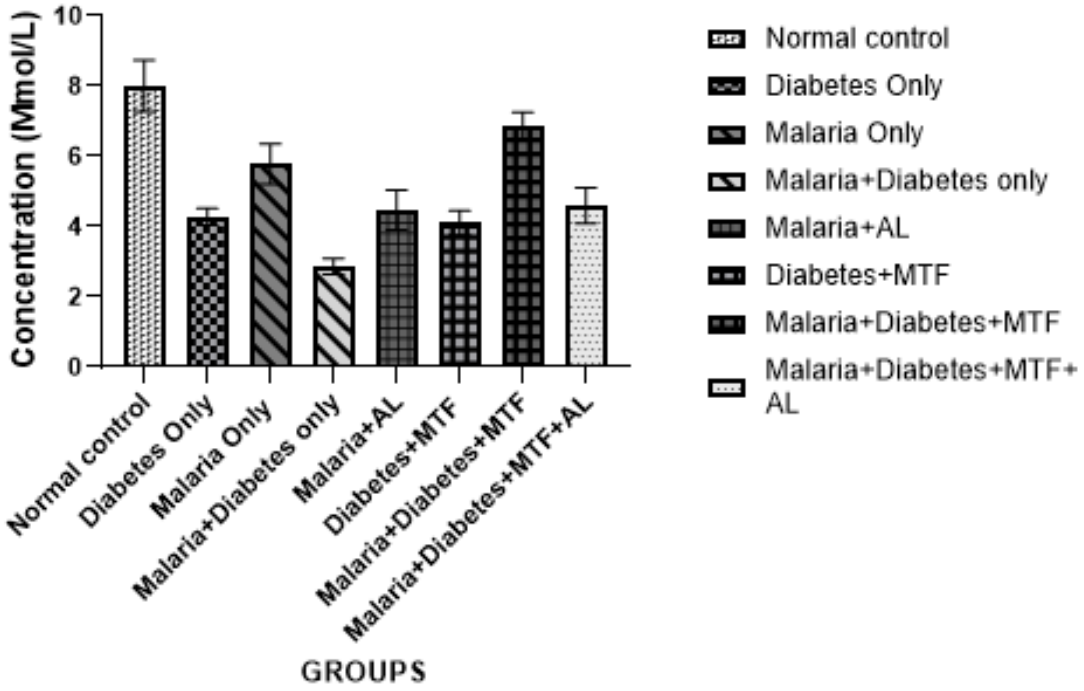

FIGURE 4.5: Urea concentration (Mmol/L) in control and test groups of experimental mice

Values are presented as mean ± SEM, std. deviation, n=4

MTF: Metformin; AL: Artemether Lumefantrine

4.6 TNF –α Assay Interpretations in pg/mL

Experimental mice were used to measure the serum levels of TNF- α, and the results showed no significant difference between any of the groups in the median concentration of TNF. Group 3 (Malaria only) had the highest TNF- α concentration reading, whereas group 5 (Malaria+ AL) had the lowest reading. However, the finding demonstrates that, in contrast to the other groups, the malaria groups have a higher concentration of TNF- α.

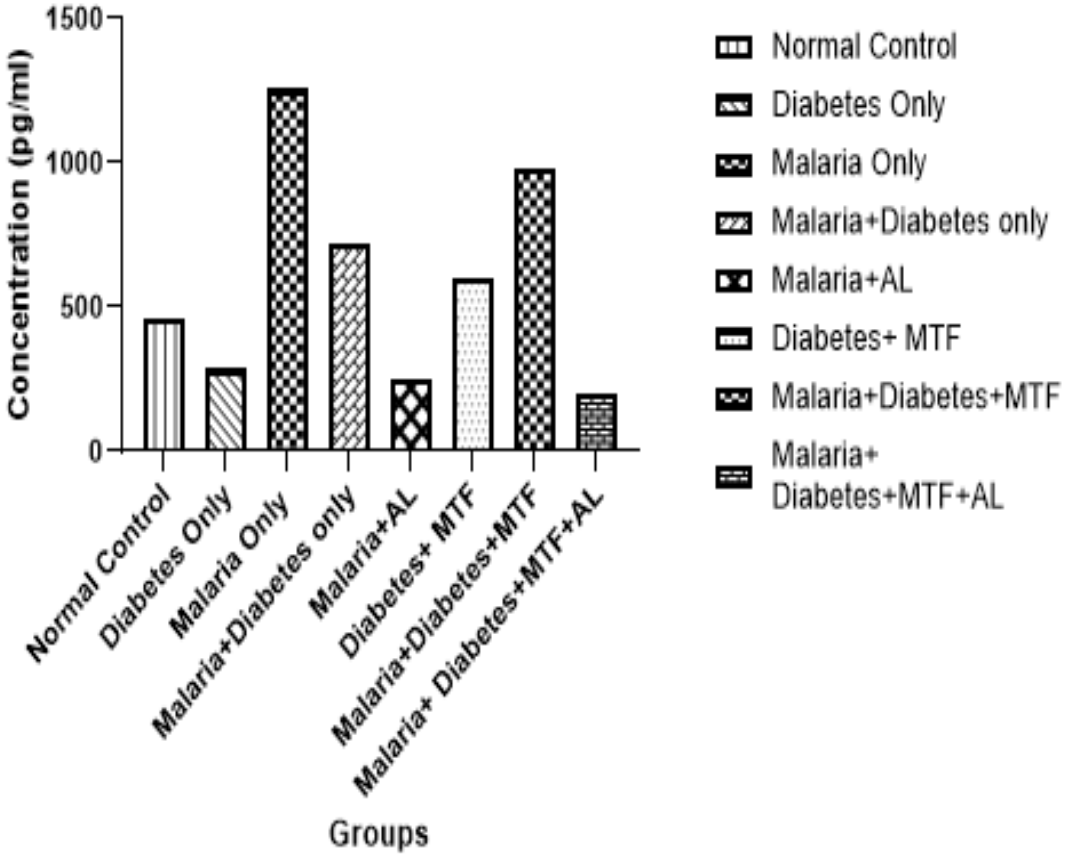

FIGURE 4.6: Graphical representation of TNF –α assay

Values are presented as median (68.3917) and interquartile range (112.2339), n=4

4.7 Interleukin-6 (IL-6) Assay Interpretations in pg/mL

According to figure 4.7, group 4 (Malaria + Diabetes Only) has the greatest mean value, whereas group 3 has the lowest value (Malaria only). Interleukin-6 levels in serum were measured in experimental mice, and the results showed that group 4 (Malaria + Diabetes Only) had significantly higher levels of IL-6 than the normal control ($p=0.046$). However, the graphical representation shown below showed that, when compared across all test groups, the IL-6 Concentration was significantly higher in all of the control groups (Diabetes Only, Malaria Only, and Malaria + Diabetes Only).

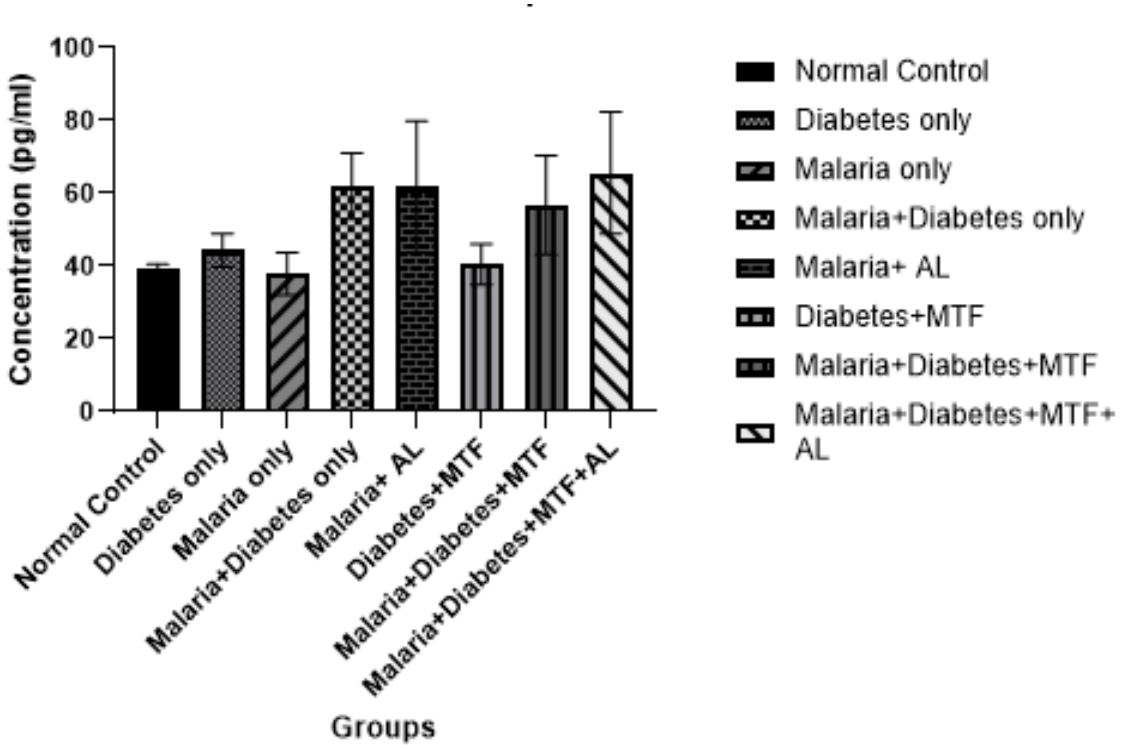

Figure 4.7: Graphical representation of the interleukin-6 assay.

Values are presented as Mean ± SEM, Std. Deviation, n=4

4.8 DISCUSSION

In this investigation, BALB/C mice's immunological and biochemical parameters were studied in relation to the impacts of diabetes and Plasmodium berghei infections.

Hyperglycemia (high blood glucose level) caused by errors in insulin secretion or irregularities in action can be a symptom of diabetes mellitus (DM), a set of metabolic diseases (Abdulqader, 2011). The protozoan parasites (genus *plasmodium*) that cause malaria are common in tropical and subtropical regions, including parts of America, Asia, and Africa. Malaria is a vector-borne infectious disease (Balogun *et al*., 2012). In Nigeria, both malaria and diabetes are common illnesses, as is their concomitant co-infection.

Infections with malaria and diabetes have effects on the weights of the host as seen in the results of the groups with single infections and co-infections of both malaria and diabetes. This study shows that there was a significant decrease in the weight of the infected groups when compared with the treated groups. This could be as a result of low food and water intake that was observed in the infected groups. Other reasons for this observed low body weight may be due to the destructive effect of the injected streptozotocin. Furman *et al*. (2021) reported selective destruction of pancreatic islet β-cells which proves the toxicity of the compound in inducing diabetes.

The findings of this study reveal that the co-infection of diabetes and malaria has effects in the parasitemia count and fasting blood glucose concentration of infected groups. It can be said that a host with diabetes can have an increased risk of developing malaria infection whereas a host with malaria infection can also have the tendencies of developing diabetes. This study also showed that as the concentration of fasting blood glucose increased in co-infected groups, the parasitemia count of that same group also increased when compared with the single infected groups. This risk is probably as a result of impaired defense against liver and/or blood-stage parasites and from prolonged persistence which rendered the host viable to becoming resistant to insulin production or abnormal functioning of insulin in order to reduce the blood sugar of the host in which will in turn reduce the risk of developing diabetes. The result gathered from this study has a very high correspondence with danquah *et al*. (2010) which inferred that increased glucose availability may increase malaria growth.

Metformin is the first-line pharmacologic treatment for type 2 diabetes and the most commonly prescribed drug for this condition worldwide, either alone or in combination with insulin or other glucose-lowering therapies (Flory and Lipska, 2019). Recent studies recorded that metformin has a potent activity against liver stage malaria *in vivo*. A study from vera *et al*. (2019), reported that metformin impairs parasite liver stage development of both rodent-infecting *Plasmodium berghei* and human-infecting *P. falciparum* parasites. This could be as a result of metformin accumulation in the liver in which was due to the high expression of organic cation transporters (OCTs) in the hepatocyte plasma membrane enabling drug import. Parasitemia levels in this study were significantly increased in the group treated with metformin which does not support the conclusion of vera *et al*. (2019).

According to the findings of this study, as compared to the group that had both malaria and diabetes, the groups with single infections had considerably greater levels of creatinine and urea in their blood. This might be due to hemolysis, which is characteristic of malaria cases in particular.Some studies have reported cases of severe malaria associated with acute kidney infections and described some of its classical clinical symptoms. The treatment of malaria and diabetes with 1.14/ 6.86 mg/kg Artemether Lumefantrine and 200 mg/kg Metformin significantly decreased the concentration of the creatinine and urea in the blood when compared with the infected groups. It can be seen from the results that the standard drugs were able to decrease the concentration of urea and creatinine in the treatment groups which means it may not have toxic effects in the liver and also indicated the efficacy of the standard drugs in treating malaria and diabetes. Kidneys maintain optimum chemical composition of the body fluids by acidification of urine and removal of metabolite wastes as urea, uric acid, creatinine and ions (Biyani *et al*., 2003). During renal disease, the concentrations of these metabolites increase in blood.

The result gathered from this study has a very high correspondence with a scientific study done on diabetic mice infected with malaria by Balogun *et al*. (2012), and the study confirmed that there was high level of urea in the malaria-infected and diabetic-induced groups. Hence, elevated creatinine and urea levels may play a part in the settlement of acute kidney injury in the co-infection of malaria and diabetes.

Tumour necrosis factor alpha (TNF- α) is implicated in systemic inflammation and activates the acute phase response (Moller, 2000). According to Langhorne *et al*. (2008), *P. falciparum*-

induced antibodies play a part in both the pathogenesis and protection against malaria. High levels of serum TNF- α are also correlated with disease severity and mortality. Results from this study show that there was a significant increase TNF- α concentration of the malaria only and co-infected group of malaria and diabetes when compared across all other groups. This is as a result of the invasion of infected sporozoites or blood stage parasite from the plasmodium berghei which resulted into an impulse of reaction that secreted pro-inflammatory cytokine such as TNF- α which supports the conclusion of Liabagui *et al*., 2017. There was no significant increase of TNF- α in the diabetes induced group but an increase was seen in the co-infected group with metformin only treated group which however, could imply that metformin has active compounds that could probably induce inflammatory responses in the process of insulin sensitization.

Based on the results from this study, the concentration Interleukin-6 was significantly higher in the diabetes only group and co-infected group of malaria and diabetes relative to all other groups when compared to the normal control. This could probably be as result of the immune system response to the release of the pro-inflammatory cytokine, in this case IL-6, to signal other cells on the invasion. A study from Akbari and Zadeh, 2018 confirmed that the increased circulating amount of IL-6 is thought to play a role in the emergence of inflammation, insulin resistance, and beta-cell dysfunction and it is also an independent predictor of Type 2 Diabetes. As observed in the study, there was an increase in Interleukin-6 concentration of the malaria group treated with Artemether Lumefantrine and also the co-infected group that was treated with both Artemether Lumefantrine and Metformin. This simply indicates that the standard drugs increased the cytokine activity with respect to inflammation.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

As an anti-malaria medication, Artemether Lumefantrine was found to have a significant impact on the decrease in parasitemia count in this investigation. The therapeutic effects of metformin on lowering blood sugar levels in the experimental mice were also validated by this study. The results of this investigation demonstrated that the therapy for malaria is not prophylactically affected by metformin. This study offers proof that people with diabetes have a higher risk of contracting *P. berghei* infection. The risk growing with rising glucose concentration is a symptom of physiological plausibility, even though the underlying causes are uncertain.

5.2 RECOMMENDATION

Future study on the co-infections of malaria and diabetes is advised in order to identify treatment approaches that could treat both co-morbidities simultaneously.

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APPENDIX

Appendix I: The Data obtained from mice study.

Selected dose	**Animal body weight (g)**	**Average weight (g)**	**Stock solution (conc.)**	**The daily dose in mL**
Group 1 (Normal Control)	1A[H] 25	26		0.5
	1B[T] 24			0.5
	1C[B] 30			0.6
	1D[Um] 24			0.5
Group 2 (Diabetes Only) (40mg/kg STZ)	2A[H] 25	25		0.5
	2B[T] 24			0.5
	2C[B] 26			0.5
	2D[Um] 26			0.5
	2E[LL] 23			0.5
	2F[RL] 23			0.5
	2G[HT] 27			0.5
	2H[RLT] 25			0.5
Group 3 (Malaria Only)	3A[H] 25	24		0.2
	3B[T] 25			0.2
	3C[B] 23			0.2
	3D[Um] 26			0.2
	3E[RL] 23			0.2
Group 4 (Malaria+ Diabetes Only) (40mg/kg STZ)	4A[H] 28	25		0.6
	4B[T] 22			0.4
	4C[B] 22			0.4
	4D[Um] 27			0.5
	4E[LL] 28			0.6
	4F[RL] 22			0.4

	4G[HT] 30			0.6
				0.5
	4H[RLT] 23			
Group 5 (Malaria+AL)	5A[H] 28	26	15 mg in 42 mLs Normal Saline for 3 days	0.5
	5B[T] 24			0.5
	5C[B] 25			0.5
	5D[Um] 27			0.5
	5E[LL] 26			0.5
	5F[RL] 26			0.5
Group 6 (Diabetes+MTF) (40mg/kg STZ) (200mg/kg MTF)	6A[H] 29	27	837 mg in 81 mLs Metformin for 7 days	0.5
	6B[T] 24			0.4
	6C[B] 27			0.5
	6D[Um] 22			0.4
	6E[LL] 28			0.5
	6F[RL] 28			0.5
	6G[HT] 30			0.6
Group 7 (Malaria+ Diabetes+MTF) (40mg/kg STZ) (200mg/kg MTF)	7A[H] 24	24	837 mg in 81 mLs Metformin for 7 days	0.5
	7B[T] 26			0.5
	7C[B] 26			0.5
	7D[Um] 23			0.5
	7E[LL] 27			0.6
	7F[HT] 23			0.5
	7G[RL] 23			0.5
				0.5
	7H[S] 23			
Group 8 a(Malaria+	8A[H] 22	25	837 mg in 81 mLs Metformin for 7	0.4
	8B[T] 24			0.5

Diabetes+ MTF +AL) (40mg/kg STZ) (200mg/kg MTF) (1.14/ 6.86 mg/kg AL)	8C[B] 27		days AL (15 mg in 42 mLs Normal Saline) for 3 days	0.5
	8D[Um] 24			0.5
	8E[LLB] 24			0.5
	8F[RL] 24			0.5
	8G[HT] 33			0.6
	8H[LAT] 25			0.5

APPENDIX II: ELISA readings of TNF-α on microplate reader

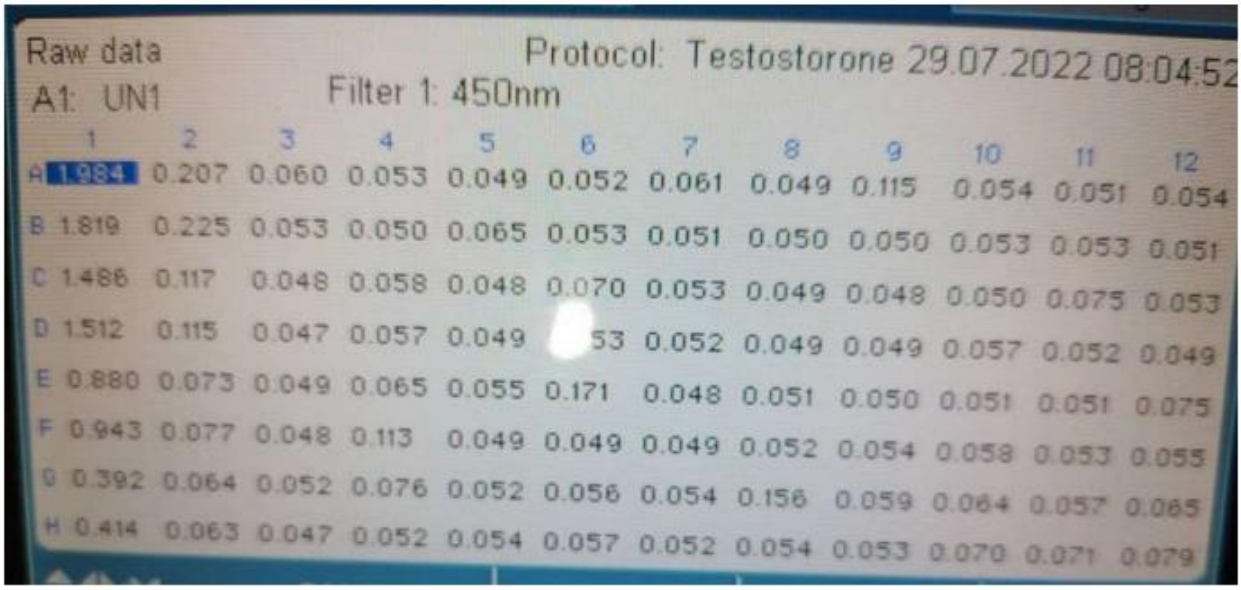
TNF-alpha

Raw data — Protocol: Testostorone 29.07.2022 06:11:16
A1: UN1 — Filter 1: 450nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.500	0.239	0.088	0.332	0.320	0.348	0.276	0.484	0.686	0.123	0.384	0.316
B	2.170	0.230	0.077	0.373	0.310	0.554	0.261	0.663	0.351	0.188	0.172	0.488
C	1.438	0.146	0.244	0.382	0.182	0.326	0.294	0.316	0.403	0.299	0.230	0.535
D	1.427	0.135	0.303	0.299	0.290	0.608	0.401	0.508	0.955	0.595	0.157	0.107
E	1.031	0.105	0.189	0.479	0.309	0.534	0.130	0.568	0.665	0.736	0.190	0.146
F	0.711	0.131	0.486	0.441	0.272	0.287	0.440	0.741	0.127	0.550	0.330	0.483
G	0.592	0.092	0.293	0.340	0.473	0.257	0.499	1.360	0.791	0.516	0.692	0.746
H	0.420	0.101	0.252	0.272	0.312	0.295	0.387	0.526	0.268	0.137	0.614	0.154

APPENDIX III: ELISA readings of IL-6 on microplate reader

IL-6



Raw data — Protocol: Testostorone 29.07.2022 08:04:52
A1: UN1 — Filter 1: 450nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.984	0.207	0.060	0.053	0.049	0.052	0.061	0.049	0.115	0.054	0.051	0.054
B	1.819	0.225	0.053	0.050	0.065	0.053	0.051	0.050	0.050	0.053	0.053	0.051
C	1.486	0.117	0.048	0.058	0.048	0.070	0.053	0.049	0.048	0.050	0.075	0.053
D	1.512	0.115	0.047	0.057	0.049	0.053	0.052	0.049	0.049	0.057	0.052	0.049
E	0.880	0.073	0.049	0.065	0.055	0.171	0.048	0.051	0.050	0.051	0.051	0.075
F	0.943	0.077	0.048	0.113	0.049	0.049	0.049	0.052	0.054	0.058	0.053	0.055
G	0.392	0.064	0.052	0.076	0.052	0.056	0.054	0.156	0.059	0.064	0.057	0.065
H	0.414	0.063	0.047	0.052	0.054	0.057	0.052	0.054	0.053	0.070	0.071	0.079