EFFECT OF COMPLEX *Moringa oleifera and* VipAlbumin *(*MOVA)TO DEVELOP T CELL AND B CELL IN DIABETES MICE

THESIS

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BRAWIJAYA UNIVERSITY

2019

APPROVAL PAGE

Thesis

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Exam Date : 21 October 2019

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Malang, November 2019

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BIOGRAPHY

Suheer Khalleefah Almabrouk, was born 10 July 1981 in Tripoli the Capital of Libya, and Studied elementary School Saif Al-Islam and then moved to a high school called 24th March , and Continued my education at Tripoli University. Faculty of Medical Technology, Department of Dentistry. Graduated in 2009 and then I got a scholarship from my country to study abroad. I studied master in Indonesia. Faculty of Mathematics and Natural Science, Brawijaya University in February 2017.

Malang , November 2019

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INTRODUCTION

Effect of Complex *Moringa oleifera* and *Albumin* to Develop T Cell and B Cell in Diabetes Mice

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BRAWIJAYA UNIVERSITY

2019

Health is the important thing for everybody, while on the other hand disease arise because of human lifestyle and also genes. One of the most common disease in the world is diabetes mellitus (DM). According to International Diabetes Federation (IDF), the number of youth (0-14 years) diagnosed with type 1 diabetes worldwide in 2013 was 497100 and the number of newly diagnosed cases per year was 78900.

Type 1 diabetes is mainly due to an autoimmune destruction of the pancreatic β cells through T-cell mediated inflammatory response (insulitis) as well as a humoral (B cell) response. This research used the leaf extract of *Moringa oleifera*. *Moringa oleifera* leaf could decrease plasma and urine glucose and improve glucose tolerance test. Hyperglycemia condition will increase the level of *Reactive Oxygen Species* (ROS) and lead to oxidative stress. However, oxidative stress can be resolved by exogenous antioxidant. One of the strong antioxidant obtained from snakehead fish (*channa striata*). This fish albumin is found to act as antioxidant and also will overflow (-SH), which serves as a binder radical that plays a role in the arrest of ROS.

The aim of this research is to find which dose of *Moringa oleifera* and Albumin can reduce blood glucose levels and inflammation in diabetic mice. Second, to analyzed if *Moringa oleifera* and Albumin extract can reduce inflamation cytokine (-TNFa,IFNg) in diabetics balb/c mice. Third aim is to analyzed if *Moringa oleifera* and Albumin influence profile changing of T-cell ($CD4^{+}CD8$), CD4 and B220, in the diabetic balb/c mice.

This research will combine *Moringa oleifera* with Snakehead fish albumin. This research will be using 35 adult balb/c rats in a plastic cage or glass. Weight 250-300 (75-90 days old), and acclimatized for a 7 days prior to experimental use. They were fed with standard pellet diet and tap water *ad libitum*. The mice were then divided into five groups. The mice were injected by streptozotocin at the dose of 150 mg/kg of the body weight intravenously. Streptozotocin induces diabetes within 3 days by destroying the beta cells.

The result of this research on the developing of T Cell on the CD4 and CD8 there is no signification (1,84±0.77%) compared to normal control. After giving *Moringa oleifera* extract and Albumin we can conclude that the small dose of *Moringa oleifera* and albumin not give significant in each doses. The result of developing of B cell in the (B220) showed

that, dose 2 with 150 mg/kg *Moringa oleifera* + 208.15 mg/kg Albumin give significant differences in decrease the relatives number of B220. The result of developing of cytokines TNF-α and IFN-γ by CD4+. STZ injection in DM positive increased relatives number of CD4TNf-α (4,2±4,21%) and decreased relatives number of CD4IFN-γ (2,05±1,24%) compared to normal control for CD4TNf-α (3,76±2,41%), while CD4IFN-γ (2,43±2,64%). It has been proven that the treatment of *Moringa oleifera* and VipAlbumin® could decrease the relatives number of T cell CD4, TNF-α, and IFN-γ but not significant.

The conclusion of this research is the right dose of *Moringa oleifera* combined with VipAlbumin® is dose 3 with 50 mg/kg body weight *moringa oleifera* + 624.375 mg/kg body weight VipAlbumin®, The Administration of *moringa oleifera* and VipAlbumin® gave a decrease significant result on pro-inflammatory cytokines. This study showed that TNF-α produced by CD4 T cells decreased compared to control positive DM in Dose 3. Based on the expression showed on the flow cytometry result, *moringa oleifera* and VipAlbumin® extract showed could reduce the inflammation caused by STZ on the diabetic mice. The number inflammation found decreased on the T-cell $CD4^+$, $CD8^+$, TNF-α, IFN-γ and B220. *Moringa oleifera* combined with VipAlbumin® gave the potential result as anti-inflammatory. *Moringa oleifera* and VipAlbumin® extract can be use to cure the inflammation in DM. The activity of *moringa oleifera* and VipAlbumin® on the right dose found decreases the relative number of macrophage cells and proinflammatory cytokines such as TNF-α, IFN-γ and CD4, CD8.

SUMMARY

Effect of Complex *Moringa oleifera* and *VipAlbumin* (MOVA) To Develop T Cell and B Cell in Diabetes Mice

Health is the important thing for everybody, while on the other hand disease arise because of human lifestyle and also genetic. One of the most common disease in the world is diabetes mellitus (DM). According to International Diabetes Federation (IDF), the number of youth (0-14 years) diagnosed with type 1 diabetes worldwide in 2013 was 497100 and the number of newly diagnosed cases per year was 78900.

diabetes is mainly due to an autoimmune destruction of the pancreatic β cells through T-cell mediated inflammatory response (insulitis) as well as a humoral (B cell) response. This research used the leaf extract of *Moringa oleifera*. *Moringa oleifera* leaf could decrease plasma and urine glucose and improve glucose tolerance test. Hyperglycemia condition will increase the level of ROS and lead to oxidative stress. However, oxidative stress can be resolved by exogenous antioxidant. One of the strong antioxidant obtained from snakehead fish (*channa striata*). This fish albumin is found to act as antioxidant , which serves as a binder radical that plays a role in the arrest of ROS.

The aim of this research is to determine which dose of *M.oleifera* and VipAlbumin (MOVA*)* can reduce blood glucose levels and inflammation in diabetic mice. Second, to analyzed if *M. oleifera* and Albumin extract can reduce inflamation cytokine ($TNFa^+$,$IFNg^+$) in diabetics balb/c mice. Third aim is to analyzed if *M. oleifera* and *VipAlbumin* influence profile changing of lymphocyte cell $CD4^+$ and $CD8^-$ and B220, in the diabetic balb/c mice.

This research will combine *M. oleifera* with *VipAlbumin.* This research will be using 25 balb/c mice in a plastic or glass cage . Weight 25-30g (6-7 weeks old), and acclimatized for a 7 days prior to experimental use. They were fed with standard pellet diet and tap water *ad libitum*. The mice were then divided into five groups. The mice were injected by streptozotocin at the dose of 145 mg/kg of the body weight intravenously. Streptozotocin induces diabetes within 3 days by destroying the beta cells.

The result of this research on the developing of T Cell $CD4^+$ and $CD8^-$ is It had become increase (2,52±0.96%) compared to normal control. After giving MOVA. we can conclude that the small dose of *M. oleifera* and *Vipalbumin* not give significant in each doses. The result of developing of B cell in the (B220) showed that, dose 2 with 150 mg/kg *M. oleifera* + 208.15 mg/kg Albumin give significant differences in decrease the relatives number of B220. The result of developing of cytokines TNF-α and IFN-γ^+ by CD4+. Can process the inflammation of $CD4^+$TNf-α (4,21±4,35%) and also $CD4^+$ IFN-γ (2,05±1,24%) in D1 and D3 compared to normal control for $CD4^+$TNf-α (3,76±2,41%), while CD4 +IFN-γ+ (2,43±2,64%) and DM positive. It has been proven that the treatment of *M .oleifera* and *VipAlbumin*® could decrease the inflammation of T cell CD4+, TNF-α, and IFN-γ after giving the treatment.

The conclusion of this research is the right dose of *Moringa oleifera* combined with VipAlbumin® to reduce blood glucose levels is dose 1 with 100 mg/kg body weight *(MO)* + 624.375 mg/kg body weight (VA), The Administration of *M .oleifera and VipAlbumin*® gave a decrease significant result on pro-inflammatory cytokines. This study showed that TNF-α produced by (CD4+) decreased compared to control positive DM and normal control in dose 1 and dose 3. Based on the expression showed on the flow cytometry result, the complex of MOVA showed could reduce the inflammation caused by STZ on the diabetic mice. The number inflammation found decreased on $CD4^{+}$, TNF-α^{+}, IFN-γ^{+}. *M .oleifera* combined with VipAlbumin® gave the potential result as anti-inflammatory. *M .oleifera* and VipAlbumin® extract can be use to cure the inflammation in DM. The activity of *M .oleifera* and VipAlbumin® on dose 2 found increase the relative number of (B220) cells, and dose 3 can decrease the relative number of $B220^{+}$.

Foreword

The first .Alhamdulillaahi Robbil alaamin for helped and succeeded. To allah all thanks and gratitude.

Alhamdulillaah I got my master degree of science in biology department faculty of mathematice and natural science brawijaya university , Malang .on this occasion the author wishes to express her thanks to persons who supported and helped her to finish this thesis.

1. Prof. Muhaimin Rifa'I, S. Si., Ph. D , MED ,Sc. As supervisor I. Thank you with all my respect for your accompanied and gave guidance and additional knowledge, suggestions to me.
2. Dr. Sri Rahayu, M. Kes As supervisor II .Thank you for guidance advices and knowledge that given to me .I really appreciate it.
3. Prof. Dr. Ir. Moch. Sasmito Djati, Ms. As Examiner I, And Dr. Sri Widyarti, M. Si, Thank you for providing guidance, advice and all the valuable information you have provided to me.
4. My Father and my Mother. Without your support I cannot succeed thank you so much for your guidance and your pray for me.
5. Almabrouk khalleefah My brother, thank you for being with me in all difficult moments to support and motivate for the better.
6. Noviana Dwi lestari, S. Si. thank you for everything that you have give, help and support in this research .and all the friends in the physiology laboratory. Mr. Bambang Pristiwanto, M. Si and Sapti puspitarini S. Si .
7. Rani Nur Arsy Ekananda. Thank you so much for your help and your support, really appreciate it.
8. My friends in Libya who supported and prayed for my success.
9. Brawijaya University Team. I really proud that I studied at this University.
10. Libya. Thank you for giving me this opportunity .hope peace will prevail in Libya

TABLE OF CONTENTS

LIST OF TABLE

Pg.

LIST OF FIGURES

Pg.

CHAPTER I
INTRODUCTION

1.1 Background.

Diabetes Mellitus (DM) referred to as one all told the foremost common illness inside the globe. Rates of this genetic defect in 1985 were calculable at 30 (thirty) million, increasing to a 135 (a hundred thirty five) million in 1995 and 217 million in 2005. This increase is believed to be primarily caused of the world population aging, a decrease in exercise, and increasing rates of fat. The five countries with the highest variety of people with polygenic disease as of 2000 are India has 31.7 million, China 20.8 million, The U.S. 17.7 million, Republic of Indonesia 8.4 million, and Japan 6.8 million. It is recognized as a worldwide epidemic by the WHO.(Gardner, et al, 2011).

Some data based on The International Diabetes Federation (IDF) state that, "the number of youth (0-14 years) diagnosed with type 1 diabetes worldwide in 2013 was 497100". IDF also giving some data about the amount of newly diagnosed of diabetes, IDF found that the new diagnosed case of diabetes mellitus count was 78900 case per year (IDF, 2013). Based on the data by IDF, there is a finding that these number not indicates the total amount of type 1 diabetes patients because of the high prevalence of type 1 diabetes in adolescence above 14 years old. In 2010, reported that about 3 million people were diagnosed with type 1 diabetes in the U.S. (Prime Group, 2010; Chiang, *et al.*, 2014). This indicates a significant increase in the amount of patients after a report in 2009 stated that the number of youth below 20 years of age diagnosed with type 1 diabetes was estimated about 166984 people (Pettitt, 2014). Although the prevalence number of type 1 diabetes in the world is still unknown, but in the United States, there was a 2.6%-2.7% relative annual increase (Dabelea, 2009; Lawrence, 2014).

Type 1 DM is mainly caused by an autoimmune devastation of the pancreatic β cells through T-cell mediated inflammatory response which dominated by mononuclear cells or insulitis as good as a humoral (B cell) response (Devendra, 2004). The diagnostic standard number of fasting plasma glucose is different, but American Diabetes Association stated a data about the diagnostic standard of DM is (a) a fasting plasma glucose level ≥ 120 mg/dL (5.6 mmol/L) for normal glucose, or (b) a 2-h plasma glucose level ≥ 125 mg/dL (5.6 – 6.9 mmol/L) for a 75 g oral glucose tolerance test, or

(c) a random plasma glucose level ≥ 200 mg/dL (11.1 mmol/L) combined with classic symptoms of hyperglycemia or hyperglycemic crisis, at this step, people diagnosed DM (Longhui, *et al*, 2015).

Nowadays, people with DM prefer traditional treatment for healing. A data from World Health Organization (WHO) found that 80% of the people in developing countries believed on traditional medicine practices (Jangir & Jain, 2016). They use plants to decrease a diabetic and provide better alternatives that are less toxic, easily available and affordable.

Since there is an indisputable fact that oxidizing agent stress concerned polygenic disorder, this analysis examines herbals that comprise medicament. One in every of the plants that used for ancient drugs to treat diabetes mellitus in most developing countries is *Moringa oleifera*. It is referred to as a quick growing tree species native to northwestern India, that is currently cultivated in several areas worldwide. It is helpful not just for human however conjointly for animals and in varied industrial applications. The leaves, fruit, flowers and immature pods of this tree square measure used as an extremely wholesome vegetable in several countries, significantly in India, Pakistan, Philippines, Hawaii and Africa (Iwara, et al, 2014). Lotseach a part of this plant, as well as root, bark, gum, leaf, fruits (pods), flowers, seed and seed oil usually used for the medication of varied ailments within the autochthonal system of medication.

This research used the leaf of *M .oleifera*. In some cases, it had been found that *M. oleifera* leaf suplementation powder will dilute blood glucose serum and LDL (Kumar & Mandapaka, 2013). *M. oleifera* leaf might decrease plasma and urine glucose and improve glucose tolerance test. A findings from other research stated that there is a hypoglycemic result proved to be related to dilute intestinal glucose uptake and speed stomachal remission time by fiber in *M. oleifera* leaf (Taweerutchana, et al, 2017).

Some studies found that hyperglycemia and a hypoglycemic insulin deficiency will influence the structure and functions of tissues, as well as the structure of proteins, also it has been stated that hyperglycemia can increase the amount of ROS and cause oxidative stress (Hidayati, et al, 2016). Hyperglycemia within the generation of oxidative stress resulting in epithelial tissue dysfunction in the blood vessels of diabetic patients. However, oxidative stress is often resolved by exogenous antioxidant. One in every of sturdier antioxidant gained from snakehead fish (Channa striata). This fish albumin is used to act as antioxidants.

Some research developed by Kharroubi and Darwish state that the autoimmune condition in type 1 diabetes was marked with the absence of insulin secretion (Kharroubi & Darwish, 2015). Another fact state that DM type 1 linked with a number of genes adjusting T, B, and innate cell Immunobiology, as are genetic variants basic to β cells, which demolish affect β cell purpose and/or responses to inflammation (Bottini, *et al.*, 2004; Dooley, *et al.*, 2016).

Xia, *et al* (2017) developed some research and stated that inflammation caused by the shortage of insulin secretion was linked to IR and could increase the number of TNF-α in adipose tissue. Furthermore, Inflammatory cytokines such as IL-1β and IFN-γ which are increased in obesity also modulate insulin signalling. The Immune system also being a key role in the pathogenesis of DM. Some research discovered that the immune system not only affected by the hypoglycemic action, but also by the simultaneously acidosis or ketosis that arises in people with DM (Daoud, *et al*, 2009). The mechanism responsible to increase the prevalence of infection is still to be determined because most of the perform on the inflammation biomarkers and change in the immune system which linked with adipose tissue in the existence of obesity (Richard, *et al*, 2017).

With the condition of hypoglycemia in the DM patients, this research examines Moringa oleifera leaves and also Vipalbumin® tested in an animal using streptozotocin (STZ) as a toxic agent to the β-cells of the pancreatic islet of Langerhans, which usually adjust blood glucose levels by producing insulin hormone (Sithole, 2009). Vipalbumin® used in this research contains of snakehead fish A research developed by Dwijayanti (2015) state that Vipalbumin® could increase the number of Regulatory T cells, decreased the relative number of Macrophage cells ($CD68^{+}$) And also decreased the Number of Proinflammatory Cytokine TNF-α, IFN-γ and IL-6.

1.2 Research Problem

1. Which dose of complex *M. oleifera* and *VipAlbumin* is able to reduce blood glucose levels in DM mice?
2. Can *M. oleifera* and *VipAlbumin* reduce inflammation cytokine (IFN-γ, TNF-α)?
3. How do *Moringa oleifera* and *VipAlbumin* influence profile changing of lymphocyte cell T-cell $CD4^{+}$ and $CD8^{-}$ and B cell $B220^{+}$?

1.3 Research Aim

1. To find which dose of complex *M. oleifera* and *VipAlbumin* can reduce blood Glucose levels.
2. To analyze if *M .oleifera* and *VIPAlbumin* can reduce inflammation cytokine (IFN-γ, TNF-α).
3. To analyze if *M.oleifera* and *VipAlbumin* influence profile changing of lymphocyte cell T-cell $CD4^{+}$ *and* $CD8^{-}$ And B cell $B220^{+}$?

1.4 Research Benefits

1. Giving an input and new information about medical research for another medical alternative of Diabetes treatment.
2. Applying *M.oleifera and VipAlbumin* as supplement of Diabetes treatment.
3. Proving that *M. oleifera* and *VipAlbumin* can serve as herbals to other inflammatory disease.

CHAPTER II
LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes Mellitus (DM) may be a worldwide illness. The amount of The prevalence of polygenic disease is increasing speedily worldwide and therefore the World Health Organization (WHO) has foretold that year by 2030 the aggregate of adults with polygenic disease would have nearly doubled worldwide. In 2000 reported that there is 177 million people were diagnosed with DM, but by 2030, the number would be doubled to 370 million. Another research reported that in 2010 the estimated worldwide prevalence of diabetes among adults was 285 million (6.4%) and this amount is predicted to rise around 439 million (7.7%) by 2030 (Shaw, *et al*, 2010). This disease generated by an affiliation of heterogeneous disorders of hyperglycemia and glucose intolerance as a result of lack of insulin, invalid insulin action or both. Another fact is that this disease accompanied by any risk disease, like cardiovascular disease, cerebrovascular disease and also peripheral vascular (Piero, *et al*, 2014).

There is a founding stated that a steady hyperglycemia in DM may induce a free radical like (ROS) reactive oxygen species and also nitrosative species (RNS). This two kind of free radical is a vital issue for DM macro- and microvessels complications. An activity decreased of antioxidant enzymes is known to be the origin of epithelial tissue dysfunction, insulin resistance, and DM complications. This condition happen because there is a production of ROS and RNS, (Roman-Pintos, *et al*, 2016).

There are two common types of DM, referred as type one DM (T1DM) and type two DM (T2DM). Type one diabetes, additionally referred as insulin dependent DM (IDDM), caused by insulin secretion deficiency by pancreatic beta cells. A number of the symptoms embrace weight loss, polyurea, polydipsia, polyphagia, constipation, fatigue, cramps, blurred vision, and mycosis (Bearse, et al., 2004). Long term type one DM patients might at risk of microvascular complications; (Hove, et al., 2004; Seki, et al., 2004; Saely, et al., 2004) and macrovascular unwellness (coronary artery, heart, and peripheral vascular diseases) (Pittas, 2009). Meanwhile, type two diabetes mellitus, additionally known as (NIDDM) noninsulin dependent diabetes mellitus. This type of DM is caused by a decrease the sensitivity of the object's tissue to insulin. The reduced sensitivity to insulin is usually referred to as insulin resistance (IR).

In each varieties of diabetes mellitus, there is a modification in the main metabolism. The basic result of insulin deficiency or insulin resistance (IR) on glucose metabolism is to avoid the absorption uptake and utilization of glucose in most cells of the body, except the brain (Guyton & Hall, 2006). As a result, blood glucose concentration will increase, cell utilization of glucose will decrease and exertion of fats and proteins will increase.

Type1 DM known as a chronic autoimmune disease which is usually related with the destruction of pancreatic β-cells filtrate of insulin-producing. Beta cell destruction is caused by an autoimmune process, and this process will lead to absolute insulin deficiency (Kumar, 2002).

The illness is characterised by the islet progressive infiltration inside the pancreas by the immune system cells with main roles of $CD8^+$and $CD4^+$T-cells, besides as macrophages. This infiltration may end up in insulitis and therefore the impairment of insulin production (Bending, et al, 2012). Many options characterize T1DM as an autoimmune disease (Raju & Raju, 2010). The presence of immuno-competent and accessory cells in infiltrated pancreatic islets association of susceptibility to illness with the class II (immune response) genes of the (MHC) major histocompatibility complex and human blood cell antigens (HLA). The presence of islet cell specific autoantibodies, transformation of immunoregulation in the mediated T cell, above all in $CD4^+$ T cell compartment, the involvement of monokines and TH1 cells producing interleukins within the illness process, response to therapy and frequent prevalence of different organ specific auto- immune diseases in affected people or in their relations.

Some founding research developed by Dooley stated that some of genes regulating T, B, cell immunobiology are also relate with T1DM, as are genetic variants to β cells, which deleteriously affect β cell function and responses to inflammation (Dooley *et al.*, 2016). Almost overall of T cells ($CD4^+$, $CD8^+$) penetrate to the islets of T1DM subjects through a (Th1) T helper 1—the effector phenotype, marked by IFNγ secretion (Walker, *et al*, 2016).

2.2 Insulin Resistance (IR)

The pancreatic β-cells autoimmune destruction, directly leads to a deficiency of insulin secretion which results in the metabolic disorder which linked with T1DM. The function of pancreatic α-cells will be abnormal and there is an excessive secretion of

glucagons in T1DM patients. This condition caused by the loss of insulin secretion. Normally, hyperglycemia leads to reduced glucagons secretion, but, some research developed by Holt (2004) found that in patients with T1DM, glucagons secretion is not suppressed by hyperglycemia. Furthermore, Holt explained that the resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin deficiency. Holt analysis conjointly expressed that hormone deficiency is that the primary defect in T1DM, however there is conjointly a defect within the administration of hormone. Deficiency in insulin results in uncontrolled lipolysis and raised a grade of free fatty acids inside the plasma, glucose metabolism in peripheral tissues like muscle (Holt, 2004). This impairs glucose utilization and insulin deficiency conjointly decreases the expression of variety of genes necessary for target tissues to reply commonly to insulin like glucokinase in liver and therefore the GLUT four category of glucose transporters in fatty tissue. Holt (2004) explained that the key metabolic derangements, that result from insulin deficiency in T1DM impaired glucose, lipid and protein metabolism.

In several patients of T1DM, relative insulin deficiency is in association with peripheral insulin resistance (IR) (Botero, 2005). IR could happen because there is a counteration to the insulin can lead to impaired insulin mediate glucose uptake within the periphery incomplete suppression by muscle and fat of hepatic glucose output and impaired triglyceride uptake also by fat.

IR may be a condition wherever a standard or elevated insulin level produces associate degree attenuated biological response (Wilcox, 2005). Furthermore, this condition refers to refers to impaired sensitivity to insulin mediating glucose removal. Another suggestion states that (IR) may be a condition in 3 primary metabolic tissue which are sensitive to insulin, namely the skeletal , white adipose tissue (WAT) and liver. Decreased sensitivity to insulin and the action of metabolism to flow below normal serum glucose concentrations are also closely related to obesity, hypertension, hyperglycemia , and metabolic syndrome (Chen, 2015).

Since obesity being the major cause of IR, there are two clinical entities linked to IR, first is an inflamation with an activated immune/repair system; and increased mental activation (Straub, 2014). in adipocytes, which activates (JNK) c-Jun N-terminal kinase and also nuclear factor. Obesity causes lipid accumulation kappa B (NF-κB) signaling pathways and subsequently can increase the production of cytokine proinflammatory like tumor necrosis factor-alpha (TNF-α^{+}) and interleukin-6 (IL-6).

Some data stated that a deficient insulin sensitivity and adiponectin levels have been found in the obese and overweight case, and it had a positive correlation. Otherwise, TNF-α receptors have a negative correlation with insulin sensitivity, this condition will lead to the conclusion that TNF-α may be related to insulin resistance (IR) through its association with substrate oxidation under conditions of hyperinsulinemia and non-oxidative glucose metabolism (Roman-Pintos, et al, 2016).

IR in muscle and adipose tissue is devloped by the glucose–fatty acid cycle. IR is an outcome of an increased presence of circulating fatty acids and ketone of the bodies that lead to failure in glucose exertion and an ever increasing insensitivity to insulin.

One findings about inflammation stated that systemic obesity-induced inflammation and insulin resistance begin in adipose tissue, furthermore it will releases cytokines, adipokines and fatty acids, flew down on the liver and muscle ensue (Shu, *et al*, 2012). Dysfunctional adipose tissue is a main cause of inflammation. Increased adipose tissue is an important scenario and source of inflammatory cells and mediators. This inflammation condition head to insulin resistance and ultimately to hyperglycemia by exhaustion of the adaptive capacity of pancreatic beta cells (Franscisco, et al, 2016).

Adipose tissue comprehend adipocytes and also a heterogeneous constellation of adipocyte precursors, nerveterminals, blood vessels, and leukocytes collectively termedthe "stromal vascular compartment" (SVC). A different types of fat will developed an adipose tissue. These findings point to visceral adipose tissue (VAT, eg benign fat or epididymis) and are found to be a high effect on insulin sensitivity, a higher advantage of major adipocytes, and also given a high number of inflammatory cells when compared to subcutaneous adipose tissue (SAT) (Shu, *et al*, 2012).

VAT inflammation in obesity is a result of tissue accumulation of pro-inflammatory immune cells that include M1 macrophages, $CD8^+$ T cells, (Th1) cells, β-cells, natural killer cells (NK), and neutrophils, and a decreased in the proportion of anti-inflammatory immune cells such as macrophage.2 (M2), regulatory T cells (Tregs), eosinophils, and also, type 2 innate lymphoid cells (ILC2s) (Winer, *et al*, 2016). Macrophages are a key role, even though many constituent of the immune system that have been play a role in promoting or attenuating adipose tissue inflammation, (Patel, *et al*, 2013).

2.3 Inflamation Process.

Obesity and adipose tissue inflammation can lead to increases in pro-inflammatory molecules like (TNF-α) tumor necrosis factor α, interleukin IL-6, resistin and free fatty acids. Proinflammatory cytokines and a phase reactants are related in several metabolic pathways that are relevant with insulin resistance (IR), including insulin regulation, reactive oxygen species (ROS), lipoprotein lipase action and adipocyte function. (Nazarro & Mora, 2005).

There is an inflammatory pathways to mediates IR in obesity. First, inflammatory signaling interferes with insulin action and mediates insulin resistance in obesity. and stimulates tyrosine phosphorylation from protein receptors to insulin receptors (IRS). Furthermore, IRS modifying enzymes, like an inflammation and stress caused by kinases Iκβ kinase-β (IKKβ) , resulting a incapacity of IRS-1 to be involved insulin receptor signaling.

TNF-α, IL-6 and resistin are cytokines that link inflammation to IR. TNF-α, IL-6 produced by classically activated or M1 macrophages. M1 macrophages are induced by interferon gamma (IFNg) and accumulate in VAT during diet-induced obesity, where they are the critical orchestrators of inflammation (Winer & Winer, 2012). M2 macrophages produce anti-inflammatory cytokines such as interleuki-10 (IL-10) and inteleukin-1 (IL-1) receptor antagonist, which protect against insulin resistance.

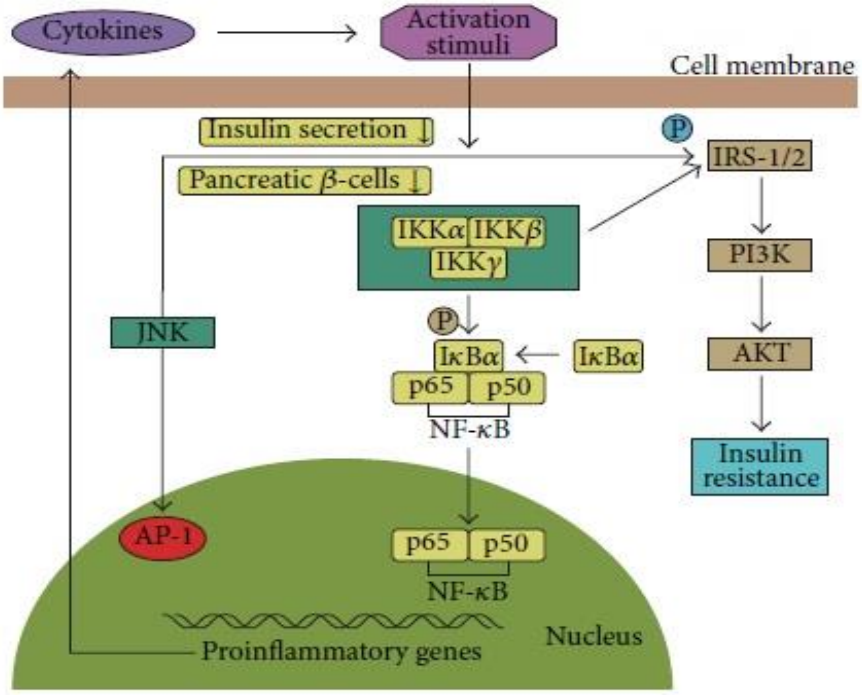
It was reported that TNF-α is an adipose tissue originated proinflammatory cytokine because of insulin resistance by enhancing lipolysis and increasing the phosphorylation (serine/threonine) of insulin receptor substrate-1.(IRS-1), while (IL-1β) is a pro-inflammatory cytokine which the secretion is regulated by inflammation activity (Chen, *et al*, 2015).

Pro-inflamatory cytokines such as TNF-α, activate IKKβ/NF-κB through receptor-mediated mechanisms which arealso activated by recognition receptors pattern, it is bound to substances as lipopolissacharide (LPS) from gram negative bacteria. These include the Toll Like Receptors (TLRs), and receptor for advanced glycation end products ((Longo, *et al*, 2014).

2.4 Involvement of T-Lymphocyte as Adaptive Immune System.

It has been suggest that activated $CD4^+$ T-cell increased the visceral adipose tissue (Xia, *et al,* 2017). $CD4^+$ effector T-cells could be divided into pro-inflamatory Th1, Th17, and anti-inflamatory Th2 and Treg. Th1 cells produce interferon- (IFN-γ),

interleukin-2 (IL-2) and tumor necrosis factor-beta (TNF-β), influence cell mediated immunity and phagocyte inflammation. While on the other hand, T helper cells 2-(TH2) produce interleukin-4 (IL-4), (IL-5), (IL-6), (IL-9), (IL-10), and interleukin-13 (IL-13) to regulate antibody responses.



Source : Chen, *et al*, 2015.

Fig. 2.1 Pathways of Inflammatory Linking Inflammation To Insulin Resistance.

Macrophage activation is affected by T-cells. Macrophages are the major inflammatory process in the adipose tissue in obesity. Adipose tissue macropages divided into two populations, pro-inflammatory M1, which is activated macrophage M2 and anti-inflammatory which is alternatively activated macrophage.

M1 macrophages release pro-inflammatory cytokines including interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-a), which is triggering local and inflammation, on the other hand M2 macrophages release IL-10, which inhibit the activity of the most pro-inflammatory cell types including M1 macrophages.

In the adipose tissue, when $CD4^+$ T cells were increased and induced the recruitment and differentiation of TNF-α and releasing M1. While from the other hand, quantity of IL-10 releasing M2 reduced in th adipose tissue.

$CD8^+$ T-cells were important for activation of macrophage induction and migration to adipose tissue by secreting MCP-1 and MCP-3. $CD8^+$ also known to be the the triggers of level produce of IFN-γ in obese individuals (Xia, *et al*, 2017).

2.5 *Moringa oleifera*

In case of Diabetes Mellitus, recent study state that, hyperglicemia-mediated oxidative stress being a key role in pathogenesis of diabetic complications such as nephropathy, thus the optimal antidiabetic medicine should combined both hypoglycemic and antioxidant properties (Tuorkey, 2016). *M. oleifera* known as *The Miracle Tree* or *horseradish-tree*, which have widely effective as treatment when used with high nutritional value (Luqman, *et al*, 2012).

Recent studies state that the leaves of *M .oleifera* is a valuable source of both macro- and micronutrients, β-carotene, protein, vitamin C, (Ca), and (K) , *Moringa oleifera* also act as natural antioxidants. Every part of *M.oleifera* such as root, bark, gum, leaf, friuts (pods), flowers, seed, and oil have been used for the medication of various medicine (Jangir & Jain, 2016).

Moringa oleifera root wood reported reduce urinary oxalate which increases and reduces constituent-forming rock deposits in calcogenic rat kidneys as a result of ethylene glycol treatment. While *Moringa oleifera* extract indicated potential benefits as antioxidant and antidiabetic activity (Al-Malky & El Rabey, 2015). Another research found that *M. oleifera* leaf suppresses the initiation and propagation of lipid proxidation and due to its phenolic content and help suppress atherosclerosis by scavenging hydrogen oxide radicals (Chumark, *et al*, 2007).

2.5.1 *Moringa oleifera* Composition

Recent study develop by Ali, *et al* (2015) explain that the flavonol quercetin is found in high concentrations as 100 mg/g of dried *M. oleifera* leaves, pre- dominantly as quercetin(-3-O-β-d-)glucoside is known as iso- quercitrin or isotrifolin. Quercetin is a potent antioxidants with multiple characteristic properties. It has shown antidyslipidemic, hypotensive, and also anti-diabetic effects in the Zucker rat model of metabolic syndrome.

Another composition find in *M. oleifera* leaf extract is Chlorogenic acid, the major phenolic acid is hydrocinnamic acid and quinic acid in *Moringa oleifera* leaves. Moreover, Chlorogenic acid can beneficially affect the process of glucose metabolism. It

has been hinder glucose-6-phosphate translocate in rat liver, lower hepatic gluconeogenesis and also glycogenolysis.

Recent study developed by Paula, *et al* (2017) found that there is a significant influence to the mice after *M. oleifera* treatment. By giving different dose of *M. oleifera* (300 and 500 mg/kg body weight), the results shown that *M. oleifera* leaves make hypoglycemic effect at both doses.

Based on the recent study, this research will use *M.oleifera* leaves to test against diabetic activity and also oxidative activity caused diabetes mellitus.

2.6 Albumin

This study will use supplement VipAlbumin® which made from Snakehead fish Albumin to reduce any diabetic and oxidative activity in the diabetic mice. The used of VipAlbumin® is based on the recent study developed by Dwijayanti, *et al*, (2015) which result is the effect of VipAlbumin® capsules. VipAlbumin® could increase the number of Treg cells and boost the healing process of DM by slowing down the inflammation that occurs in pancreas (Dwijayanti, et al, 2015). VipAlbumin® also contains of vitamin C and D which affect the activation of T reg cells.

This study will also combine *M. oleifera* and VipAlbumin® to reduce diabetes mellitus characterize by hyperglicemia, and proof that the combine of *M. oleifera* and albumin could be a treatment against diabetes mellitus.

2.7 Animals and Diabetogenic Agents

2.7.1 Balb/c mice

Recents study have found 99% of mouse genes have human counterparts. Mouse and its brothers rat, are moderately inexpensive to breed and to maintain. They can reproduce quickly, thus enable researchers to study the function of particular genes through several generations of offsprings during a period of time (Johnson, 2012).

Balb/c mice are an innate strain of albino, immunodeficiency. The main characteristic of this mice is an easy breeding and have minimal weight variations between males and females. The Jackson Laboratory stated that this mice produced plasmacytoma if its injected by mineral oil. This strain is known to have a high level of anxiety and is quite resistant to atherosclerosis triggered by food, so these mice are useful for cardiovascular research (Jax Mice Literature, 2006).

This research will be using 25 adult balb/c mice in a plastic cage or glass. Weight 25-30 (6-7 weeks old), and acclimatized for a 7 days prior to experimental use. And feed them with standard pellet and tap water *ad libitum*. The mice were then divided into five groups.

2.7.2 Diabetonic Agent

Streptozocin (STZ) is a glucosamine-nitrosourea compound that has been in clinical trial since 1967 (Singh & Pathak, 2015). and mediated by reactive oxygen species (ROS) that caused severe health problems figured in the increase of serum glucose and increase of glycosylated hemoglobin (Al-Malky & El Rabey, 2014). STZ damages pancreatic β cells, resulting in hyperglycemia and hypoinsulinemia (Graham, et al, 2011). STZ can induce a diabetic condition in 2 ways, and it was depend on doses. The selectivity for β cells related with preferential accumulation of the chemical in β cells after entry the GLUT2 glucose transporter receptor.

High-dose STZ injections have been shown to damage pancreatic β cell function, causing insulin secretion, which is thought to take after T1DM. While on the other hand, low-dose STZ injections have been reported to induce a gradual decrease in insulin secretion, which is similar to the natural development of DM in humans. (Qian, *et al*, 2015).

Some study reported that Intra-venous 60 mg/kg injection of streptozotocin in adult mice causes swelling of pancreas followed by decreasing of Langerhans islet beta cells that will stimulates diabetes mellitus in 2 or 4 days. Three days after beta cell decrease , diabetes will induced in the mice. Histopathological effects on beta cells that may be an intermediate for diabetes induction caused by Nicotinamide-adenine dinucleotide (NAD) in pancreatic islet beta cells.

The newly research shown that STZ induced for diabetes mellitus related to Nitric Oxide (NO) in β-cell pancreas. NO causes disfunction of β-cell pancreas, through necrosis and apoptosis. The disruption of β-cell pancreas through the DNA damage. STZ damage pancreatic beta cells selectively involving uptake by glucose transporter-2 It also generates reactive oxygen species (ROS) which contribute to DNA fragmentation other changes in the beta cells pancreas (Jangir & Jain, 2016).

This research will induce 145 mg/kg body weight STZ into Balb/c mice to prevent initial drug induced hypoglycemic mortality. Diabetes Development was after one week of STZ injection by measuring blood glucose level (Jangir & Jain, 2016).

2.8 Glucometer

The glucometers are the device developed to measure glycemia of capillary blood (Borin, *et al,* 2012), obtained through digital or heel puncture using a lance orhypodemic needle. This device is automatic and easy to use and determine the blood glucose contents by means of chemical reactions calculated from arranging reagent strips impregnated with glucose oxidase, peroxidase and chromogemy.

The main advantage of using glucometer are the small blood amounts required (1-5 µL of blood). The quickness results are given for 5-25 seconds and this device is also low cost.

2.9 Flow Cytometry Analysis

Flow Cytometry is a technology that can measure a single particle simultaneously then provides multiple physical characteristics, because the particle flows in a fluid flow through a beam light. A flow cytometer is built up of three main systems first, fluidics, which transports particles in a stream to laser beam for investigation. Second system is the optics system which consists of lasers to given a light to the particles in the hallway stream and optical filters to point resulting light signals to appropriate detectors. Third is electronics system, it will transform the detected light signals into electronic signals computer processed. For some instruments equipped with a sorting feature, the capable of initiating sorting decisions is electronics system to charge and deflect particles.

In this research, flow cytometry used to identify and quantify cellular antigens in the surface of cells or inside. Process involves the use of antibodies (immunoglobulins) to identify cell antigens. An important principle of cytometry flow data analysis is the gating procedure. this procedure visualizes the target cells selectively and removes the results from unwanted particles eg. dead cells and debris.

2.10 Conceptual Framework

Injections of high doses of STZ critically damages pancreatic β-cell functioning. STZ contains glucose molecules in the form of deoxy associated with methylnitrosourea which is highly reactive which is thought to have a cytotoxic effect of STZ, while the glucose portion directs chemicals to pancreatic β cells. Absolute insulin deficiency caused by progressive infiltration of islets pancreas assisted by cells of the immune system which cause the destruction of pancreatic cells producing insulin insulin

selectively. In the infiltration that result in insulitis and the impairment of insulin production. The damaged pancreatic β cells result in hypoinsulinemia and hyperglycemia. Cell damage caused by hyperglycemia increases production of (ROS) reactive oxygen species. Moreover, STZ also (ROS) generates that contribute to DNA fragmentation and evokes other changes in the beta cells pancreas. Along with overproduction of ROS, a reduction of the activity of antioxidant enzymes is known to cause endothelial dysfunction, insulin resistance, and DM complications.

Following that, defective central tolerance allows islet-reactive $CD4^+$ and $CD8^+$ T cells come out of the thymus and will reach the lymph nodes of the pancreas. In pancreatic lymph nodes, autoreactive CD4 + T cells interact with cells that form small island antigens and become helper cell as (TH1), T Follicular Helper (TFH), and Regulatory T Cells . TFH cells help β-cells produce composite islet-specific antibodies. TH1 cells activate dendritic cells and enhance antigen presentation to islet-specific $CD8^+$ T cells to induce effector $CD8^+$ T cell skewing. TH1 cells traffic to the pancreas, secreted pro-inflammatory cytokines such as interferon gamma (IFNγ) and TNFα, and induce beta cell death. TH1-derived IFNγ and TNFα stimulate M1 macrophages to produce reactive oxygen species (ROS) in the islets. TNFα, and IL-1β, which turn amplify beta cell death cycle. Resulting inflammation leads to increased T cell $CD8^+$, direct beta cell killing via perforin and granzyme B and attempts by nTregs and pTregs to dampen this response via TGFβ and IL-10. Beta cell death is mediated through cytokine production by T cell $CD4^+$ and $CD8^+$ within pancreatic islets. cytokines such as IFN-γ and TNF-α are directly toxic to beta cells.

Moringa oleifera is a rich source of β-carotene, protein, vitamin C, calcium (Ca), and potassium (k) ,and also act as natural antioxidants. *M. oleifera* suppressed the production level of TNF-α, IL-1β, IL-6. *Moringa oleifera* hydroethanolic bioactive extract of leaves considerably suppressed iNOS and COX-2 pro-inflammatory mediators, the density ratio of iNOS, COX-2, and NFκB-p65 significantly is decreased after treatment by *M. oleifera*. *Meanwhile*, Albumin contained ω-3 polysaturated fatty acids that regulate prostaglandin synthesis and also influence the immune system, furthermore,

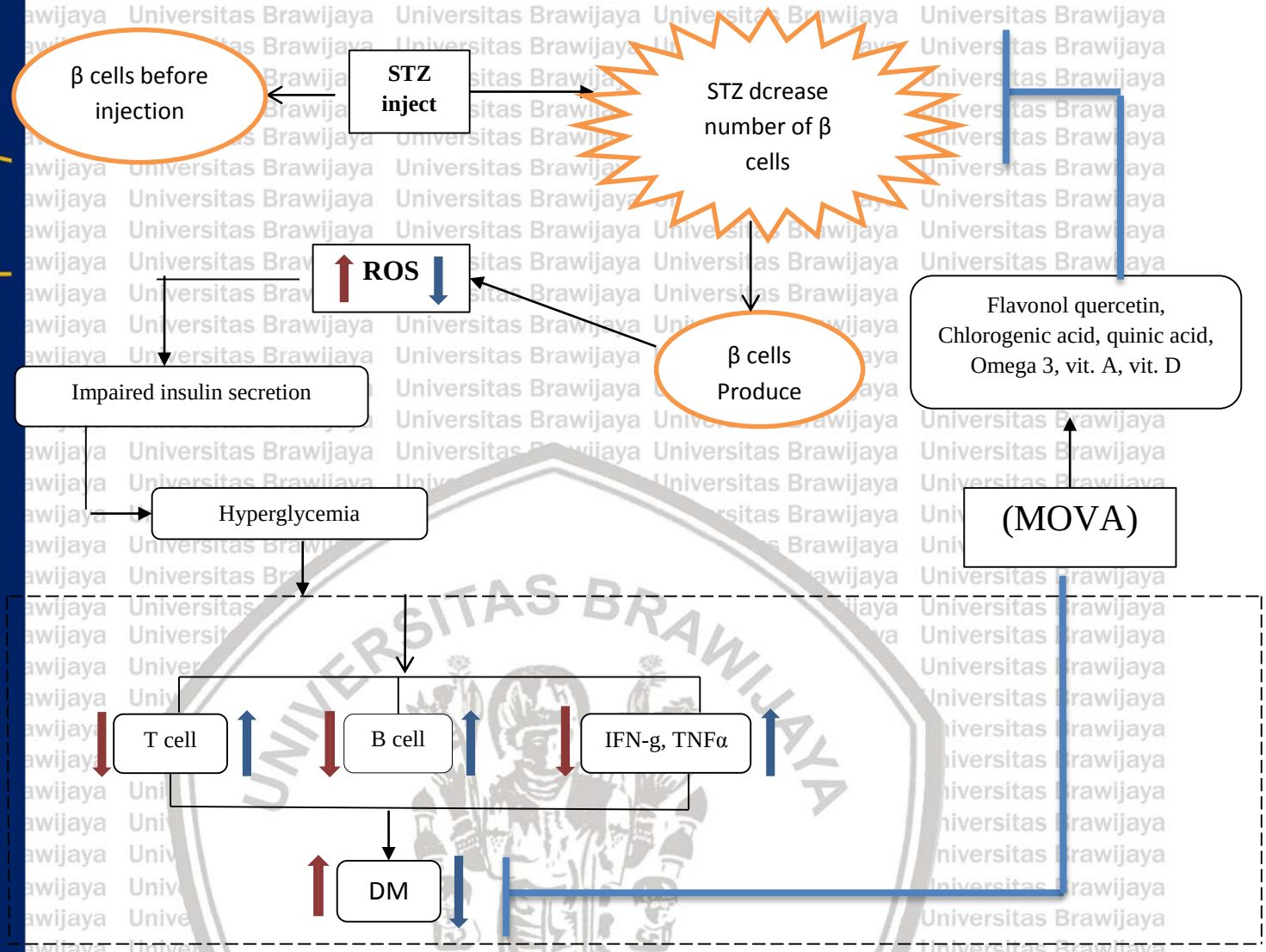


Fig. 2.2 Moringa Oleifera and Albumin Framework against IR and islet inflammation

Note :

: Causes : increase

: Blockade

: Observed area : decrease

The amino acid composition in Albumin has also been analyzed and was reported to play a role to process of wound healing. VipAlbumin® could increase the number of Treg cells and boost the healing process of DM by slowing down the inflammation that occurs in pancreas. The conceptual framework is described in Figure 2.2 as follows.

2.11 Hipotheses

1. Complex *Moringa oleifera and Albumin* could reduce blood glucose levels.
2. *Moringa oleifera and Albumin* complex could reduce pancreatic β-cells destruction
3. *Moringa oleifera and Albumin* complex could reduce Inflamation in the female Balb/c mice with DM pancreas.

CHAPTER III
MATERIAL AND METHOD

3.1 Research Time and Place

This research started in March 2018 and ended in October 2018. It also took place in Animal Physiology Laboratory of Mathematic and Science Faculty of Brawijaya University.

3.2 Operational Framework

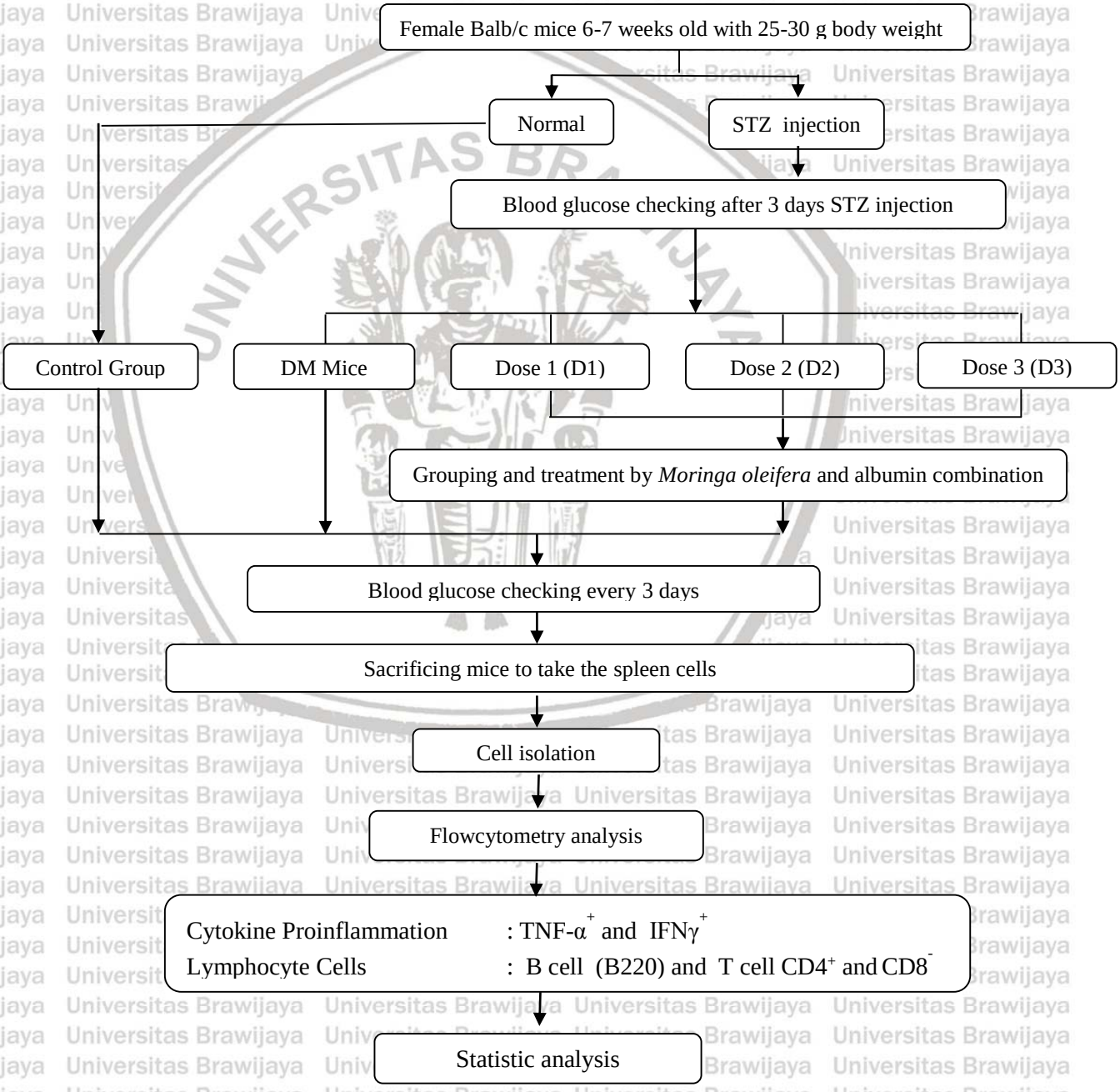


Figure 3.1 Operational Framework

3.3 Research Procedure

3.3.1 Animal Description

Animals used in this research were female Balb/c mice about 6-7 weeks old with body weight of ± 25-30 grams and in a healthy condition (move active and full furry). In the experiment, Balb/c mice were acclimated for seven days and fed with standard feed *ad libitum*. This research divided the mice into five groups with total 25 mice used.

This mice were housed in a plastic cage under standart husbandry conditions (12 hours light / dark cycle; 25±3°C temperature).

3.3.2 Treatment Description

This research undertook experimental research in Animal Physiology Laboratory and divided the Balb/c mice into five groups. They were observed in five time repeated treatment. This research divided the group of mice in healthy/normal mice group (-), control positive (DM mice) without treatment, and the groups with the treatment including D1, D2 and D3. The dose its mean from combination between *Moringa oleifera* (MO) and Vipalbumin®(V) its also called MOV

Table 3.1 Treatment Description

No	• Treatment Group	145 mg/kg Weight STZ	100 mg/kg (Mo) + 416.25 mg/kg (V)	150 mg/kg (Mo) + 208.15 mg/kg (V)	50 mg/kg (Mo) + 624.375 mg/kg (V)
1	Control positive (-)	-			
2	Control Positive (+)	-			
3	D1 (+)	+	+		
4	D2 (+)	+		+	
5	D3 (+)	+			+

Note :

(+) : With Treatment.

(-) : No Treatment.

3.3.3 Streptozotocin Solution and Induced Protocol .

The preparation of STZ solution began with dissolving 145 mg STZ into 10 ml citric buffer 0.1M until STZ solution with pH (4.5) was obtained. STZ solution was homogenized, then poured into 15 ml bottle, covered with aluminium foil, and then kept at the temperature of 4°C.

25 female balb/c mice about 6-7 weeks old were induced with STZ with intraperitoneal (IP) way at a dose of 145 mg/kg body weight. Blood glucose was checked every 3 days after STZ induction. The mice were reported to suffer from type 1 diabetes when its blood glucose levels exceeded 200 mg dL−1 .

3.3.4 *Moringa oleifera* Extract

Moringa oleifera samples were taken from about 100 gram leaves, then dried and powdered using blender before mixed with 100 mL of distilled water for 24 hours and then stored at 4 °C. Afterward, the mixture was filtered twice through a 2-µm pore filter paper, and then stored at 4 °C for 5 days until experiment.

3.3.5 Albumin Preparation.

Albumin used in this research was VipAlbumin®, which contained 500 mg of Snakehead fish extract and 30, 20% albumin, and also vitamin A, vitamin D, and calcium.

3.3.6 Balb/c mice Treatment

After seven days acclimation, Balb/c mice were induced with 145 mg/kg body weight of streptozotocin in intraperitoneal. Afterward, mice were incubated for 14 days to give treatment of MOV base on the D1, D2 and D3 for 14 days. In incubation period of treatment, the blood glucose of the mice was observed in day 3,6,9, and 14 days after induction of STZ. Blood glucose was measured by glucometer.

3.3.7 Blood Sampling and Blood Glucose Measure

The mice were prepared in the cage with tail leads outside the cage. The blood sample was taken from the tail by cutting the tails about 0.5cm (lateral veins). Afterward, the blood was taken and dropped in glucometer stick. The glucometer showed the blood

glucose level in mg/dl denomination. Blood sampling and blood glucose measurement was taken before *Moringa oleifera* and Albumin theraphy.

3.3.8 Spleen Isolation Cell

The spleen was taken from the mice by dislocation in mice neck. The spleen was separated from the mice body and washed with sterile PBS solution. It was layed down on the sterile petri dish containing 2 ml of sterile PBS. Afterward, the spleen was pureed with the spuit on one way. The homogenate was strained with a strainer, put in the 15 ml sterile propilene tube, and then centifugated in 2500 rpm at 4°C for 5 minutes, afterward, the pellete was resuspensed with 1 ml sterile PBS.

3.3.9 Flowcytometry Analysis .

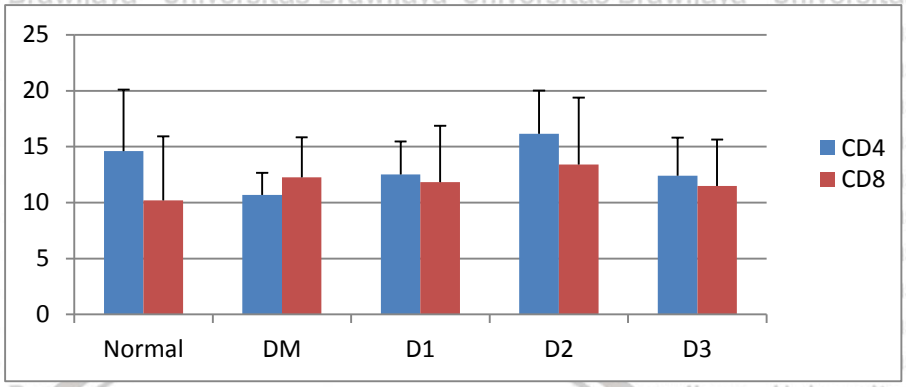
To analyze the mixture of *Moringa oleifera* and Albumin extract against pro-inflamatory cytokines, the isolated cells were taken for 200 µl, placed on the sterile microtube and centrifugated in 2500 rpm at 4°C for 5 minutes. Following that, the supernatant was separated and the pellete was added with 40 µl of antibody staining and incubated for 15-20 minutes in dark condition icebox. The cells were then added with 300 µl of PBS sterile and placed into flow cytometry and ready for running.

3.3.10 Data Analyzing.

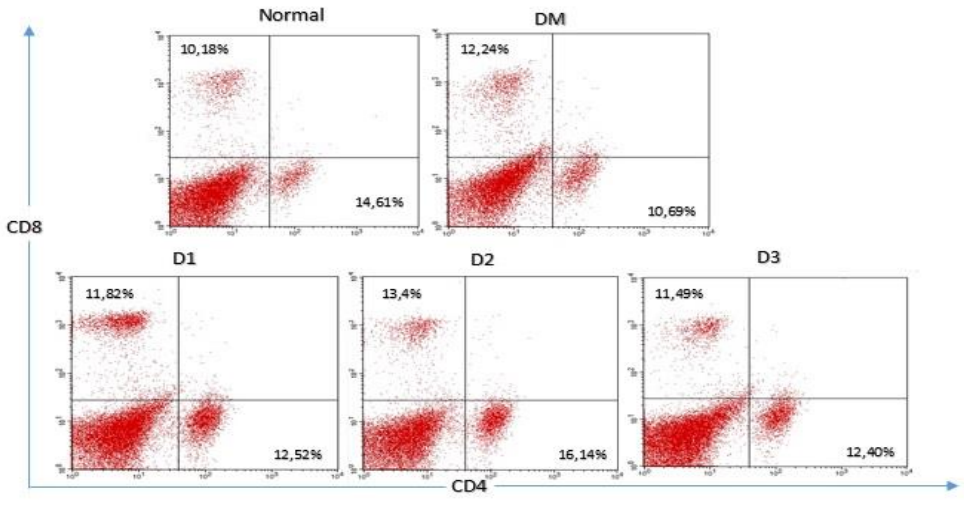
The results from this experiment were noted as data and were analyzed statistically with one way ANOVA with significance $p < 0.05$ using SPSS to discover the relationship between variables and continued with Tukey test to discover the most involving variable.

CHAPTER IV
RESULT AND DISCUSSION

4.1 Profile of $CD4^+$ and $CD8^-$ T cell in spleen .



(a)



(b)

Figure 4.1 Expression result for $CD4^+$ and $CD8^-$ T-cells from Balb/c mice spleen. (a) Result graphic calculation for $CD4^+$ T-cells expressed $CD8^-$ in a **Normal** mice without STZ injection and Moringa Oleifera + VipAlbumin, **DM positive** mice injected by STZ without M.oleifera + VipAlbumin treatment, **D1** with STZ injection and 100 mg/kg *MO* + 416.25 mg/kg VA , **D2** with STZ injection and 150 mg/kg *Moringa oleifera* + 208.15 mg/kg Albumin, **D3** with STZ injection and 50 mg/kg *M.oleifera* M.+ 624.375 mg/kg Albumin. (b) Flowcytometry analysis result, shows an significant in each doses of *M. oleifera* and *Vipalbumin* compare with normal control and DM positive mice.

Both $CD4^+$ and $CD8^-$ cells can play distinct and highly pathogenic roles mediating diabetogenesis (Wagner, 2011). The insulitic infiltrates are mainly comprised of both $CD4^+$ and $CD8^+$ lymphocytes, with a predominance of cytotoxic T cells Furthermore, the higher representation of cytotoxic T lymphocytes among the

inflammatory infiltrates is probably because of the fact that these cells might be the main effectors responsible for the destruction of the insulin-producing beta cells (Calvino, *et al,* 2017). There are several research findings that report that, in many cases, viral infections cause insulitis associated with interferon responses to hyperexpression of HLA class I molecules. Furthermore, hyperexpression of HLA class I molecules provides assigntments of autoantigen epitopes to $CD8^+$ T cells. Islet-infiltrating $CD8^+$ T cells also target viral epitopes, $CD4^+$ T cells may react with antigens provides by APCs in the pancreatic lymph node and in the islets (Pugliese, 2017).

Built upon statistical data analysis, (Fig. 4.1) shows there is not significant on T-cell $CD4^+$ compared with normal control (14,61±5.47%) and DM (10,69±1.95%). This test begin with dose 1 (D1) with 50% *M. oleifera* extract and 50% VipAlbumin. The result shows a decrease number but not significant for $CD4^+$ in D1 (12,52±2.94%). This D1 result will compared with another dose of MOVA. D2 contains of 75% of *M. oleifera* extract and 25% VipAlbumin shows an increase result (16,14±3.87%) compared to DM positive. While another dose of MOVA contains of 25% *Moringa oleifera* and 75% VipAlbumin shows a small number, (12,40±4.41%) compared to normal control and DM.

Based on flowcytometry analysis for $CD8^-$, the result finding for normal control is (10,18±5.73%) and DM, (12,24±3.59%). After giving MOVA this test begin with the dose 1 (D1) with 50% *M. oleifera* and 50% *VipAlbumin* shows an increase and decrease number compared to normal control and DM (11,82±5.02%). This result also compared with another dose of (MOVA). D2 with (13,4±5.97%). While D3 with, (11,49±4.15%).

Based on the flowcytometry analysis and statistic, we can conclude that the treatment of (MOVA) is not given effect in each different dose. This not significant result cause by the low dose of (MO) used in this research is 100 mg/kg , So the doses that we used in this research is not high to effect on cell proliferation. it has been reported that M.oleifera have immunomodulatory activity which present in CD8 cell. The low dose of M.oleifera can push the highest cells proliferation. CD4+ also affect with a high number of CD8 cells cause it secrete IFN-γ and IL2 to induce the proliferation of CD8 T cell (Rachmawati & Rifa'i, 2014).

Furthermore, the low dose stimulate the highest cell proliferation but in higher doses, the number of cell decreased It denoted that absolute cells number of $CD4^+$ is

affected by the treatment in each difference doses increasing number of CD4+ will affect the number of $CD4^+$ cell itself and also $CD8^+$ or $CD25^+$ through the cytokine secretion. And stimulates B cell to secrete antibody (Rachmawati & Rifa'i, 2014).

$CD4^+$ T cells are responsible for 'licencing' $CD8^-$ T cell activation (Walker and Herrath, 2015) .The important role that T cell $CD4^+$ have is obesity. and obesity-induced insulin resistance. A recent study develop by Xia, et al (2017) that found $CD4^+$ effector T cells Could be into proinflammatory Th1, Th17, and anti-inflammatory Th2 and Foxp3+ regulatory T cell (Treg) subtypes based on their functionality and cytokine production. Furthermore, Xia report that when Th1 and Th2 cells activated, it show many of the significant signs of inflammation, such as releasing large amount of cytokines. Xia also completed the report with an information that Th1 cells could produce interferon-g (IFN-g) and tumor necrosis factor- (TNF-) beta. Th1 also reported being a trigger for cell, mediated immunity and -dependent inflammation. Th2 cells, otherwise, produce interleukin-4 (IL-4), interleukin-5 (IL-5), and (IL-6), (IL-9), (IL-10), and also interleukin-13 (IL-13), this kind of cells used to adjust antibody responses.

$CD8^-$ T cells are very important for the induction of macrophage activation and migration to adipose tissue by issuing MCP-1, MCP-3, and regulated on activation normal T cells are expressed and secreted (RANTES) (Xia, et al, 2017). Several findings have suggested a crucial role for the $CD8^-$ T cell subset in the early induction phase of IDDM. It has been proposed that. B cells are destroyed by CD8 cytotoxic T cells, lead to release of pancreatic Ags. These Ags are picked up and presented on professional class II-positive APCs, which can subsequently activate $CD4^+$ T cells. can expand or recruit other $CD8^-$ T cells. Thus, cytotoxicity by $CD8^-$ T cells is believed to be critical to the onset of diabetes (Kreuwel et al, 1999).

4.2 Developing of (B220) .

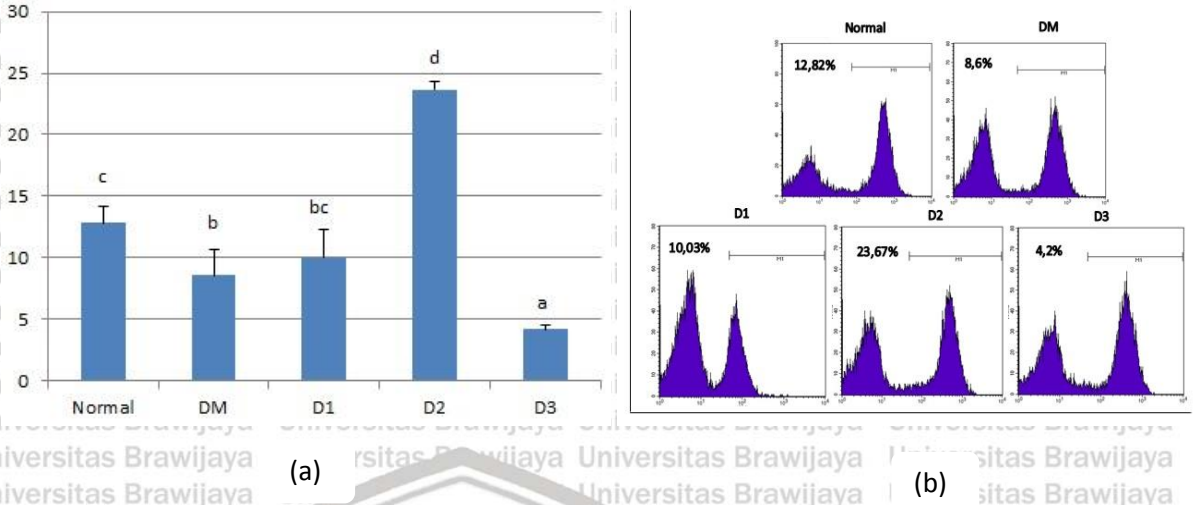


Figure 4.2 Expression result for B220 cells from Balb/c mice spleen . (a) Result graphic calculation for B220 in a **Normal** mice without STZ injection and (MOVA), **DM positive** mice injected by STZ without Moringa oleifera + Albumin treatment , **D1** with STZ injection and 100 mg/kg *Moringa oleifera* + 416.25 mg/kg Albumin, **D2** with STZ injection and 150 mg/kg *Moringa oleifera* + 208.15 mg/kg Albumin, **D3** with STZ injection and 50 mg/kg Moringa oleifera + 624.375 mg/kg Albumin. (b) Flowcytomery analysis result, shows increase and a decrease results in B cell by *Moringa oleifera* and albumin treatment compare with normal control and DM positive mice.

Based on the data of some previos research findings, β-cells on the immune system will produce $B220^+$ surface marker during the formation of plasma cells. In addition, the increasing relative number of $B220^+$ cells caused by STZ injected nto the mice body will cause free radical establishment in the body exercise. the Free radical in the body will cause oxidative reactions and inflammation, in some case this inflammation happen in the adipose tissue and pancreas, (Rochmatika & Rifa'i, 2015).

Flow cytometry result for the B220 cells shows a significant increase and decrease the relative number of B cells ,like in normal group the number was 12,82±1,414%. While after STZ injection the number was significantly decrease became 8,6±2,03% if compared with control treatment on healthy mice *(P<0,05).* After treatment by MOVA simultaneously, dose 1 result was (10,03±2,27%) but not significant compared with normal control and DM positive group, dose 2 was (23,67±0,65%) a significant number compared to normal control and DM positive and other doses D1, D3,so dose 2 increasing the number of B cell, while dose 3 gave a significant decrease result (4,2±0,31%) compared with normal control and DM positive and other doses D1, D2.

These result showed that dose 1 contains 50% of *Moringa oleifera* + 50% VipAlbumin® not give effect, and dose 3 contains 25% 0f Moringa oleifera + 75%

VipAlbumin® could decrease number of B cell (B220). However, reviewed from dose for DM mice, the right dose was dose 2 (D2) because it was proven give significant differences to increase the relatives number of B cell (B220) and the dose contains 75% of *(MO)* and 25% (VA) .

Based on the results D2 able increase the relative number of B cell and D3 can decrease the number of the cell because the (MOVA) has immunomodulatory effect that can cause increase or decrease at the same time. the extract of *M.oleifera* leaf shows immunostimulant activity in B cells (B220). When the cells expressed high relative number of $CD4^+$, it showed the lowest number of B220 expression. Otherwise, when the number of $CD4^+$ is low, then the number of B220 cell is high (Rachmawati & Rifa'i, 2014). Furthermore, Rachmawati & Rifa'i state that B220 is a marker for naive B cells. that have not activated by the presence of antigens. Naive B cells formed from IL-2 secreted by $CD4^+$ help the activation of B cells resulting in higher IL-2 secretion, so the surface expression of B220 cells will be reduced. This is because B cells that are activated will have decreased B220 expression.

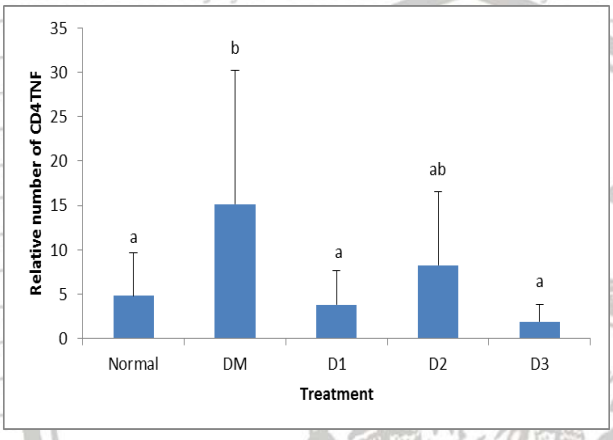
The profile of B cell could be utilized by different condition with interferin-g the growth process of cells in bone marrow (Rifa'i, et al, 2013). The immune system of B cell will produce B220 (surface marker) during the formation of plasma cells (Lehner, et al, 2001).

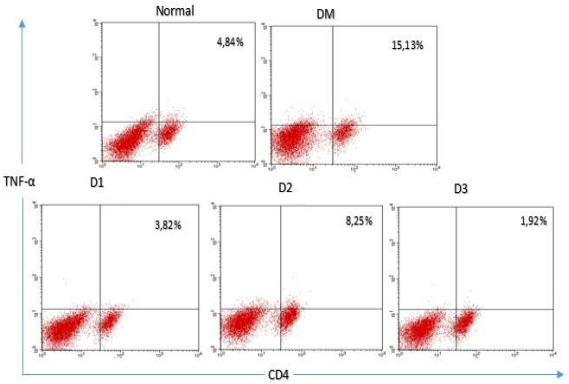
B220 is a full-length of CD45 expressed in the B-cell lineage. The predominant CD45 isoform on thymocytes is CD45RO, which do not have exons 4±6.9. Otherwise, The isoform 220,000-MW of CD45 which is contained all three exons labelled as `B220' because it was first considered a marker of B-cell lineages. B220 expression peripheral T cells activated by staphylococcal B enterotoxin, concanavalin A or -CD3 monoclonal antibody (mAb), B22O cell expression on mature T cells prelude apoptosis following the activation of these cells. Furthermore, B220 is centrally expressed within thymocytes and the dormant B220 may be exposure to the surface of cells. It has been reported that B220 is accidentally expressed in apoptotic cells from T-cell lineages, B220 expression in these cells probably play a role in the process of a apoptosis. The implication of B220 expression in apoptosis remains to explained (Oka, *et al*, 2000).

4.3 Developing of Cytokines Proinflammatory After Giving MOVA .

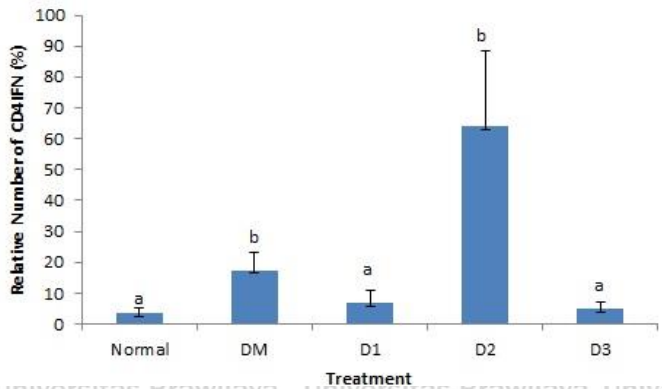
Based on statistical result in figure 4.3, it showed that there is significant different number on each doses to decrease the inflammation of cytokine TNF-α, and IFN-γ in T cell Compared to normal control and DM positive .

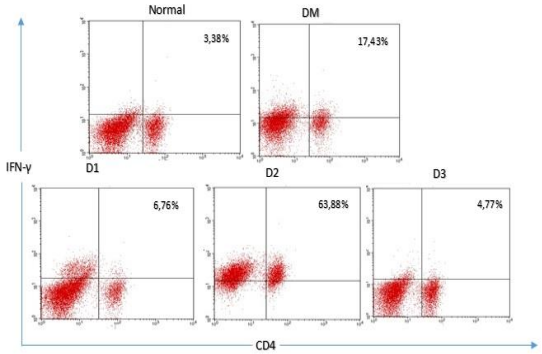
TH1 cytokines (IL-2, IFN-γ, TNF-β) cause the development of the disease, whereas TH2 or Th3 cytokines (IL-4, IL-10, TGF-β) prevent the disease. However, cytokines had a very complex role in type 1 diabetes pathogenesis . Treatment of NOD mice with anti-IFN-γ antibody prevented the development of diabetes, and transgenic expression of IFN-γ resulted in the development of diabetes in diabetes-resistant mice (Yun & Joon, 2005).





(a)





(b)

Figure 4.3 (a) Developing of Cytokines TNF-α by CD4 in T cell with CD4 TNF-α total percentage. (b) developing of cytokines IFN-γ by CD4 in T cell with CD4 IFN-γ total percentage. (**Normal** = Healthy mice; **DM** = Diabetes mice with STZ injection; **D1** = 100 mg/kg *M.oleifera* + 416.25mg/kg VA D2 = 150 mg/kg MO 208.15 mg/kg VA; **D3** = 50 mg/kg MO+ 624.375 mg/kg VA .

The graphic (a) on the figure 4.3 about expression TNf-α^+ by $CD4^+$ shown that there is significant results after gave MOVA combination treatment simultaneously.

(b)

Dose 1 treatment (3,82±1,44%) can reduce the inflammation compared with normal control and DM positive, dose 2 treatment (8,25±4,06%), does not given effect compared to normal and DM positive. and dose 3 treatment (1,92±0,58%) showed a significant decrease results compared with normal control mice (4,84±1,84%) and DM positive mice (15,13±3,55%) so D3 also can reduce inflamation.

The graphic (b) about expression IFN-γ by $CD4^+$ had shown there is a significant different between normal control and DM positive group with the other doses after gave MOVA treatment simultaneously. Dose 1 treatment (6,76±4,14%), dose 2 treatment (63,88±24,42%), and dose 3 treatment (4,77±2,36%). So the treatment of (MOVA) in dose 1 gave effect to process inflammation in $CD4^+$ that expression TNF-α and IFN-γ compared with normal control and DM positive. And also dose 3 can process inflammation compared with normal and DM .

The expression of TNF-α, IL-1*β and IL-6* occurred in a significantly lower level in the serum of control rats treated with moringa aqueous extract than in controls treated with saline (Azevedo, et al, 2018). It has been shown that *M. oleifera* leaf extract and quercetin regulate the expression of IFN-γ , iNOS and C-reactive protein and decrease TNF-α and IL-6 release, in rats (Brilhante, et al, 2017).

TNF-α known as an effective inflammatory mediator. It has been reported that monocytes, macrophages, $CD4^+$ and $CD8^+$ T cells, B cells, lymphokine-activated killer (LAK) cells, NK cells, endothelial cells, and a number of non-haematopoietic tumour cell lines and also other sources such as neutrophils upon stimulation, produced (TNFa) While interferon-gamma (IFN-γ), also known as type II interferon or macrophage-activating factor (MAF), was initially identified because of its antiviral activity (Bazzaz, et al, 2014).

It has been documented that the destructive form of insulitis is related with pro-inflammatory overexpression such as interleukin-1 (IL-1) and (TNFa) Tumor Necrosis Factor , Interferon-γ (IFN-γ) and type 1 cytokines such as (IFN-γ, TNF-β, IL-2 and IL-12), while up-regulation of type 2 cytokines such as IL-4 and IL-10 and also type 3 cytokines (TGF-β1) are reported as benign insulitis, which describe the role of cytokines as regulators and mediators immune response (Bazzaz, et al, 2014).

Many compounds found in (*M. Oleifera)* leaves might be involved in glucose homeostasis. For example, isothiocyanates have been reported to reduce insulin resistance as well as hepatic gluconeogenesis. Phenolic acids and flavonoids affect glucose homeostasis, influencing β-cell mass and function, and increasing insulin

sensitivity in peripheral tissues. Antihyperglycemic and Hypoglycemic activity of *Moringa oleifera* leaves might be due to the presence of terpenoids, which are involved in the stimulation of β-cells and the subsequent secretion of insulin. Also, flavonoids could play an important role for hypoglycemic action (Jimenez, et al, 2017).

4.4 Blood Glucose Analysis .

Hyperglycemia or an increase blood glucose levels is characteristic of diabetes mellitus. Hyperglycemia is caused by a deficiency or decrease in the effectiveness of insulin and disruption of insulin production by the pancreas in the body (Maritim, et al., 2003). Insulin is a protein synthesized by β-pancreatic cells that functions in response to various stimuli such as glucose, sulfonylureas, and arginine but glucose is the main one (Joshi, et al., 2007). The results showed that the condition of glucose levels in healthy mice during the treatment period fluctuated, but did not exceed ≥200 mg/dl. Glucose levels of healthy mice during treatment ranged from 124-169 mg/dl. DM mice have initial glucose levels after STZ injection ranging from 459 mg/dl, and the end of treatment ranges from 376 mg/dl. According to Martha et al 2009 stated that mice experience DM when they have blood glucose levels ≥200 mg/dl. So that this study proves that STZ injection with a dose of 145 mg/kg BW can produce DM models of mice. Increased blood glucose levels due to STZ induction are caused by damage to the β-pancreatic cells, so that insulin production is impaired. STZ penetrates beta langerhans cells through the GLUT2 glucose tansporter. Intracellular STZ action results in changes in pancreatic beta cell DNA. DNA alkylation by STZ through the nitrosourea group results in damage to the pancreatic beta cells (Szkudelski, 2001).

According to the graph shows that mice giving D1, D2 and D3 has an effect on decreasing blood glucose levels during treatment. Initial glucose levels after STZ injection in D1 ranged from 500 mg/dl, D2 at 454 mg/dl and D3 at 555 mg/dl. This condition experienced fluctuations in decreases and increases during the treatment period (Figure 4.7). So the graph shows results of blood glucose analysis in each treatment that D1 can reduce blood glucose levels compared to normal control and DM positive The results showed that after 13 days of treatment, each doses in the treatmant D1, D2 and D3 showed decline compared to the initial treatment D1 around 203 mg/dl,

D2 around 294 mg/dl, and D3 around 401 mg/dl. The decline is close to the normal condition if compared to healthy mice is D1 So in this case, dose1 is the right dose to reduce the condition of blood glucose level to near normal .

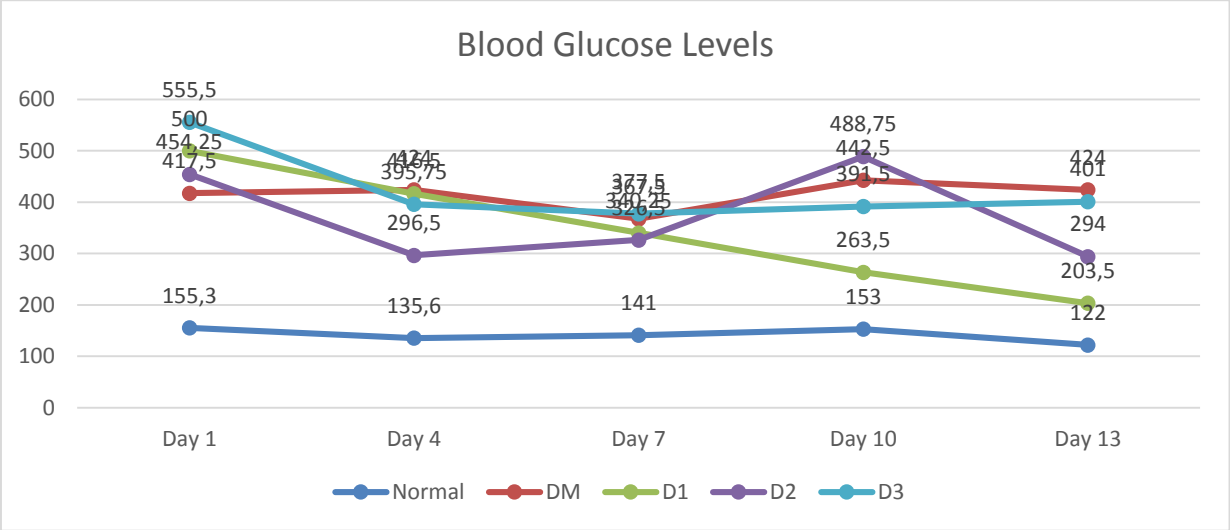


Figure 4.4 The average blood glucose level of mice in each treatment. (Normal = Healthy mice; DM = Diabetes mice with STZ injection; D1 = 100 mg/kg Moringa oleifera + 416.25 mg/kg Albumin; D2 = 150 mg/kg Moringa oleifera + 208.15 mg/kg Albumin; D3 = 50 mg/kg Moringa oleifera + 624.375 mg/kg Albumin

A decrease in blood glucose levels after treatment shows that the moringa-albumin combination can be used as an anti-hyperglycemic drug. Based on the research of Ples and Ho (2009) the consumption of *Moringa* leaves is able to control the condition of blood sugar levels. *Moringa* leaf consumption in normal patients does not affect normal glucose levels. Whereas when consumed by people with hyperglycemia, blood sugar levels decrease significantly close to normal conditions. *Moringa* has anti-inflammatory, antimicrobial, antioxidant, antitumor, anticancer, cardiovascular, antihyperglycemic and diuretic effects (Moyo et al., 2012; Toma & Deyno, 2014). According to Dinia et al. (2016) albumin can trigger lymphocyte proliferation and maintain homeostasis in the DM-2 model. Albumin can synergize with Zn minerals which are needed for cell development and the formation of new cell tissues. Cork albumin can suppress the secretion of proinflammatory molecules so that tissue and organ level damage can be prevented. Based on the graphic analysis shows that the

nutrient content of the albumin-moringa is able to synergize in reducing blood glucose levels.

4.5 Body Weight Profile .

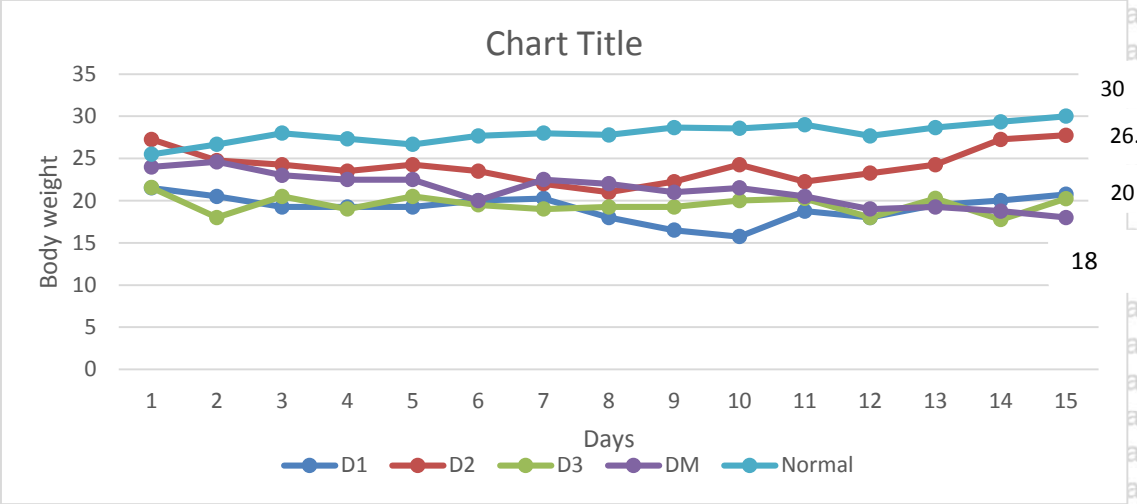


Figure 4.5 The average body weight level of mice in each treatment. (Normal = Healthy mice; DM = Diabetes mice with STZ injection; D1 = 100 mg/kg Moringa oleifera + 416.25 mg/kg Albumin; D2 = 150 mg/kg Moringa oleifera + 208.15 mg/kg Albumin; D3 = 50 mg/kg Moringa oleifera + 624.375 mg/kg Albumin

Based on the analysis for normal control. On the 2nd day mice 2 gave a increase number of body weight from 25gr run up into 27gr. All of the mice gave a decrease number of body weight on day 9th from 27gr each mice into 24gr, 22gr and 23gr.

DM positive control on the 2nd day mice 2 gave a decrease number of body weight from 24gr run down into 18gr, while mice two has a stable body weight condition. On the 7th day mice two has an increase number of body weight, from 20gr became 23gr and has a decrease result on te 8th day become 21gr.

Mice one, on the other hand always has a decrease number of body weight but on the 10th day it increase from 21gr into 23gr and got decrase on the day 11th but got increase on the day 12th and 13th became19 gr and 18gr.

D1 group Diabetes Mice Threat with 100 mg/kg *Moringa oleifera* + 416.25 mg/kg VipAlbumin®. On the 1st day the average number of all mice in this group was between 20-27gr, this number has been decrease until day 5th. On the 5th day, mice 1 and 4 has a decrease number of body weight, from 25gr became 22gr.

On the 8th day all of the mice has a decrease number of body weight but nor for mice 4, it has increase from 18gr to 22gr. On the 11th day all of the mice has an increase number of

body weight especially for mice 3, which has a lowest number of body weight on day 10th 13gr, and became high in day 11th became 22gr and from day 11th to day 12th, 13th ,14th, 15th showing increasing the body weight.

D2 group Diabetes Mice Threat with 150 mg/kg *Moringa oleifera* + 208.15 mg/kg VipAlbumin®. On the 1st day the average number of all mice in this group was between 24-29gr, heavier than average number for D1 on the 1st day. This number has been decrease until day 4th. On the 4th day, mice 3 has an increase number of body weight, from 24gr became 25gr. On the 5th day, mice 2 has an increase body weight number from 22gr became 23gr.and then became high in day 10th became 25gr and from day 11th to day 12th, 13th ,14th, 15th showing that the line show is close to normal control that is mean all the mice in dose 2 can lifting the body weight.

D3 group Diabetes Mice Threat with 50 mg/kg *Moringa oleifera* + 624.375 mg/kg VipAlbumin®. On the 1st day the average number of all mice in this group was between 23-29gr, lighter than average number for D2 on the 1st day.

On the 2nd day all of the mice showed decreased in its body weight an became increase on the 3rd day. A significant decrease happned to mice 3 on the 4th day, from 19gr to 15gr, this condition was stable for mice 3 until 7th day, while others have unstable condition every measure day. On the 10th day mice 3 has a very low body weight during measurement day it has 13gr body weight and keep going up and down until 13th day.

Mice 1 also has the lowest number of body weight on the 10th day, it was 16 gr but got increase in day 11th it was into 20gr.

CHAPTER V
CONCLUSION

5.1 Conclusion

The result from this research are :

1. The right dose of complex *Moringa oleifera and VipAlbumin*® (MOVA) is dose 1 with 100 mg/kg BW *moringa oleifera* + 624.375 mg/kg BW VipAlbumin®, The Administration of *moringa oleifera* and VipAlbumin® in this dose gave a decrease significant result to reduce blood glucose level compared to control positive DM .
2. Based on the expression showed on the flow cytometry result, *moringa oleifera* and *VipAlbumin*® complex showed could reduce the inflammation caused by STZ on the diabetic mice. inflammation found decreased on dose 1 and dose 3 in the $CD4^{+}$TNF-α, IFN-γ .
3. The treatment of *Moringa oleifera* and *VipAlbumin*® (MOVA) can influence profile changing of lymphocyte cell, ($CD4^{+}$) and ($CD8^{-}$) in dose 2 but not significant maybe because the low dose of (MO) used in this research is 100 mg/kg. on the other hand the treatment can influence profile changing of B cell in dose 2 and dose 3. The activity of the treatment on the right dose found decreases and increases the relative number of B cell such as B220.

1. ETHICAL CLEARENCE

2. PLAGIARISM CERTIFICATE

3.The Dosage Formulation As Follow :

Dose 1 50% MO (100 mg/kg body weight) : 50% A (416.25 mg/kg body weight)

Dose 2 75% MO (150 mg/kg body weight) : 25% A (208.15 mg/kg body weight)

Dose 3 25% MO (50 mg/kg body weight) : 75% A (624.375 mg/kg body weight)

Calculated :

Dose 1 :

50% MO = 100 mg/kg body weight

50% A = 416,25 mg/kg body weight

To make Dose 2 and Dose 3 based on Dose 1

Dose 2 (75% MO : 25% A) :

50% MO = 100 mg/kg body weight : 50% A = 416,25 mg/kg body weight

75% MO = X	25% A = X
X x 50 = 100 x 75	X x 50 = 416,25 x 25
50X = 7500	50X = 10406,25
X = 150 MO	X = 208,15 A

So, dose 2 is 75% MO (150 mg/kg body weight) : 25% A (208.15 mg/kg body weight)

Dose 3 (25% MO : 75% A) :

50% MO = 100 mg/kg body weight : 50% A = 416,25 mg/kg body weight

25% MO = X	75% A = X
X x 50 = 100 x 25	X x 50 = 416,25 x 75
50X = 2500	50X = 31218,75
X = 50 MO	X = 624,375 A

So, dose 3 is 25% MO (50 mg/kg body weight) : 75% A (624.375 mg/kg body weight)

5. Flow cytometry Test Result

1. T Cells CD4^{+}AND CD8^{-}

Normality Test

One-Sample Kolmogorov-Smirnov Test

		CD4	CD8
N		25	25
Normal Parameters[a]	Mean	13,2760	11,8300
	Std. Deviation	3,90973	4,66971
Most Extreme Differences	Absolute	,125	,111
	Positive	,125	,111
	Negative	-,091	-,097
Kolmogorov-Smirnov Z		,625	,557
Asymp. Sig. (2-tailed)		,830	,916

a. Test distribution is Normal.

Homogenity Test

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
CD4	,761	4	20	,563
CD8	,637	4	20	,642

ANOVA Test

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CD4	Between Groups	90,007	4	22,502	1,626	,207
	Within Groups	276,856	20	13,843		
	Total	366,863	24			
CD8	Between Groups	27,249	4	6,812	,275	,891
	Within Groups	496,100	20	24,805		
	Total	523,348	24			

Post Hoc Test

Post Hoc Test

Multiple Comparisons

Dependent Variable		(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
CD4	Tukey HSD	Normal	DM	3,91800	2,35311	,476	-3,1234	10,9594
			D1	2,09000	2,35311	,898	-4,9514	9,1314
			D2	-1,53000	2,35311	,965	-8,5714	5,5114
			D3	2,21200	2,35311	,878	-4,8294	9,2534
		DM	Normal	-3,91800	2,35311	,476	-10,9594	3,1234
			D1	-1,82800	2,35311	,934	-8,8694	5,2134
			D2	-5,44800	2,35311	,181	-12,4894	1,5934
			D3	-1,70600	2,35311	,948	-8,7474	5,3354
		D1	Normal	-2,09000	2,35311	,898	-9,1314	4,9514
			DM	1,82800	2,35311	,934	-5,2134	8,8694
			D2	-3,62000	2,35311	,551	-10,6614	3,4214
			D3	,12200	2,35311	1,000	-6,9194	7,1634
		D2	Normal	1,53000	2,35311	,965	-5,5114	8,5714
			DM	5,44800	2,35311	,181	-1,5934	12,4894
			D1	3,62000	2,35311	,551	-3,4214	10,6614
			D3	3,74200	2,35311	,520	-3,2994	10,7834
		D3	Normal	-2,21200	2,35311	,878	-9,2534	4,8294
			DM	1,70600	2,35311	,948	-5,3354	8,7474
			D1	-,12200	2,35311	1,000	-7,1634	6,9194
			D2	-3,74200	2,35311	,520	-10,7834	3,2994
	Games-Howell	Normal	DM	3,91800	2,60137	,600	-6,5156	14,3516
			D1	2,09000	2,78151	,936	-8,2690	12,4490
			D2	-1,53000	3,00097	,984	-12,1835	9,1235
			D3	2,21200	2,88822	,932	-8,2507	12,6747
		DM	Normal	-3,91800	2,60137	,600	-14,3516	6,5156
			D1	-1,82800	1,58052	,774	-7,4940	3,8380
			D2	-5,44800	1,94090	,148	-12,7647	1,8687

			D3	-1,70600	1,76156	,861	-8,1903	4,7783
		D1	Normal	-2,09000	2,78151	,936	-12,4490	8,2690
			DM	1,82800	1,58052	,774	-3,8380	7,4940
			D2	-3,62000	2,17641	,505	-11,2723	4,0323
			D3	,12200	2,01810	1,000	-6,8877	7,1317
		D2	Normal	1,53000	3,00097	,984	-9,1235	12,1835
			DM	5,44800	1,94090	,148	-1,8687	12,7647
			D1	3,62000	2,17641	,505	-4,0323	11,2723
			D3	3,74200	2,31123	,526	-4,2729	11,7569
		D3	Normal	-2,21200	2,88822	,932	-12,6747	8,2507
			DM	1,70600	1,76156	,861	-4,7783	8,1903
			D1	-,12200	2,01810	1,000	-7,1317	6,8877
			D2	-3,74200	2,31123	,526	-11,7569	4,2729
CD8	Tukey HSD	Normal	DM	-2,05800	3,14992	,964	-11,4837	7,3677
			D1	-1,63800	3,14992	,984	-11,0637	7,7877
			D2	-3,21200	3,14992	,843	-12,6377	6,2137
			D3	-1,30200	3,14992	,993	-10,7277	8,1237
		DM	Normal	2,05800	3,14992	,964	-7,3677	11,4837
			D1	,42000	3,14992	1,000	-9,0057	9,8457
			D2	-1,15400	3,14992	,996	-10,5797	8,2717
			D3	,75600	3,14992	,999	-8,6697	10,1817
		D1	Normal	1,63800	3,14992	,984	-7,7877	11,0637
			DM	-,42000	3,14992	1,000	-9,8457	9,0057
			D2	-1,57400	3,14992	,986	-10,9997	7,8517
			D3	,33600	3,14992	1,000	-9,0897	9,7617
		D2	Normal	3,21200	3,14992	,843	-6,2137	12,6377
			DM	1,15400	3,14992	,996	-8,2717	10,5797
			D1	1,57400	3,14992	,986	-7,8517	10,9997
			D3	1,91000	3,14992	,972	-7,5157	11,3357
		D3	Normal	1,30200	3,14992	,993	-8,1237	10,7277
			DM	-,75600	3,14992	,999	-10,1817	8,6697
			D1	-,33600	3,14992	1,000	-9,7617	9,0897
			D2	-1,91000	3,14992	,972	-11,3357	7,5157
	Games-Howell	Normal	DM	-2,05800	3,02706	,955	-13,0162	8,9002
			D1	-1,63800	3,41132	,987	-13,4726	10,1966

	D2	-3,21200	3,70293	,901	-16,0098	9,5858
	D3	-1,30200	3,16730	,993	-12,5079	9,9039
DM	Normal	2,05800	3,02706	,955	-8,9002	13,0162
	D1	,42000	2,76425	1,000	-9,3784	10,2184
	D2	-1,15400	3,11699	,995	-12,5190	10,2110
	D3	,75600	2,45678	,998	-7,7746	9,2866
D1	Normal	1,63800	3,41132	,987	-10,1966	13,4726
	DM	-,42000	2,76425	1,000	-10,2184	9,3784
	D2	-1,57400	3,49137	,990	-13,7215	10,5735
	D3	,33600	2,91716	1,000	-9,8301	10,5021
D2	Normal	3,21200	3,70293	,901	-9,5858	16,0098
	DM	1,15400	3,11699	,995	-10,2110	12,5190
	D1	1,57400	3,49137	,990	-10,5735	13,7215
	D3	1,91000	3,25336	,973	-9,6677	13,4877
D3	Normal	1,30200	3,16730	,993	-9,9039	12,5079
	DM	-,75600	2,45678	,998	-9,2866	7,7746
	D1	-,33600	2,91716	1,000	-10,5021	9,8301
	D2	-1,91000	3,25336	,973	-13,4877	9,6677

2. B Cells B220.

Normality Test

One-Sample Kolmogorov-Smirnov Test

		B220
N		25
Normal Parameters[a]	Mean	11,8632
	Std. Deviation	6,80852
Most Extreme Differences	Absolute	,172
	Positive	,172
	Negative	-,148
Kolmogorov-Smirnov Z		,859
Asymp. Sig. (2-tailed)		,451

a. Test distribution is Normal.

Homogenity Test

Test of Homogeneity of Variances

B220

Levene Statistic	df1	df2	Sig.
1,845	4	20	,160

ANOVA Test

ANOVA

B220

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1065,049	4	266,262	112,125	,000
Within Groups	47,494	20	2,375		
Total	1112,543	24			

Post Hoc Test

Multiple Comparisons

Dependent Variable:B220

	(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Normal	DM	4,22000*	,97462	,003	1,3036	7,1364
		D1	2,79200	,97462	,065	-,1244	5,7084
		D2	-10,84800*	,97462	,000	-13,7644	-7,9316
		D3	8,62000*	,97462	,000	5,7036	11,5364
	DM	Normal	-4,22000*	,97462	,003	-7,1364	-1,3036
		D1	-1,42800	,97462	,595	-4,3444	1,4884
		D2	-15,06800*	,97462	,000	-17,9844	-12,1516
		D3	4,40000*	,97462	,002	1,4836	7,3164
	D1	Normal	-2,79200	,97462	,065	-5,7084	,1244
		DM	1,42800	,97462	,595	-1,4884	4,3444
		D2	-13,64000*	,97462	,000	-16,5564	-10,7236
		D3	5,82800*	,97462	,000	2,9116	8,7444
	D2	Normal	10,84800*	,97462	,000	7,9316	13,7644
		DM	15,06800*	,97462	,000	12,1516	17,9844
		D1	13,64000*	,97462	,000	10,7236	16,5564
		D3	19,46800*	,97462	,000	16,5516	22,3844

	D3	Normal	-8,62000*	,97462	,000	-11,5364	-5,7036
		DM	-4,40000*	,97462	,002	-7,3164	-1,4836
		D1	-5,82800*	,97462	,000	-8,7444	-2,9116
		D2	-19,46800*	,97462	,000	-22,3844	-16,5516
Games-Howell	Normal	DM	4,22000*	1,10875	,037	,2731	8,1669
		D1	2,79200	1,19979	,245	-1,5592	7,1432
		D2	-10,84800*	,69670	,000	-13,5183	-8,1777
		D3	8,62000*	,64816	,001	5,8719	11,3681
	DM	Normal	-4,22000*	1,10875	,037	-8,1669	-,2731
		D1	-1,42800	1,36714	,829	-6,1657	3,3097
		D2	-15,06800*	,95653	,000	-18,9652	-11,1708
		D3	4,40000*	,92178	,036	,3997	8,4003
	D1	Normal	-2,79200	1,19979	,245	-7,1432	1,5592
		DM	1,42800	1,36714	,829	-3,3097	6,1657
		D2	-13,64000*	1,06073	,000	-18,0261	-9,2539
		D3	5,82800*	1,02949	,020	1,3393	10,3167
	D2	Normal	10,84800*	,69670	,000	8,1777	13,5183
		DM	15,06800*	,95653	,000	11,1708	18,9652
		D1	13,64000*	1,06073	,000	9,2539	18,0261
		D3	19,46800*	,32518	,000	18,2335	20,7025
	D3	Normal	-8,62000*	,64816	,001	-11,3681	-5,8719
		DM	-4,40000*	,92178	,036	-8,4003	-,3997
		D1	-5,82800*	1,02949	,020	-10,3167	-1,3393
		D2	-19,46800*	,32518	,000	-20,7025	-18,2335

*. The mean difference is significant at the 0.05 level.

3. TNF-α and IFN-γ

Normality Test

One-Sample Kolmogorov-Smirnov Test

		CD4TNF	CD4IFN
N		25	25
Normal Parameters[a]	Mean	6,7924	19,5348
	Std. Deviation	5,32489	25,54980
Most Extreme Differences	Absolute	,203	,259
	Positive	,203	,259
	Negative	-,151	-,241
Kolmogorov-Smirnov Z		1,015	1,296
Asymp. Sig. (2-tailed)		,254	,069

a. Test distribution is Normal.

.Homogenity Test

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
CD4TNF	12,419	4	20	,000
CD4IFN	5,928	4	20	,003

ANOVA Test

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CD4TNF	Between Groups	540,293	4	135,073	19,267	,000
	Within Groups	140,215	20	7,011		
	Total	680,508	24			
CD4IFN	Between Groups	13045,399	4	3261,350	24,880	,000
	Within Groups	2621,622	20	131,081		
	Total	15667,020	24			

Post Hoc Test

Multiple Comparisons

Dependent	(I)	(J)	Mean	Std. Error	Sig.	95% Confidence Interval

Variable		PERLAKUAN	PERLAKUAN	Difference (I-J)			Lower Bound	Upper Bound
CD4TNF	Tukey HSD	Normal	DM	-10,29000*	1,67460	,000	-15,3010	-5,2790
			D1	1,02000	1,67460	,972	-3,9910	6,0310
			D2	-3,41400	1,67460	,284	-8,4250	1,5970
			D3	2,92200	1,67460	,431	-2,0890	7,9330
		DM	Normal	10,29000*	1,67460	,000	5,2790	15,3010
			D1	11,31000*	1,67460	,000	6,2990	16,3210
			D2	6,87600*	1,67460	,004	1,8650	11,8870
			D3	13,21200*	1,67460	,000	8,2010	18,2230
		D1	Normal	-1,02000	1,67460	,972	-6,0310	3,9910
			DM	-11,31000*	1,67460	,000	-16,3210	-6,2990
			D2	-4,43400	1,67460	,099	-9,4450	,5770
			D3	1,90200	1,67460	,786	-3,1090	6,9130
		D2	Normal	3,41400	1,67460	,284	-1,5970	8,4250
			DM	-6,87600*	1,67460	,004	-11,8870	-1,8650
			D1	4,43400	1,67460	,099	-,5770	9,4450
			D3	6,33600*	1,67460	,009	1,3250	11,3470
		D3	Normal	-2,92200	1,67460	,431	-7,9330	2,0890
			DM	-13,21200*	1,67460	,000	-18,2230	-8,2010
			D1	-1,90200	1,67460	,786	-6,9130	3,1090
			D2	-6,33600*	1,67460	,009	-11,3470	-1,3250
	Games-Howell	Normal	DM	-10,29000*	1,79437	,007	-17,0170	-3,5630
			D1	1,02000	1,04942	,860	-2,6576	4,6976
			D2	-3,41400	1,99686	,496	-11,0913	4,2633
			D3	2,92200	,86791	,097	-,6173	6,4613
		DM	Normal	10,29000*	1,79437	,007	3,5630	17,0170
			D1	11,31000*	1,71863	,005	4,5634	18,0566
			D2	6,87600	2,41658	,116	-1,5080	15,2600
			D3	13,21200*	1,61421	,005	6,2267	20,1973
		D1	Normal	-1,02000	1,04942	,860	-4,6976	2,6576
			DM	-11,31000*	1,71863	,005	-18,0566	-4,5634
			D2	-4,43400	1,92909	,281	-12,1755	3,3075
			D3	1,90200	,69801	,174	-,8352	4,6392
		D2	Normal	3,41400	1,99686	,496	-4,2633	11,0913
			DM	-6,87600	2,41658	,116	-15,2600	1,5080

		D1	4,43400	1,92909	,281	-3,3075	12,1755
		D3	6,33600	1,83667	,105	-1,6598	14,3318
	D3	Normal	-2,92200	,86791	,097	-6,4613	,6173
		DM	-13,21200*	1,61421	,005	-20,1973	-6,2267
		D1	-1,90200	,69801	,174	-4,6392	,8352
		D2	-6,33600	1,83667	,105	-14,3318	1,6598

CD4IFN	Tukey HSD	Normal	DM	-15,51400	7,24102	,242	-37,1819	6,1539
			D1	-3,38000	7,24102	,990	-25,0479	18,2879
			D2	-60,50000*	7,24102	,000	-82,1679	-38,8321
			D3	-1,39000	7,24102	1,000	-23,0579	20,2779
		DM	Normal	15,51400	7,24102	,242	-6,1539	37,1819
			D1	12,13400	7,24102	,470	-9,5339	33,8019
			D2	-44,98600*	7,24102	,000	-66,6539	-23,3181
			D3	14,12400	7,24102	,325	-7,5439	35,7919
		D1	Normal	3,38000	7,24102	,990	-18,2879	25,0479
			DM	-12,13400	7,24102	,470	-33,8019	9,5339
			D2	-57,12000*	7,24102	,000	-78,7879	-35,4521
			D3	1,99000	7,24102	,999	-19,6779	23,6579
		D2	Normal	60,50000*	7,24102	,000	38,8321	82,1679
			DM	44,98600*	7,24102	,000	23,3181	66,6539
			D1	57,12000*	7,24102	,000	35,4521	78,7879
			D3	59,11000*	7,24102	,000	37,4421	80,7779
		D3	Normal	1,39000	7,24102	1,000	-20,2779	23,0579
			DM	-14,12400	7,24102	,325	-35,7919	7,5439
			D1	-1,99000	7,24102	,999	-23,6579	19,6779
			D2	-59,11000*	7,24102	,000	-80,7779	-37,4421
	Games-Howell	Normal	DM	-15,51400*	2,68440	,014	-26,5900	-4,4380
			D1	-3,38000	2,00258	,509	-11,2429	4,4829
			D2	-60,50000*	10,94869	,023	-108,9364	-12,0636
			D3	-1,39000	1,29938	,816	-6,0003	3,2203
		DM	Normal	15,51400*	2,68440	,014	4,4380	26,5900
			D1	12,13400*	3,17565	,035	,8896	23,3784
			D2	-44,98600	11,22266	,060	-92,3672	2,3952
			D3	14,12400*	2,78619	,017	3,2121	25,0359
		D1	Normal	3,38000	2,00258	,509	-4,4829	11,2429
			DM	-12,13400*	3,17565	,035	-23,3784	-,8896
			D2	-57,12000*	11,07937	,027	-105,0016	-9,2384
			D3	1,99000	2,13709	,876	-5,8784	9,8584
		D2	Normal	60,50000*	10,94869	,023	12,0636	108,9364
			DM	44,98600	11,22266	,060	-2,3952	92,3672

		D1	57,12000*	11,07937	,027	9,2384	105,0016
		D3	59,11000*	10,97409	,025	10,7893	107,4307
	D3	Normal	1,39000	1,29938	,816	-3,2203	6,0003
		DM	-14,12400*	2,78619	,017	-25,0359	-3,2121
		D1	-1,99000	2,13709	,876	-9,8584	5,8784
		D2	-59,11000*	10,97409	,025	-107,4307	-10,7893

*. The mean difference is significant at the 0.05 level.

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REFERENCES

Akram T. K. and Hisham M. D. (2015). *Diabetes mellitus: The epidemic of the century*. World J Diabetes. Jun 25; 6(6): 850–867.

Ali, F., T., Nahla S. H., and Rehab R. A.(2015). *Potential activity of Moringa Oleifera leaf extract and some active ingredients against diabetes in rats*. International Journal of Scientific & Engineering Research. Vol. 6 (5).

Al-Malki, A.L., H. A. El-Rabey.(2015). *The Antidiabetic Effect of Low Dose of Moringa Oleifera Lam. Seeds on Streptozotocin Induced Diabetes and Diabetic Nephropathy in Male Rats.* Biomed Research International of Hindawi Publishing Corporation.

Badawi, A., A. Klip, P. Haddad**,** D. E. C. Cole, B. G. Bailo, A. El-Sohemy, M. Karmali. (2010). *Type 2 diabetes mellitus and inflammation: Prospects for biomarkers of risk and nutritional intervention.* Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. Vol.(2010) (3) : 173–186.

Barrett J. C., Clayton D. G., Concannon P., Akolkar B., Cooper J. D., Erlich H. A. (2009). *Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes.* Nat Genet. Vol. 41(6):703–707.

Bearse M. A., Jr, Han Y., Schneck M. E., Barez S., Jacobsen C. (2004). *Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy.* Invest Ophthalmol Vis Sci. Vol. 45: 3259-3265.

Bending, D., P. Zaccone, and A. Cooke. (2012). *Inflammation and Type One Diabetes.* International Immuonology. Vol. 24 (6) : 339-346.

Botero D., and Wolfsdorf J. I. (2005). *Diabetes mellitus in children and adolescents.* Arch Med Res. Vol. 36: 281-290.

Bottini N., Musumeci L., Alonso A., Rahmouni S., Nika K., Rostamkhani M.(2004). *A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes.* Nat Genet. Vol. 36(4):337–338.10.1038/ng1323

Chiang J., L., Kirkman M.,S., Laffel L.,M., Peters A.,L. (2014). *Type 1 Diabetes Sourcebook Authors. Type 1 diabetes through the life span: a position statement of the American Diabetes Association.* Diabetes Care.(2014), Vol.37 : 2034–2054.

Chinedu, A. A., S.O. Alani, A.O. Olaide. (2014). *Effect Of The Ethanolic Leaf Extract Of Moringa Oleifera On Insulin Resistance In Streptozotocin Induced Diabetic Rats.* Journal of Plant Sciences. Vol. 2 (6-1) : 5 – 12.

Daoud, A. K. M. A. Tayyar, I. M. Fouda & N. Abu Harfeil. (2009). *Effects Of Diabetes Mellitus Vs. In Vitro Hyperglycemia On Select Immune Cell Functions.* Journal of Immunotoxicology. Vol. 6 (1), 36-41. https://doi.org/10.1080/15476910802604564

Dabelea D., Mayer-Davis E. J., Saydah S., Imperatore G., Linder B., Divers J., Bell R., Badaru A., Talton J. W., Crume T.(2014). *Prevalence of type 1 and type 2 diabetes among children and adolescents from(2001) to(2009.)* JAMA.(2014) Vol. 311:1778–1786.

Devendra D., Liu E., Eisenbarth G. S. (2004). *Type 1 diabetes: recent developments.* BMJ. Vol. 328:750–754.

De Carvalho Leited, N., E. G. Montes, S. V. Fisher, C. R. C. Cancian, J. C. Oliveira, M. C. Martins-Pinge, C. C. Kanunfre, K. L. A. Souza, S. Grassiolli. (2015). Splenectomy attenuates obesity and decreases insulin hypersecretion in hypothalamic obese rats. Metabolism, Vol. 64 (9), September 2015, Pg. 1122-1133. https://doi.org/10.1016/j.metabol.2015.05.003

Dooley J., Tian L., Schonefeldt S., Delghingaro-Augusto V., Garcia-Perez J. E., Pasciuto E. (2016). *Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes.* Nat Genet. Vol. 48(5):519–527.

Dwijayanti D. R., M. S. Djati, M.Ibrahim and M. Rifa'i. (2015). *The Potential of VipAlbumin to Chronic Inflammation in Type 2 Diabetes Mellitus Balb/C Mice Model.* American Journal of Immunology.

García-Compeán, D., J. A. González-González, F. J. Lavalle-González, E. I. González-Moreno, H. J. Maldonado-Garza,* J. Z. Villarreal-Pérez. *The treatment of diabetes mellitus of patients with chronic liver disease.* Annals of Hepatology. November-December, Vol. 14, (6) : 780-788.

Guyton A. C., Hall J. E. (2006). Textbook of Medical physiology. 11th Edition. Elsevier Inc, New Delhi.

Hidayati, D., A. Faizah, E.N. Prasetyo, N. Jadid, and N. Abdulgani. (2018). *Antioxidant Capacity of Snakehead Fish Extract (Channastriata) at Different Shelf Life and*

Temperatures. International Conference on Statistics, Mathematics, Teaching, and Research. Conf. Series **1028** (2018) 012021. doi :10.1088/1742-6596/1028/1/012021

Holt, R. I. (2004). *Diagnosis, epidemiology and pathogenesis of diabetes mellitus: an update for psychiatrists.* Br J Psychiatry Suppl. Vol. 47: S55-63.

Hove M. N., Kristensen J. K., Lauritzen T., Bek T.(2004). *The prevalence of retinopathy in an unselected population of type 2 diabetes patients from Arhus County, Denmark.* Acta Ophthalmol Scand. Vol.82: 443-448.

International Diabetes Federation. IDF Diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; (2013).

Iwara, I. A., G.O. Igile, I.P. Ogar, O.E.Mboso, U.P. Ujong, E.U. Eyong, P.E. Ebong.(2014). *Anti-Lipidemic Effect of Combined Leaf Extract of Moringa Oleifera and Peristrophe Bicalyculata in Alloxan-Induced Diabetic Rats.* Pharmacology and Pharmacy of Scientific Research. Vol. 5 : 340-348.

Jangir, R. N., and G. C. Jain. (2016). *Antidiabetic and Antioxidant Potential of Hydroalcoholic Extract of moringa Oleifera Leaves in Streptozotocin-Induced Diabetic Rats*. European Journal of Pharmaceutical and Medical Research. Vol. 3(9) : 438-450.

Joshi, S. R., R. M. Parikh, A. K. Das. 2007. *Insulin-History, Biochemistry, Physiology and Pharmacology.* Supplement of Journal of The Association of Physicians of India (JAPI). Vol. 55 : 19-25.

Lawrence J. M., Imperatore G., Dabelea D., Mayer-Davis E. J., Linder B., Saydah S., Klingensmith G. J., Dolan L., Standiford D. A., Pihoker C.(2014). *Trends in incidence of type 1 diabetes among non-Hispanic white youth in the U.S., (2002-2009).* Diabetes. Vol. 63:3938–3945.

Loghmani, Emily. (2005). *Diabetes Mellitus : Type 1 and Type 2.* Guidelines For Adolecent Nutrition Services. 167-182.

Longhui, C., Y. Zemin, C. Weiwen, L. Ruliu, L. Chuanquan, G. Lihua, Z. Zhangzhi, C. Ruifang, L. Saimei, Z. Lingbo, Z. Jinhao, W. Jianhua. 2015. *Differential expression of immune-related genes between healthy volunteers and type 2 diabetic patients with spleen-deficiency pattern.* Journal of Traditional Chinese Medicine. 646-652.

Luqman S., S. Srivastava, R. Kumar, A. K. Maurya, and D. Chanda. 2012. *Experimental Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and*

Scavenging Potential Using In Vitro and In Vivo Assays. Hindawi Publishing Corporation.

Kumar, P., R.T. Mandapaka, (2013). *Effect Of Moringa Oleifera On Blood Glucose, LDL Levels In Types II Diabetic Obese People.* Innovative Journal Of Medical And Health Science. Vol. 3, no. 1.

Kumar P. J. And Clark M. (2002) Textbook of Clinical Medicine. Pub: Saunders, London, UK. 1099-1121.

Pettitt D. J., Talton J., Dabelea D., Divers J., Imperatore G., Lawrence J. M., Liese A. D., Linder B., Mayer-Davis E. J., Pihoker C.(2014). *Prevalence of diabetes in U.S. youth in(2009) : The SEARCH for diabetes in youth study.* Diabetes Care. Vol. 37:402–408.

Phillips J. M., Parish N. M., Raine T.(2009). *Type 1 diabetes development requires both CD4+ and CD8+ T cells and can be reversed by non-depleting antibodies targeting both T cell populations.* Rev. Diabet. Stud. Vol. 6: 97.

Pickup, John C.(2004). *Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabetes.* Diabetes Care. Vol. 27 : 813-823.

Piero, M. N., G.M. Nzaro, J.M. Njagi. (2015). *Diabetes Mellitus – A Devastating Metabolic Disorder.* Asian Journal of Biomedical and Pharmaceutical Sciences. Vol. 04(40) : 1-7.

Pitocco, D., M. Tesauro, R. Alessandro, G. Ghirlanda, and C. Cardillo.(2013). *Oxidative Stress in Diabetes: Implications for Vascular and Other Complications.* International Journal of Molecular Sciences. Vol. 14 : 21525-21550.

Pittas A. G. (2009). *Diabetes Mellitus, Diagnosis and Pathophysiology.* Tufts University; (2005-2009).

Prime Group for JDRF. JDRF: Type 1 Diabetes; 2011

Raju S. M., Raju B .(2010). *Illustrated medical biochemistry. 2nd Edition.* Jaypee Brothers Medical Publishers ltd, New Delhi, India.

Rehman, K., and M.S.H. Akash. (2016). *Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked?.* Journal of Biomedical Science. Vol. 23 : 87.

Richard, C., M. Wadowski, S. Goruk, L. Cameron, A. M. Sharma, C. J. Field. (2017) *Individuals With Obesity And Type 2 Diabetes Have Additional Immune Dysfunction Compared With Obese Individuals Who Are Metabolically Healthy*. BMJ Open Diabetes Research and Care. Vol. **5** : e000379. doi:10.1136/ bmjdrc(-2016)-000379

Roman-Pintos, L.M., G. Villegas-Rivera, A. D. Rodríguez-Carrizalez, A. G. Miranda-Díaz, and E. G. Cardona-Muñoz. (2016). *Diabetic Polyneuropathy in Type 2 Diabetes Mellitus: Inflammation, Oxidative Stress, and Mitochondrial Function*. Hindawi Publishing Corporation Journal of Diabetes Research.

Rowley W. R., and Bezold C. (2012). *Creating public awareness: state (2025) diabetes forecasts.* Population Health Management. 15.

Saely C. H., Aczel S., Marte T. (2004). *Cardiovascular complications in type 2 diabetes mellitus depend on the coronary angiographic state rather than on the diabetes state.* Diabetologia. Vol. 47: 145-146.

Seki M., Tanaka T., Nawa H., Usui T., Fukuchi T. (2004). *Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin- induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factors for dopaminergic amacrine cells.* Diabetes. Vol. 53: 2412-2419.

Shaw J. E., Sicree R. A., Zimmet P. Z.(2010). Global estimates of the prevalence of diabetes for (2010) and(2030). Diabetes Res. Clin. Pract. 87:4-14.

Sithole, H.L., (2009). *A Review Of The Use Of Streptozotocin (STZ) In The Induction Of Diabetes In Rats And Subsequent Ocular Tissue Changes.* S Afr Optom, (2009) : Vol. 68(2), 82-88.

Straub, Rainer H. (2014). *Insulin Resistance, Selfish Brain, And Selfish Immune System: An Evolutionarily Positively Selected Program Used In Chronic Inflammatory Diseases.* Arthritis Research and Therapy. Vol. 16 (2).

Taweerutchana R., N. Lumlerdkij, S. Vannasaeng, P. Akarasereenont, and A. Sriwijitkamol, (2017). *Effect of Moringa oleifera Leaf Capsules on Glycemic Control in Therapy-Naïve Type 2 Diabetes Patients: A Randomized Placebo Controlled Study.* Evidence-Based Complementary and Alternative Medicine. Volume (2017). https://doi.org/10.1155/2017/6581390

Tuorkey, Moubarak J.(2016). *Effects Of Moringa Oleifera Aqueous Leaf Extract In Alloxan Induced Diabetic Mice*. Interventional Medicine & Applied Science, Vol. 8 (3) : 109–117

Walker L. S., and von Herrath M.(2016). *CD4 T cell differentiation in type 1 diabetes*. Clin Exp Immunol. Vol. 183(1):16–29

Wilcox, Gisela. (2005). *Insulin and Insulin Resistance*. The Clinical Biochemist Reviews. Vol.26 : 19-39.

Xia, Chang, Xiaoquan Rao, and Jixin Zhong. (2017). *Role of T Lymphocytes in Type 2 Diabetes and Diabetes-Associated Inflammation*. Hindawi Journal of Diabetes Research.