

Effect of Genistein on Interleukin 1 β and Transforming Growth Factor β in Diabetic Rat

Putri Dafriani^{1,*}, Nur Indrawati Lipoeto², Honesty Diana Morika³, Eliza Arman¹, Weni Sartiw³, Annita¹

¹Biomedic, Sekolah Tinggi Ilmu Kesehatan Syedza Saintika, Padang, Indonesia

²Nutrition, Universitas Andalas, Padang, Indonesia

³Nursing, Sekolah Tinggi Ilmu Kesehatan Syedza Saintika, Padang, Indonesia

*Corresponding author: putridafrianiabd@gmail.com

Received January 18, 2019; Revised March 17, 2019; Accepted April 05, 2019

Abstract One of diabetic complications is diabetes nephropathy. Nephropathy is caused by oxidative stress. Oxidative stress causes an inflammatory response that continues to the kidney fibrosis. Genistein can reduce proinflammatory cytokines. This study aims to examine the effect of genistein on levels of IL-1 β , TGF- β , HSP 47 and type IV collagen. This study was experimental. This study use 25 male wistar rats as experimental animals. Rats were divided into five groups, normal rats, hyperglycemic rats, hyperglycemic rats with administration of genistein 0.5 mg/kgw, hyperglycemic rats with administration of genistein 1 mg/kgw, hyperglycemic rats with genistein 2 mg/kgw. Streptozotocin given 65 mg/kgw by intraperitoneal injection. The duration of administration of genistein is 4 weeks. The average levels of IL-1 β in non-diabetic control group was 181.436 pg/ml, diabetic control 359.303 pg/ml, STZ; G 0.5 mg/kgw 265.088 pg/ml, STZ; G 1 mg/kgw 240.088 pg/ml and STZ; G 2 mg/kgw 120.344 pg/ml with p value of 0.000 ($p < 0.05$), it means that there is the effect of genistein on the levels of IL-1 β in diabetic. The average levels of TGF- β in non diabetic control group was 1411.462 pg/ml, diabetic control 8492.520 pg/ml, STZ; G 0.5 mg/kgw 7334.098 pg/ml, STZ; G 1 mg/kgw 6134.568 pg/ml and STZ; G 2 mg/kgw 5012.616 pg/ml with p value of 0.000 ($p < 0.05$), which means that there is the effect of genistein on the levels of TGF- β . Genistein can reduce fibrosis by reducing levels of IL-1 β and TGF- β .

Keywords: *genistein, interleukin 1 β , TGF- β , diabetic nephropathy*

Cite This Article: Putri Dafriani, Nur Indrawati Lipoeto, Honesty Diana Morika, Eliza Arman, Weni Sartiw, and Annita, "Effect of Genistein on Interleukin 1 β and Transforming Growth Factor β in Diabetic Rat." *Journal of Food and Nutrition Research*, vol. 7, no. 4 (2019): 287-290. doi: 10.12691/jfnr-7-4-5.

1. Introduction

Hyperglycemia is a manifestation of diabetes mellitus (DM). The prevalence is increasing in developed countries. This disease is ranked fourth of the five causes of death in developed countries. One of the complications of diabetes is diabetes nephropathy. Diabetes nephropathy is one of the causes of end-stage failed disease [1]. Diabetes nephropathy occurs in 30-40% of all diabetes cases [2]. Diabetic nephropathy is characterized by impaired renal structure [3], expansion of mesangial cells and thickening of the glomerular basement membrane [4].

Hyperglycemia causes oxidative stress. It damages proteins, carbohydrates, fats and DNA. Oxidative stress can increase proinflammatory cytokines. Control of inflammatory factors will inhibit process of fibrosis [5]. Oxidative stress causes changes in transcription factors of genes [6]. Nuclear factor kappa beta (NF κ B) activates mesangial cells [7]. NF κ B also stimulates the transcription factor of endothelin-1 genes (ET-1), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), interleukin 6, interleukin 1 and tumor necrosis

α (TNF- α) which increases the development of diabetic nephropathy [8]. TNF- α and IL-1 β cause increased expression of chemotactic factor (MCP-1), stimulating kidney cells to produce growth factors transforming factor beta (TGF- β), connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), increases vascular endothelial permeability and stimulates proliferation of mesangial cells and increases extracellular matrix formation [9].

The expression of TGF- β is enhanced by glomerular mesangial cells of DM and endothelial cells. Inhibition of TGF- β prevents fibrosis in DM conditions. Treatment in DM rats by providing antibodies that fight increased expression of TGF- β can reduce glomerular hypertrophy and kidney due to DM. TGF- β can stimulate excessive production of extracellular matrix proteins [10], resulting in expansion of mesangial cells consisting of fibrinectin and collagen type IV [11].

Genistein is one type of isoflavone whose highest level in soybeans. It is an inhibitor of the enzyme α glucosidase [12]. Glucosidase is an enzyme that plays a role in the digestion of carbohydrates. The administration of an α glucosidase enzyme inhibitor effectively improves hyperglycemic conditions and diabetes nephropathy.

Genistein also inhibits NfκB activation in renal inflammation of rats fed a fructose diet. It can reduce the levels of proinflammatory cytokines so inhibit the inflammatory process and fibrosis in early nephropathy conditions [13]. This study aimed to study the effect of genistein soybean on levels of IL-1β and TGF-β.

2. Materials and Methods

2.1. Chemicals

Genistein and streptozotocin (STZ) were obtained from Sigma Aldrich. Kits for the assay of IL-1β and TGF-β are obtained from elabscience. All chemicals for this study were obtained from pharmaceutical laboratories, Andalas University, Indonesia.

2.2. Diet

The diet for the control group consisted of starch. Diet for the treatment group consisting of genistein. Genistein is given orally at a dose of 0.5 mg / kgw / day, 1 mg / kgw / day, 2 mg / kgw / day. The concentration of the genistein solution is made into 1mg / 1ml. The solution was given with a pipe stomach for 30 days.

2.3. Animals

The population of experimental animals was male white rats (*Rattus Novergicus*) strain Wistar strain, aged 8 weeks with a weight of 180-200 grams. The rat was obtained from Sawahlunto. Humidity cages are between 20-22°C and automatic lighting (alternation 12-periods of light and dark). Experimental procedures were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Ethical Committee of Animal Care, Andalas University, Indonesia (NO: 104 / KEP / FK / 2015).

2.4. Experimental Design

The samples were 25 rats divided into 5 groups. Group 1 (negative control), group 2 (diabetes group), group 3 (diabetes with genistein 0.5 mg/kgw), group 4 (diabetes with genistein 1 mg/kgw) and group 5 (diabetes with genistein administration) 2 mg/kgw). Streptozotocin (Sigma Chemical Co. St.Louis.M.O. USA) is used to induce diabetes. Streptozotocin given 65mg/kgw (in the preliminary study) by Intraperitoneal injection. Rats are given 5% glucose so there is no decrease in blood sugar at night. The rats were given genistein for 30 days, then after 30 days their blood and kidneys were taken to examine IL-1β and TGF-β. Blood samples are taken through the orbital vein. All samples are stored -700 C.

2.5. Assay of Inflammatory Markers

2.5.1. Interleukine 1β

The procedure for examining IL-1β use the ELISA Indirect (Sandwich) technique with the Quantikine HS kit

reagent. Microplate is coated with murine monoclonal antibody that is specific to IL-1β in its wells, inserted 100μL of the standard diluent HDIC and 150μL (standard IL-1β) or sample into each well, then it covered with adhesive cover. IL-1β will be bound by antibodies dicoated in wells. After incubation for 14-20 hours at room temperature, then it washed 4 times, added 200μL conjugate solution containing polyclonal antibody labeled with specific enzymes for IL-1β into each well, covered with a new adhesive cover, then incubated for 45 minutes at room temperature, this well was not washed, but immediately added 50μL of the amplifier solution, covered with new adhesive, then incubated for 45 minutes at room temperature. Adding an amplifier will start the color. Added 50μL stop solution (2N sulfuric acid) to each well. The addition of stop solution will not affect the color in the well. Then it is read by a microplate reader at a wavelength of 450nm (in 30 minutes). Readings are corrected at a wavelength of 650nm or 690nm.

2.5.2. TGF-β

The blood is centrifuged to get the serum. Prepare the materials and tools used in the condition of the room and prepare the material for the standard according to the desired standard concentration. Take the well plate enter the 59μl assay diluent to all the wells (blanks, standards and samples). Then enter 50μl of the standard solution, control and sample according to the well plate sample. The well plate is placed on top of the screw and moved at 50 rpm for 3-5 minutes so that the samples and antibodies in the well are mixed well. After sekher the well plate is closed with adhesive plastic, then incubated at room temperature for 2 hours. After incubation, the solution is well sucked using a multichannel pipette. Then the well plate is washed by entering a 300μl / well washing buffer and then the sacher is carried out for 2 minutes at a speed of 50-100 rpm. The solution on the plate is well sucked using a multichannel pipette. When sucking the washing buffer, it must be careful so that no damage to the base of the well plate containing antibody antigen reactions will occur. Do activities up to 4x. In the 4th washing, turn the dialed well plate with a tissue and tap it slowly so that the washing liquid has been completely wasted. Add the 100μl conjugate enzyme to all wells except the blank (filled with washing buffer 100μl) then cover with plastic adhesive film then incubate for 2 hours at room temperature. After incubation of conjugate fluid that does not react with antibody antigens on the bottom of the well is removed using a multichannel pipette. Perform washing like at number 8. Add 100 μl of the solution substrate made from color reagent A and B which is mixed momentarily doing this step to each well, incubating for 30 minutes at room temperature in dark conditions. The addition of the solution substrate will give a sky blue color. Add a 100μl stop solution to each well and color changes from blue to yellow. Insert the well plate into the ELISA microplate reader then set it at a wavelength of 450nm. The measurement results come out in the form of absorbance and concentration and the results of the measurements used are concentration.

2.6 Statistical Analysis

Data is analyzed using a computer. The data in this study were normally distributed, using parametric analysis with ANOVA to determine the mean differences in IL-1 β and TGF- β between the negative control group, the diabetes group, the diabetes group with the administration of genistein 0.5 mg/kgw/day, the diabetes group with administration of genistein 1 mg/kgw/day and diabetic group with genistein 2 mg/kgw/day. Furthermore, a posthoc test was performed with a bonferroni test to determine which groups had significant differences and which groups did not have significant differences.

3. Results

3.1 Effect of Genistein on Interleukine 1 β

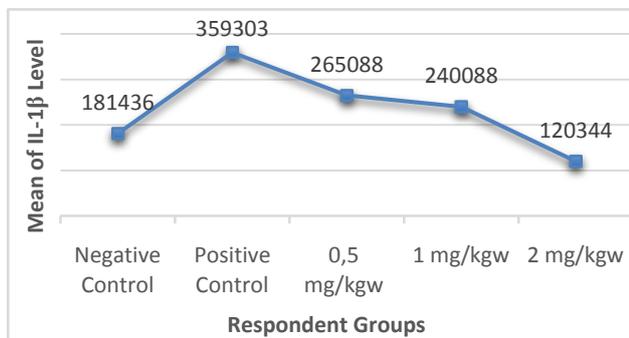


Figure 1. Effect of Genistein on Interleukine 1 β

The average levels of IL-1 β in non-diabetic control group was 181.436 pg/ml, diabetic control 359.303 pg/ml, STZ: G 0.5 mg/kgw 265.088 pg/ml, STZ; G 1 mg/kgw 240.088 pg/ml and STZ; G 2 mg/kgw 120.344 pg/ml. The test results obtained ANOVA p value of 0.000 ($p < 0.05$), which means that there is the effect of genistein on the levels of IL-1 β in diabetic.

3.2. Effect of Genistein on TGF- β

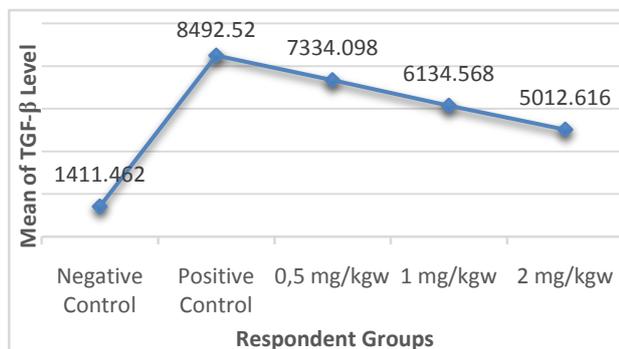


Figure 2. Effect of Genistein on TGF- β

The average levels of TGF- β in non diabetic control group was 1411.462 pg/ml, diabetic control 8492.520 pg/ml, STZ; G 0.5 mg/kgw 7334.098 pg/ml, STZ; G 1 mg/kgw 6134.568 pg/ml and STZ; G 2 mg/kgw 5012.616 pg/ml. From the test results obtained ANOVA p value of 0.000 ($p < 0.05$), which means that there is the effect of genistein on the levels of TGF- β .

4. Discussions

4.1. Effect of Genistein on Levels of Interleukine 1 β

Levels of IL-1 β STZ group 359,303 pg/ml higher than the levels of IL-1 β non diabetic group 181,436 pg/ml. This shows that Hyperglycemia can cause inflammation. Hyperglycemia can stimulate macrophages to produce proinflammatory cytokines such as IL-1 β , TNF- α , and interferon gamma (INF- γ). they can stimulate cells resident in the kidneys produce various chemokines. Macrophages activation responsible for the occurrence of fibrosis [14].

Genistein may improve inflammatory conditions because of hyperglycemia [15]. Genistein can reduce inflammatory mediators like TNF- α , IL-1 β and IL-6. Genistein can protect cells from the pathological processes [16]. NF κ B can bind the inflammatory gene promoter. NF κ B is a transcription factor gene encoding IL-1 β . Genistein can inhibit the formation of IL-1 β by inhibiting the activation of NF κ B [17]. Genistein can reduce cell viability macrophages so reduce the proinflammatory cytokines [18].

Protein Tyrosine kinases are protein signaling for the production of proinflammatory cytokines. Protein tyrosine kinases play an important role in inflammation in the kidney. Genistein can improve kidney from hyperglycemia effect. Genistein can inhibit the action of protein tyrosine kinase that can be used as anti-inflammatory with high therapeutic index and a very low toxicity. Giving of genistein in the long term can improve complications in blood vessels because of hyperglycemia [7].

4.2. Effect of Genistein on Levels of TGF- β

Levels of TGF- β STZ group 8492.520 pg/ml higher than the levels of TGF- β non diabetic group 1411,262 pg/ml. This suggests that hyperglycemia can lead to increased levels of TGF- β . Hyperglycemia conditions can stimulate the secretion of TGF- β by cells and mesangial cells in kidney Podosit [19]. TGF- β is one of the proteins that are important in the occurrence of fibrosis in the kidney. Excessive expression in renal hypertrophy will trigger and stimulate the formation of extracellular matrix [20]. TGF- β is produced by various cell types in the kidney.

Early development of diabetic nephropathy is associated with higher levels of TGF- β . Active TGF- β mediated gene transcription factor fibronectin and collagen that stimulates formation of extracellular matrix [21]. Increased levels of TGF- β stimulates the progression of kidney disease. TGF- β action on epithelial cells stimulates epithelial cell apoptosis in renal tubular and glomerular cells podosit [22].

Genistein can decrease levels of TGF- β on uterine myoma [17]. Icariin can improve the condition of nephropathy [23]. Icariin, which is a major flavonoids isolated from Epimedium can reduce levels of TGF- β hyperglycemic mice. Giving of genistein in rats fed fructose for 6 weeks also can decrease the expression of TGF- β [24]. Based on the results of the study decreased levels of TGF- β in a dose of 0.5 mg/kgw, 1 mg/kgw and 2 mg/kgw significantly.

5. Conclusion

The administration of genistein can reduce levels of IL-1 β and TGF- β . Based on the research results, the suggestion of this study are: observation on histological glomerular

Conflict of Interest

There were no conflict of interest found among the authors in this work.

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