The Potential of VipAlbumin® to Chronic Inflammation in Type 2 Diabetes Mellitus Balb/C Mice Model

Dinia Rizqi Dwijayanti, M. Sasmito Djati, Mansur Ibrahim and Muhaimin Rifa’i

Department of Biology, Brawijaya University, Malang, Indonesia

Abstract: Diabetes Mellitus (DM) is one of diseases which have increasing number of sufferers every year. Almost all DM patients are type 2 DM. One of the causes of type 2 DM is a chronic inflammation due to an increase of circulating proinflammatory cytokines such as TNF-α, IFN-γ and IL-6. VipAlbumin® from snakehead fish extract is expected to be useful as an alternative treatment of type 2 DM because of its proinflammatory activity. The aim of this study was to determine the effect of VipAlbumin® on regulatory T cell activation, macrophage cells, proinflammatory cytokines and NF-κB. The experiments were done by inducing mice model of type 2 DM with STZ 100 mg/kg BW and then gave them oral administration of VipAlbumin® 0 mg/g BW, 0.01664 mg/g BW, 0.0416 mg/g BW and 10.4 mg/g BW for 14 days. The data were statistically analyzed with one way ANOVA with significance value 0.05% and Tukey test using SPSS version 16 for Windows. The results showed the decreasing number of regulatory T cells in type 2 DM mice, increasing number of macrophage cells and proinflammatory cytokines TNF-α, IFN-γ and IL-6 as well as the increasing number of NF-κB as a transcription factor of inflammatory mediators in CD4+ T cells, CD8+ T cells and macrophages (CD68+) compared to healthy mice (p<0.05). Oral administration of VipAlbumin® for 14 days was proven to cure type 2 DM mice (K+) by increasing the number of regulatory T cells, decreasing the number of macrophage cells and proinflammatory cytokines TNF-α, IFN-γ and IL-6 and inhibiting NF-κB in T lymphocytes CD4+, CD8+ and macrophages at the level equivalent to healthy mice and significantly different (p<0.05) compared with K+ group.

Keywords: Inflammation, Ophiocephalus Stiatus, Regulatory T Cells, Type 2 DM, VipAlbumin®
Production of cytokines in β cells blocks the function of β cell due to the activation of NF-κB, transcription factor to proinflammatory cytokine (Weaver, 2012). According to Donath and Shoelson (2011), CXCL8 and CXCL10 and other chemokine produced by β cells are chemotactic for monocytes and macrophages. Brooks-Worrell et al. (2011) mentions that in the peripheral blood of patients with type 2 DM, it was also found 42% increase in T cells that react against β cells. These indicate that either innate or adaptive immune response is considered as one of the factors associated with type 2 diabetes.

Basically, the treatment of type 2 DM can be done directly by inhibiting proinflammatory cytokines and activation of regulatory T cells (Treg). Treg cell functions to regulate the performance of effector T helper cells (Th) and T cell cytotoxic (Tc). That regulation causes effector cells do not work reactivity and do not secrete cytokines (Corthay, 2009; Sakaguchi et al., 2009; Rifa‘i, 2014). However, a synthetic drug used to treat type 2 DM only focuses on increasing the level of insulin and its long-term use can cause side effects such as liver and kidney damage. Hence, it needs alternative treatments from natural substance to reduce these side effects.

Snakehead fish (Ophiocephalus stiatus) is one of Indonesia’s natural resources that contain proteins, including albumin, which is higher compared to the other kind of fish. Several recent studies reveal many efficacies of albumin such as increasing Hb level, accelerating wound healing, increasing the number of serum albumin, anti-cancer, anti-inflammatory and so on (Mustafa et al., 2012). One of the supplements gotten from albumin snakehead fish is VipAlbumin® which is believed to be useful in the process of therapy of type 2 DM. Therefore, the aim of this study was to determine the effect of VipAlbumin® to regulatory T cells, macrophage cells, proinflammatory cytokines produced by immunocompetent cells and NF-κB on effector cells.

Materials and Methods

Induction of Type 2 DM Mice Model

Mice model of type 2 DM were obtained by injecting STZ at neonatal mice 5 day old at a dose of 100 mg/kg BW intraperitoneal. The mice were allowed to grow, fed and watered ad libitum. Blood glucose levels were measured with a glucometer after 4 weeks old. Mice were declared to suffer from type 2 diabetes when their blood glucose levels exceed 200 mg dL⁻¹.

Oral Administration of VipAlbumin® to Type 2 DM Mice Model

Positive diabetic type 2 mice were given VipAlbumin® orally at a dose of 0 mg/g BW (K+); 0.01664 mg/g BW (D1); 0.416 mg/g BW (D2); and 10.4 mg/g BW (D3). The dose is obtained from the conversion of the human dose that is 33.3 mg/kg BW. The administration of VipAlbumin® was conducted for 14 days. Blood glucose levels were measured every 3 days during the administration of VipAlbumin® to determine the change of blood glucose level.

Cell Isolation from Spleen

Mice were dislocated and dissected. Spleen was isolated and washed in petri dish containing PBS. Cells from mice spleen were isolated by crushing spleen in PBS. Homogenates of cell were centrifuged with a speed 2500 rpm, 10°C, for 5 min. Pellet was resuspended in 1 mL of PBS.

Immunocytochemistry and FACS Analysis

Spleen cell suspensions were divided into micro tubes A, B, C, D and E and then centrifuged with a speed of 2500 rpm, 10°C, for 5 min. Pellets were stained with antibodies. The combinations of antibodies used were A: FITC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse CD25 and PE/Cy5-conjugated rat anti-mouse CD62L; B: FITC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse CD8 and PE/Cy5-conjugated rat anti-mouse NF-κB; C: FITC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse TNF-α and PE/Cy5-conjugated rat anti-mouse IFN-γ; D: FITC-conjugated rat anti-mouse CD68 and PE/Cy5-conjugated rat anti-mouse NF-κB; E: FITC-conjugated rat anti-mouse CD68 and PE/Cy5-conjugated rat anti-mouse IL-6.

Extracellular staining was done to cell in micro tube A. Cells were stained with 1 µL of antibody that had been diluted with 50 µL of PBS 10% FBS and then incubated for 20 min in ice box at 4°C. Intracellular staining was done to cell in micro tube B, C, D and E. These cells were added with extracellular antibody for 20 min in ice box. After that, cells were added with 50 µL fixative solution cytofix/cytoperm and incubated for 20 min in ice box. Fixative solution was removed by 500 µL washing solution washperm and then centrifuged with a speed of 2500 rpm, 10°C, for 5 min. Pellets were stained with intracellular antibodies then incubated for 20 min in the ice box. After extracellular and intracellular staining procedure, cells were added with 500 µL of PBS and transferred into a flow cytometry cuvet. Each sample was analyzed with flow cytometer.
Experimental Design

This study is an explorative experiment and consists of five treatments. They are negative control (healthy mice and without VipAlbumin® administration), positive control (type 2 DM mice model and without VipAlbumin® administration), D1, D2 and D3 (type 2 DM mice model and with different concentration of VipAlbumin® administration). Each treatment was repeated 5 times.

Data Analysis

The data were analyzed by using BD cellquest PRO™ software then tabulated using Microsoft Excel and analyzed statistically. Statistical analysis used was a parametric one-way ANOVA with significance of 0.05% and followed with Tukey test.

Results

VipAlbumin® Increased the Number of Regulatory T Cells

The administration of VipAlbumin® can increase significantly (p<0.05) the relative number of regulatory T (Treg) cells in type 2 DM mice models compared to control (Fig. 1). The number of Treg cells in control group of type 2 DM mice models (K+) significantly decreased (p<0.05) compared to healthy mice (K-) from 53.24% became 43.79%. Relatively, the number of type 2 DM mice models which were given orally VipAlbumin® was increasing and it became 63.54% in D1, 64.40% in D2 and 65.55% in D3 group.

VipAlbumin® Decreased the Relative Number of Macrophage Cells (CD68+)

It is found out that the number of macrophage cells increases in type 2 DM patients, especially in pancreas tissue. It happens because macrophage plays an important role in chronic inflammation as a proinflammatory cytokine producer. In this study, the number of macrophages cells increased significantly in mice models of type 2 DM (K+) compared to healthy mice (K-), 4.09% doubling to 8.23%. The administration of VipAlbumin® in mice model of type 2 DM, among D1, D2 and D3, significantly decreased the number of macrophages cells compared to controls (K+) became 1.64, 1.57 and 1.44% (Fig. 2).

VipAlbumin® Decreased the Number of Proinflammatory Cytokine TNF-α, IFN-γ and IL-6

Proinflammatory cytokines produced by immune cells is one of chronic inflammation mediators in pancreas that causes pancreatic β cells damage. These proinflammatory cytokines include TNF-α, IFN-γ and IL-6. In vivo test, it showed a decrease in the relative number of TNF-α produced by CD4+ T cells in mice models of type 2 DM after oral administration of VipAlbumin® for 14 days (Fig. 3). Relative number of TNF-α produced by CD4+ T cells in healthy mice (K-) was 1.49% and increased significantly (p<0.05) became 6.93% in mice models of type 2 DM (K+). The administration of VipAlbumin® in group D1, D2 and D3 significantly decreased the relative number of TNF-α produced by CD4+ T cells (p<0.05) than K+ group became 1.11%, 1.89% and 0.39%.

VipAlbumin® reduced the relative number of cytokine IFN-γ t produced by CD4+ T cells. This study showed the increase of relative number of IFN-γ secreted by CD4+ T cells in type 2 DM mice model (K+) compared to healthy mice (K-). 1.12% became 4.63% or 4 times higher than normal conditions (Fig. 4). Mice model of type 2 DM which were given oral administration of VipAlbumin® showed that it was like a control (K-) relative number; they were 0.84% (D1), 2.18% (D2) and 1.64% (D3). These numbers were significantly different (p<0.05) with K+ group.

Cytokines IL-6 detected in this study was IL-6 produced by macrophages (CD68+). Relative number of cytokine IL-6 produced by macrophages in normal conditions (K-) was 1%. In mice models of type 2 DM (K+) this number increased significantly (p<0.05) became 4.6%. On the other hand, oral administration of VipAlbumin® D1, D2 and D3 in type 2 DM were proven significantly reduced that number (p<0.05) became 2.26, 1.47 and 1.55%. The numbers of IL-6 produced by macrophages were not significantly different from normal conditions (Fig. 5).

VipAlbumin® Suppressed NF-kB in T Lymphocyte and Macrophage Cells

Proinflammatory cytokine produced by immune cells is played by NF-kB, the transcription factor for proinflammatory cytokines. In this study, NF-kB in T cells and macrophages in type 2 DM mice model (K+) were significantly increased compared with healthy mice (K-). VipAlbumin® suppressed NF-kB among in lymphocytes CD4+, CD8+ T cells and macrophages (CD68+). Oral administration of VipAlbumin® in type 2 DM had suppression activity to relative number of CD4+NF-kB+ in VipAlbumin® treatment group (D1, D2, D3) significantly (p<0.05) compared with control (K+), 9.42% became 1.51% in D1, 2.32% in D2 and 1.94% in D3, close to normal conditions (K-) was 1.18% (Fig. 6).
Fig. 1. The administration of VipAlbumin® in type 2 DM mice model can increase relatively good number of T regulatory cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). T regulatory cell (CD4+CD25+CD62L+) were presented in relative number. The data are mean±SD in each group with p value <0.05.

Fig. 2. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of macrophage cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). Macrophage (CD68+) cells were presented in relative number. The data are mean±SD in each group with p value ≤0.05.
Fig. 3. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of TNF-α produced by CD4⁺ T cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). TNF-α produced by CD4⁺ T cells was presented in relative number. The data are mean±SD in each group with p value < 0.05.

Fig. 4. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of IFN-γ produced by CD4⁺ T cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). IFN-γ produced by CD4⁺ T cells was presented in relative number. The data are mean±SD in each group with p value < 0.05.
Fig. 5. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of IL-6 produced by CD68⁺ cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). IL-6 produced by macrophage cells were presented in relative number. The data are mean±SD in each group with p value <0.05.

Fig. 6. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of NF-κB on CD4⁺ T cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). NF-κB on CD4⁺ T cells were presented in relative number. The data are mean±SD in each group with p value <0.05.
Fig. 7. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of NF-κB on CD8⁺ T cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). NF-κB on CD8⁺ T cells were presented in relative number. The data are mean±SD in each group with p value < 0.05.

Fig. 8. The administration of VipAlbumin® in type 2 DM mice model can decrease relative number of NF-κB on macrophage cells. K- is healthy mice group. Mice model of type 2 DM were treated with VipAlbumin® 0 mg/gr BW (K+); 0.01664 mg/gr BW (D1); 0.416 mg/gr BW (D2); and 10.4 mg/gr BW (D3) for 14 days. After 14 days, mice were dissected, cells were isolated from spleen and then analyzed using flow cytometry (a) and tabulated using Microsoft Excel (b). NF-κB on macrophage cells were presented in relative number. The data are mean±SD in each group with p value < 0.05.
VipAlbumin® also showed suppression activity to NF-kB in CD8+ T cells. Relative number of CD8’NF-kB’ T cells in treatment group was lower than control group (K+) and significantly different (p<0.05) from healthy mice group (K-), which was 0.57 (Fig. 7). The efficacy of VipAlbumin® in increasing the number of Treg cells in type 2 DM can help the healing process of type 2 DM by slowing down the chronic inflammation that occurs in pancreas. Components contained in VipAlbumin® may bind to receptors on the surface of T cells that have an impact on the activation of PKC-θ, Bcl-10, CARMA1 or IKK2. Four of these molecules are signaling pathway from TCR to NF-kB activation that leads the role in the process of differentiation of naïve T cells into Treg cells (Feuerer et al., 2009).

VipAlbumin® components that affect the activation of Treg cells are vit.A and Vit.D. Main metabolite of Vit.A, ATRA can change the naïve CD4+Foxp3+ T cells into Treg cells FoxP3+. Histone acetylation in the FoxP3 gene promoter is induced when ATRA binds to the nuclear retinoic acid receptor α, resulting in the expression of FoxP3 protein in CD4+ T cells and becomes Treg cells. ATRA also can prevent the conversion of Treg cells into Th17 proinflammatory cells induced by IL-6 (Issazadeh-Navikas et al., 2011). Besides vit.A, Vit.D especially D3 also affects the activation of Treg cells. Gregori et al. (2002) and Issazadeh-Navikas et al. (2011) explains that the active form of Vit.D3, 1,25-(OH)2D3, can induce an increase in Treg cells.

Alpan et al. (2004) and Rifa‘i and Widodo (2011) explained that the role of Treg cells is to create the tolerance conditions and homeostasis in immune system because Treg cells is able to regulate and control the effector cells that have been activated to avoid autoreactivity of effector cells, including the ability of effector cells to secrete proinflammatory cytokine (Yamazaki et al., 2003). Suppressive activity of Treg cells on effector cells can be through four main mechanisms. First, Treg cells have capability to secrete cytokines that can inhibit the function of effector cells directly, such as IL-10, TGF-β and IL-35. These cytokines are classified as inhibitors cytokine that contribute to the inhibition of effector cell function directly (Collison et al., 2007). Second, it acts as competitors for effector cells in utilizing IL-2 because Treg cells express three molecules which have a high affinity for IL-2, namely CD25 (receptor α chain IL-2), CD122 and CD132 (Yu et al., 2009). Third, activated Treg cell has a function as cytotoxic cells that secrete granzyme A and granzyme B and some other types of perforin that cause apoptosis of effector cells directly (Grossman et al., 2004; Gondek et al., 2005). The last, Treg cells can express molecules such as galectin-1 which is one of a family of β-galactosidase binding protein that will cause cell cycle arrest and apoptosis in effector cells (Garin et al., 2007). These mechanisms can suppress the activity of effector cells especially in production of proinflammatory cytokines.

Macrophages are phagocytic cells that are most responsible for chronic inflammatory process, especially in type 2 DM because macrophages can produce proinflammatory cytokines (Scull et al., 2010). This study proved that the number of macrophages (CD68+) in type 2 DM mice model is higher than normal condition and VipAlbumin® can reduce this number almost equivalent to healthy mice. This decreasing number can occur because activated Treg can suppress macrophage cells through four main mechanisms which have been described previously. On the other hand, the content of VipAlbumin® also acts directly as an anti-inflammatory and thus inhibits the activation of macrophage cells. Fujiwara and Kobayashi (2005) also explain that macrophages can be deactivated by an anti-inflammatory agent.

As mentioned before, one of the causes of type 2 DM is a systemic inflammation that occurs in β cells, adipose tissue, liver and muscle (Morino et al., 2006). The main trigger in β cells inflammation is the same as fatty tissue, liver and muscle as they are the excessive of saturated Free Fatty Acids (FFA), lipid mediators such
as 12 (S)-hydroxyeicosatetraenoic acid [12(S)-HETE], an increase of glucose level and proinflammatory cytokines and chemokines such as TNF-α, IFN-γ and IL-6 (Yumi et al., 2013). The results of this study are in line with the theory stating that proinflammatory cytokine TNF-α, IFN-γ and IL-6 significantly increase in mice model of type 2 DM compared to the control.

In the case of type 2 DM, proinflammatory cytokines secreted by immunocompetent cells is one of the inflammatory mediators that cause β cell dysfunction. VipAlbumin® was proven to reduce the number of proinflammatory cytokines TNF-α, IFN-γ and IL-6 in mice models of type 2 DM at an equal level to the number of cytokine in healthy mice.

Fig. 9. The mechanism of VipAlbumin® in curing type 2 DM. Interleukin-1β is a pro-inflammatory cytokines that can activate NADPH oxidase and Divalent Metal Transporter 1 (DMT1); both lead to an increase in Reactive Oxygen Species (ROS) and oxidative stress. ROS activates JNK, p38 MAPK or nuclear factor NF-κB. Inflammation in pancreatic β cells will lead to the recruitment and activation of immune cells in pancreas tissues such as CD4 T cells, CD8 T cells and macrophages (CD68+). These immune cells will secrete pro-inflammatory cytokines. Cytokines produced locally or from circulation such as TNF-α, IFN-γ and IL-6 also activate inflammatory pathways via specific cytokine receptors in β cells. Insulin secretion is further reduced by this chronic inflammation. VipAlbumin is expected to activate regulatory T cell, inhibit proinflammatory cytokines and suppress activation of NF-κB in effector cells. The reduction of proinflammatory cytokines will reduce chronic inflammation on pancreatic β cells so pancreatic β cells can secrete insulin normally.
In effector cells, NF-κB is a transcription factor that binds to the promoter gene of inflammatory mediators that affect the production and secretion of proinflammatory cytokines (Silverman and Maniatis, 2001; Caamano and Hunter, 2002; Liang et al., 2004; Yumi et al., 2013). The decrease of NF-κB level will decrease proinflammatory cytokine such as TNF-α, IFN-γ and IL-6 (Huang et al., 2002; Lawrence, 2009). This study also showed that NF-κB played an important role in the inflammatory process in type 2 DM due to an increase in NF-κB significantly in mice models of type 2 DM compared to controls. VipAlbumin® in this study can decrease the number of NF-κB in CD4+ and CD8+ T cells and macrophages cells at levels which are not significantly different from healthy mice.

Besides containing albumin which has long been known to have benefit as an anti-inflammatory (Mustafa et al., 2012), VipAlbumin® taken from a crude extract from snakehead fish also contains other complex protein, omega-3 fatty acids, amino acids glycine, histidine, cysteine, glutamine and tryptophan and the mineral magnesium which also has a function as an anti-inflammatory. According to Simopoulos (2002) and Wall et al. (2010), Omega-3 fatty acids, especially those derived from fish, act as anti-inflammatory and they are proven to suppress a number of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. Several types of amino acids such as glycine, histidine, cysteine, glutamine and tryptophan have anti-inflammatory effects by preventing the production of cytokines TNF-α, IFN-γ, IL-6, IL-1, preventing activation of effector T cells and macrophages, as well as inhibiting NF-κB activation (Wheeler and Thurman, 1999; Stachlewitz et al., 2000; Son et al., 2005; Liboni et al., 2005; Hasegawa et al., 2011). Magnesium also plays a role as an anti-inflammatory due to lack of magnesium in the body can cause several metabolic disorders such as type 2 DM (Schulze et al., 2007; Villegas et al., 2009).

The efficacies of VipAlbumin® in increasing the number of Treg cells, decreasing the number of macrophage cells and proinflammatory cytokine and inhibiting NF-κB in immunocompetent cells proved that it had a direct impact on reducing inflammation and further will impact on the reparation of pancreatic β-cells, so they can secrete insulin normally (Fig. 9). Furthermore, the inflammation that occurs in other tissues such as the liver and muscle will slow down, so that insulin receptors on this tissue will work normally. The healing process of mice model of type 2 DM after oral administration of VipAlbumin® was in line with a decrease of blood glucose levels closer to normal condition < 200 mg dL−1 (Fig. 10).

Conclusion

Potential of VipAlbumin® as an anti-inflammatory can be used as a supplement to cure the chronic inflammation, especially type 2 DM. Healing activity that occurs through the activation of Treg cells decreases the relative number of macrophage cells and proinflammatory cytokines TNF-α, IFN-γ and IL-6 and inhibits NF-κB in CD4+, CD8+ T lymphocytes and macrophage cells.

Acknowledgement

The authors would like to say the deepest thanks to all Laboratory of Animal Physiology team for their support in conducting this research especially to Diabetes Mellitus team Ganys Trisilvana, S.Si. and Andi Rizky, S.Si.

Funding Information

This research was funded by PT. Royal Medicalink Pharmalab-Makassar Indonesia and Prof. Muhaimin Rifa'i.
Authors’ Contributions

Dinia Rizqi Dwijayanti: Wrote the manuscript and participated in the study design.
M. Sasmito Djati: Participated in statistical analysis.
Mansur Ibrahim: Participated in the study design.
Muhammad Rifa'i: Revised the manuscript and the study design.

Ethics

This article is original research paper and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved this manuscript and there are no ethical issues involved.

References

Dula, S.B., M. Jecmenica, R. Wu, P. Jahanshahi and G.M. Verrilli et al., 2010. Evidence that low-grade systemic inflammation can induce islet dysfunction as measured by impaired calcium handling. Cell Calcium, 48: 133-142. DOI: 10.1016/j.ceca.2010.07.007
Weaver, J.R., 2012. Integration of pro-inflammatory cytokines, 12-lipoxygenase and NOX-1 in pancreatic islet beta cell dysfunction. Molecular Cellular Endocrinol., 358: 88-95. DOI: 10.1016/j.mce.2012.03.004