Effectivity Combination Of Moringaoleifera And Ifalmin To Control The Expression Of Nf-Kb And Pro-Inflammatory Cytokines Production By Cd4 T Cell In Diabetic Mice Model

NovianaDwi Lestari, MuhaiminRifa’i, Nashi Widodo, Sri Rahayu, WahyuIsniaAdharini, Ruri Vivian Nilamsari
Biology Department
Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia

ABSTRACT

Objectives: Diabetes mellitus is a metabolic disease characterized by hyperglycemia. Hyperglycemia leads to an increase in the level of ROS. The high level of ROS can cause oxidative stress that triggers the activation of inflammatory factors such as NF-kB and pro-inflammatory cytokines production. The goal of this research was to analyze the effectivity of MOLI to control NF-kB and pro-inflammatory expression in DM mice model.

Materials and Methods: The research was conducted using five different groups as follows normal healthy mice, DM mice, and three different doses to treatment in DM mice. STZ was used to induce DM in BALB/c mice (145 mg/kg BW). MOLI was orally administered during 2 weeks at dose 1 (800 mg/kg BW MOL : 800 mg/kg BW I), dose 2 (615 mg/kg BW MOL : 615 mg/kg BW I), and dose 3 (800 mg/kg BW MOL : 615 mg/kg BW I). Splenic cells from mice were isolated on day 15 after treatment, and the profile of CD4+NF-kB+, CD4+TNF-α+, CD4+IFN-γ+, and CD4+IL-17+ were analyzed by flow cytometry. The data were statistically analyzed with one way ANOVA (p≤0.05) and Tukey test using SPSS version 16 for Windows.

Results: The results of this research declared that MOLI administered in dose 1 more effective to suppress the expression of NF-kB and reduce TNF-α, IFN-γ, and IL-17 by CD4 T cells compared to another dose.

Conclusion: In conclusion, administered of MOLI with a certain dose had the potential to be used as an alternative medicine for DM.

Keywords: Diabetes mellitus, MOLI, NF-kB, pro-inflammatory, ROS

INTRODUCTION

Diabetes mellitus (DM) is the one most popular disease in the world. International Federation (2019) declared that in 2019 the suffering of DM reach up to 463 million people and it will be increased to 700 million in 2045. This disease is caused by metabolic disorders due to a lack of insulin production and/or the inability of peripheral tissues to respond to insulin marked by hyperglycemia. Hyperglycemia in DM causes an increase in reactive oxygen species (ROS). ROS is basically the result of metabolic processes in cells, which at high concentrations can cause oxidative stress. The production of oxidative stress can trigger the activation of inflammatory factors such as nuclear factor-κB (NF-κB). The high levels of NF-κB can trigger the production and secretion of cytokines, if not controlled will cause inflammation in DM to cause complications.

NF-κB is a transcription factor for pro-inflammatory cytokines, but on other pathways NF-κB is also a promoter for cell proliferation. Based on that, the alternative treatments for DM should surely only inhibit NF-κB in the inflammatory pathway to reduce activation cytokines production.

DM is a complex disease linked with multiple factors such as insulin resistance, low insulin production due to pancreatic B cell damage and uncontrolled secretion of pro-inflammatory cytokines. Therefore, DM treatment must be able to control all of these factors. However, current treatment is more focused on raising insulin levels and using pharmacotherapy, as we know consumption of these drugs causes unwanted side effects like weight gain uncontrolled, hypoglycemia, liver and kidney damage, ketoacidosis, cardiovascular risk and others. We known that the use of these drugs only focuses on one target, even though DM is a complex disease. So that the treatment is needed multitarget treatment. The use of herbal medicines made from natural ingredients such as plants and animals is generally a multitarget treatment. It is be assumed the herbs have substance a hundred of active compounds that may be potentially useful for the development of therapeutic agents. Wherein the whole part that is used consists of various bioactivity compounds that can work more than one target. The multitarget effect of natural compounds may be produced from the synergy of various compounds contained in one herb or the synergy of several natural materials such as plants and animals combined in polyherbal form.
In our research, we have purpose to evaluate and prove the combined effect of Moringaoleifera LAM (MOL) and albumin from Toman fish (Channamicropeltes) in the IFALMIN (I) product, also namely MOLI to treatment DM. MOL, is called as Kelor in Indonesia. The tree has been studied for its traditional medicine, attributed to the numerous bioactive compounds. Previous studies have identified that compounds in MOL is one of the traditional medicine that has anti-inflammatory activity. MOL leaves are the most widely studied and they have shown to be beneficial in several chronic conditions, caused by inflammation. Ples and Ho (2009) declare that consuming MOL has an ability to stabilize and maintain the normal blood glucose level. Hyperglycemia is a characteristic of DM. Albumin sources were obtained from IFALMIN product from PT Ismut manufactory, Indonesia. The contain of IFALMIN consist of Toman fish. Toman fish with high albumin compound. Albumin has a sulfhydryl (-SH) group that has an ability as a free-radical scavenger, so it plays a role in reducing the ROS in the body. The combination is expected to work synergistically to be used as a treatment for DM. The parameter we see is the expression of NF-κB as a transcription factor, and cytokine pro-inflammatory such as TNF-α, IFN-γ, and IL-17 that expresse by CD4 T cells. Uncontrolled expression of NF-κB and various of cytokine can cause severe DM disease. Thus, this parameter can be used as evidence that the MOLI combination can be used as an alternative to DM therapeutic drugs.

MATERIALS AND METHODS

Experimental design
This study was conducted at Animal Physiology Laboratory, University of Brawijaya. This study was categorized as experimental research using in vivo procedure. A total of 25 normal BALB/c male mice were used. Male BALB/c mice were classified into five groups as follows; normal groups (N) as a healthy mice, DM groups as a mice STZ injected and DM mice with three different doses of MOLI (DM-D1; DM-D2; DM-D3). The dosage MOLI are as follow; Dose 1 = 800 mg/kg BW MOL : 800 mg/kg BW I, Dose 2 = 615 mg/kg BW MOL : 615 mg/kg BW I, Dose 3 = 800 mg/kg BW MOL : 615 mg/kg BW I

Animal model-streptozotocin (STZ) induction for DM mice model
Total of 25 male mice BALB/c with the ages of 8-10 weeks were used in this study. All of the procedures were approved by The Ethical Committee of Brawijaya University (Reg. No. 1180-KEP-UB). DM mice model were induced by STZ according to DiaComp Protocols with modification. Based on the total male mice that we use, 20 rats were injected with STZ while five mice were without injection. Mice were fasted for 4-6 hours prior to STZ injection. The dose 145 mg/kg BW of STZ was used for injection of DM mice model intraperitoneally. Pasca-injection of STZ, around 4-7 days, the blood glucose levels were measured with a glucometer. Mice were declared to suffer DM when the levels of blood glucose ≥200 mg dL−1.

Preparation and oral treatment of MOL-IFALMIN combination (MOLI)
MOL as we know kelor in Indonesia were used and combined with IFALMIN to treatment. MOL was obtained from MateriaMedicaBatu, East Java, Indonesia in the form of leaves dried powder. The leaves powder of MOL was extracted with sterile aquades by mixing 50 g MOL then boiled in 500 mL aquades for 5 minutes. The mixture was then filtered using filter paper to separate the substrate. The aqueous extract solution obtained was stored at -80°C. The frozen extract was then evaporated with a freeze dryer to get pellets ready to be used for treatment. IFALMIN is a product from PT Ismut manufactory, Indonesia. The content of IFALMIN are Toman fish (Channamicropeltes) with high Albumin compound in powder form. The dosage combination was giving as according to Food Drug Administration (FDA). Diabetic mice were given MOLI orally with dose 1 (800 mg/kg BW MOL : 800 mg/kg BW I), dose 2 (615 mg/kg BW MOL : 615 mg/kg BW I) and dose 3 (800 mg/kg BW MOL : 615 mg/kg BW I). The administration of MOLI was conducted for 14 days. On day 15, a total of 25 mice were dislocation to spleen organ isolation

Spleen cells isolation
Dislocation of neck and dissection method was done to isolate the spleen organ in each treatment mice. The spleen was isolated and washed with sterile PBS in a petri dish. Spleen organ was homogenated using syringe holder to come into single cell suspension in sterile PBS. The resulted suspension was transferred to a 15 mL propylene tube and centrifuged at 2500 rpm, at 10°C temperature for 5 minutes. Pellet was resuspended in 1 mL of PBS for antibody staining.
Antibody staining and flow cytometry analysis

Pellet was stained with extracellular and intracellular antibody staining. Extracellular antibody staining was done by suspending the pellet with 50 µL of antibodies in 10% FBS containing PBS. Intracellular staining was done by standard methods according to company protocol. Subjected cells were added with 50 µL cytofix-cytosperm. Then, added with 500 µL washperm and centrifuged 2500 rpm, 4°C, for 5 min. Pellet was resuspended with 50 µL of appropriated antibodies.

All antibodies we are use were purchased from BioLegendInc (San Diego, CA). The cells that had been stained with extracellular and intracellular antibodies were added with PBS of 300-500 mL and transferred to cuvettes to analyzed with flow cytometry (BD FACS CaliburTM). The program is set as an acquisition. Gated made tailored to the type of cells to be identified and analyzed.

Statistical analysis

Statistical analysis was done using SPSS version 16 for windows. Data were analyzed using one-way analysis of variance (ANOVA) (p≤0.05). The data with significance between treatments to the parameters, followed by the Tukey test.

RESULTS AND DISCUSSIONS

The expression of NF-kB showed that there were significant differences between normal and DM (p≤0.05). The relative number of CD4+NF-κB+ in normal mice (4.35%) was lower than DM mine (17.37%). Giving all MOLI doses had a significant effect (p≤0.05) to reduce or control NF-kB expression by CD4 T cells. These results indicate that the presentation of the relative number of administration of all doses is close to or significantly the same as the presentation of the relative number of normal mice without treatment. The relative number of CD4+NF-κB+ with DM mice models which were given orally MOLI was decreasing and it became 4.26% in D1, 8.26% in D2 and 6.86% in D3 group.

The analytical of CD4+TNF-α+ was done by flow cytometry. The relative number of CD4+TNF-α+ in normal was significantly different compared to DM. In normal, the relative number of CD4+TNF-α+ are 11.28% and in DM are 19.48% (p≤0.05). These results declare that the relative number of CD4+TNF-α+ in normal mice was lower than in DM mice. The administration of MOLI dose 1 (10.4%) contributes to decrease the relative number of CD4+TNF-α+ significantly compared to other doses. Whereas, dose 2 (15.74%) and dose 3 (13.79%) did not significantly increase or decrease a relative number of CD4+TNF-α+

The effect of MOLI administration after 2 weeks treatment in dose 1 significantly could decrease the relative number of CD4+IFN-γ+ (p≤0.05). Groups of DM had the highest IFN-γ expression compared normal. It has been proven that STZ-induced can cause DM, finally was able to increase the expression of pro-inflammatory cytokine. The average relative number of IFN-γ on Normal, DM, dose1, dose2, and dose3 were 7.44%, 16.75%, 7.74%, 14.32% and 17.99% respectively.

The expression of IL-17 by CD4 T cell as a cytokine after analysis on flow cytometric showed that there were significant differences between normal and DM. The expression of IL-17 on DM (16.83%) was significantly (p≤0.05) higher than normal (6.17%). MOLI administration at dose1, dose 2, and dose 3 was given different effect to control expression IL-17. Dose 1 was able to decrease IL-17 significantly compared with other doses (p≤0.05). so, dose1 had the best impact on diabetic treated mice compared with another dosage.

DM is a metabolic diseases characterized by hyperglycemia caused by deficiency or decreased effectiveness of insulin due to damage to the cells of the pancreas and/or insulin receptor resistance. Hyperglycemia is an abnormally condition from blood glucose characterized by increasing blood glucose levels to exceed normal limits. Long term hyperglycemia condition in finally is associated with macro- and micro-vascular complications leading to kidney disease, stroke, blindness, and heart diseases.

In a state of hyperglycemia, there will be an increase in the polyol pathway, an increase in the formation of non-enzymatic glycation proteins and an increase in the glycosylation process, which causes an increase in oxidative stress. Increased oxidative stress occurs through the formation of Advanced Glycation End Products (AGEs) which can induce the synthesis of various pro-inflammatory cytokines, resulting in the activation of protein kinase-C and NF-κB. NF-κB functions in controlling the expression of genes that encode pro-inflammatory cytokines, chemokines and adhesion molecules also participate in inflammasome regulation. In DM have shown
increased secretion of pro-inflammatory molecules, such as Interferon-gamma (INF-γ) and Tumor necrosis factors-alpha (TNF-α) and decreased secretion of interleukin-10 (IL-10). It is similar to the results of the analysis of TNF-α and INF-γ on DM mice in our study. Wherein DM mice have a high level of pro-inflammatory secretion than normal mice significantly. In addition, there was an increase in IL-17 which is also a pro-inflammatory molecule. Coaction between TNF-α and IL-17 can induce nitric oxide (NO) more than higher level compared to individual cytokine induced alone. In addition, the act of them be able to increase prostaglandin-2 production. It is proved that could be to magnify inflammatory responses. TNF-α is known to have a role to inhibit insulin signaling, and causing insulin resistance by activating c-Jun amino-terminal kinase and an inhibitor of NF-κB kinase, leading to serine phosphorylation of insulin receptor substrate-1. Furthermore, the high-level pro-inflammatory cytokines have been demonstrated to recruit more inflammatory cells to adipose tissue and pancreatic islets, promote hepatic fatty acid syntheses and induce the liver to produce more acute-phase proteins. IL-17-secreting Th17 cells through mediate inflammation have been known contributed to T1D development by stimulating pro-inflammatory cytokines production and countering cytokines functions that mediate immune tolerance. In addition, IL-17 also associated with T2D pathogenesis which up-regulates pro-inflammatory cytokine genes expression through by activation NF-κB pathway. So, in this case, the main function of IL-17 is believed could to increase responses of inflammatory and elevate autoimmune destruction of insulin-producing β-cells in the pancreas. Furthermore, the collaboration between IL-17 with IFN-γ and IL-1β potentiated the upregulation of COX-2, the expression of which is associated with β cell dysfunction. Downregulation BCL-2 as a gene of anti-apoptotic was seen as well mouse insulinoma cells and apoptosis increase in human islet cells when treated with IL-17 with other inflammatory cytokines, such as IFN-γ and IL-1β. So, the coproducers of IL-17 and IFN-γ may be of clinical importance as implicated in animal models of autoimmune diabetes, seen from the synergistic effect of IFN-γ and IL-17 in the induction of apoptosis in the human islet cells and inflammation.

In our research, a single large dose of STZ (145 mg/kg BW) is used for experiments to get DM mice models by direct to virulence β cells. Previous study examines mice that indicated DM had blood glucose levels ≥ 200 mg/dl. Next, DM mice were treated with MOLI with three different doses. After 2 weeks of treatment, analysis of transcription factor (NF-κB) and cytokine pro-inflammatory (TNF-α, INF-γ, and IL-17) were expressed by CD4 T cells was done by flow cytometry. The results showed that there was a significant role of MOLI to regulate the transcription factor (NF-κB) expression and pro-inflammatory cytokines, as indicated by the decrease in the relative number of these parameters in treated mice compared to non-treatment DM mice.

MOLI consists of MOL and IFALMIN product. MOL is a plant as we know have various benefit to traditional medicine. MOL contains many active compounds including flavonoids, phenolics, carotenoids, ascorbic acid, mineral, vitamin, amino acids and a lot of other compounds. Beside that, MOL have an anti-inflammatory effects, anti-diabetic, anti-microbial, anti-oxidant, anti-tumor, anticancer, cardiovascular, anti-hyperglycemic, and diuretic. MOLI also contains of albumin from IFALMIN product. The albumin obtained from Toman fish or called Giant snakeheads. In Central Kalimantan, Toman fish is believed as a therapeutic agent to accelerate recovery and restore stamina by post-partum mothers. IFALMIN contains albumin, zinc, omega-6, omega-3, and amino acids. Toman fish are one of the fish species of family Channidae that is protein source, has high albumin content. Albumin from fish can be used as an alternative to fulfill albumin in the body, also is expected to become as an alternative for substitute Human Serum albumin (HSA). Albumin has a group sulphydryl (-SH), which functions as a free-radical scavenger. Based on the explanation above, we assumed the combination from MOL and IFALMIN, has potential as anti-diabetic and anti-inflammation.

MOL showed as an anti-inflammatory activity when consuming in mice model DM. MOLI reduced the level of NF-κB and cytokine pro-inflammatory that produced by CD4 T cells. NF-κB is widely have a play an important role in different biological proses in eukaryotic cells, including inflammation, stress response, apoptosis and immunity. So, in this case, NF-κB as regulator genes to control cell proliferation and cell survival. NF-κB signaling is critical in modulating the immune response through the transcriptional regulation of chemokine and cytokine expression. The composition of MOLI showed effective to decline the transcription factor of inflammation in DM. The flow cytometry analysis shows that decline the relative number of CD4+NF-κB+ in all dose treatment is assumed as due to the presence of flavonoids from MOLI.
Flavonoids have the function to inhibit cell proliferation. Flavonoids have an action to suppress NF-κB, decline the production of ROS and inhibit the expression of topoisomerase I and topoisomerase II enzymes that play a role in catalysis of DNA. In addition, besides flavonoids, the content of MOLI also contains the amino acid glutamate which can enhance glutamine synthesis. Glutamine has an anti-inflammatory effect by inhibiting the activation of NF-κB. The decrease of NF-κB level will decrease pro-inflammatory cytokine.

The decrease relative number of CD4+TNF-α+, CD4+IFN-γ+, and CD4+IL-17+ in DM mice compared to normal mice prove that DM conditions can increase the level of pro-inflammatory cytokines expressed by CD4 T cells. The high level of them is assumed due to hyperglycemia condition as an effect STZ-induction. STZ is a substance having a potent alkylating agent known to directly methylate DNA to targeted β cells damage, is used to trigger the initial cell death. Administration dose 1 has a potential to decrease level cytokines pro-inflammatory expression by CD4 T cells is assumed as due to the presence of many compounds from MOLI like albumin and quercetin works synergistically in reducing pro-inflammatory cytokines expression. Albumin has a sulfhydryl (-SH) group that acts as a natural antioxidant to cleaning and catching ROS in hyperglycemia. Albumin involved in reducing ROS formation by binding to metal ions through copper bonds with high affinity. Albumin as an antioxidant is capable of destroying ROS which was made from oxidation process capturing oxygen, decomposing hydrogen peroxide radical, so that ROS can be neutralized. Furthermore, quercetin has an ability as anti-inflammatory action by blocking IkB kinase so that there is no degradation of IkB which can prevent the activation of NF-κB, and the end can suppress NF-κB expression, as a transcription factor. Uncontrolled NF-κB expression can lead to elevated levels of TNF which can cause inflammation. NF-κB becomes active due to a stimulus from ROS agents that cause DNA damage. MOLI with a certain dose was proven to decrease the number of pro-inflammatory cytokines TNF-α, IFN-γ, and IL-17 in DM mice models at an equal level to the relative number of cytokine in normal/healthy mice.

CONCLUSION

In conclusion, this study demonstrated that MOLI has a role in controlling the development of NF-κB as a factor transcription and pro-inflammatory cytokine (TNF-α, IFN-γ, and IL-17) expression in DM. Based on the result, dose 1 from all the treatments more effective to decrease the parameter we use compared to another dose. Although our investigations provide information about the possible use of MOLI as an alternative therapeutic medicine for DM.

ACKNOWLEDGMENTS

The author would like to thank for all of thecolleaguesin the Laboratory of Animal Anatomy and Physiology, Department of Biology, Brawijaya University for assistance in conducting research.

REFERENCES


42. Feng J, Lu S, Ou Bet al., The Role of JNk Signaling Pathway in Obesity-Driven Insulin Resistance, Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 2020;13:1399-1406.
45. Sarkar SA, Kutlu B, Velmurugan K et al., Cytokine-mediated induction of anti-apoptotic genes that are linked to nuclear factor kappa-B (NF-kappaB) signalling in human islets and in a mouse beta cell line, Diabetologia, 2009;52(6):1092–1101.


53. Bolzan AD, Bianchi MS, Genotoxicity of Streptozotocin, Mutation Research/Reviews in Mutation Research, 2002;512(2-3):121-134.


Figures

Figure 1.

Figure 2.

Figure 3.
Figure 1. The administration of MOLI in DM mice model can reduce relative number of CD4$^{+}$NF-$\kappa$B$. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$.

Figure 2. The effect of MOLI administration can manage the relative number of CD4$^{+}$TNF-$\alpha$. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$.

Figure 3. The impact of MOLI administration to control the expression of IFN-$\gamma$ on CD4 T cell. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$.

Figure 4. MOLI administration can regulate the expression IL-17 on CD4 T cells after 2 weeks administration. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$. 

---

**LEGEND FOR FIGURES**

Figure 1. The administration of MOLI in DM mice model can reduce relative number of CD4$^{+}$NF-$\kappa$B$. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$.

Figure 2. The effect of MOLI administration can manage the relative number of CD4$^{+}$TNF-$\alpha$. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$.

Figure 3. The impact of MOLI administration to control the expression of IFN-$\gamma$ on CD4 T cell. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$.

Figure 4. MOLI administration can regulate the expression IL-17 on CD4 T cells after 2 weeks administration. Normal = healthy mice (STZ uninduced); DM = DM mice without treatment; DM-D1 = DM + dose 1 (800 mg/kg BW (MOL) : 800 mg/kg BW (I)); DM-D2 = DM + dose 2 (615 mg/kg BW (MOL) : 615 mg/kg BW (I)); DM-D3 = DM + dose 3 (800 mg/kg BW (MOL) : 615 mg/kg BW (I)). Mice were sacrificed and analyzed using flow cytometry (left) on day 15 and tabulated into Microsoft Excel (right). The data are mean+SD in each group with $\rho \leq 0.05$. 

---