Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(9); 1206-1212
doi: 10.25258/phyto.v9i09.10307

ISSN: 0975-4873

Research Article

Alpha Mangostin and Xanthone from Mangosteen (*Garcinia mangostana* l.) Role on Glucose Tolerance and Glucose Transporter-4 in Diabetes Mellitus

Ratwita W*, Sukandar E Y, Adnyana I K, Kurniati N F

Department of Pharmacology and Toxicology, School of Pharmacy, Bandung Institute of Technology, Indonesia.

Received: 8th Jul, 17; Revised 22nd Aug, 17, Accepted: 14th Sept, 7; Available Online:25th Sept, 17

ABSTRACT

Objective: This research elaborated role of alpha mangostin and xanthone on glucose tolerance and Glucose Transporter (GLUT)-4 by measuring blood glucose level and GLUT-4 expression on cardiac cell musce and adipocyte cell culture. Methods: Glucose tolerance test were conducted using male wistar rat divided into 9 groups, which were normal, control (D-Glucose induced only), glibenclamide, various doses of α-mangostin and xanthone (5, 10, 20 mg/kgbw). All group induced by D-glucose 3 g/kg orally 30 minutes later. Blood glucose levels changes were observed at 90th and 150th minute. GLUT-4 study conducted for 3 weeks on mice that divided into 10 groups which were normal, diabetic control mice (alloxan induced only), metformin, glibenclamide, various doses of α-mangostin and xanthone (5, 10, 20 mg/kgbw). GLUT-4 expression than observed on cardiac cell muscle. While other study observed GLUT-4 expression on adipocyte cell culture that treated with α-mangostin/xanthone/pioglitazone. Results: Normal group (non-diabetic) responds slightly to the administration of glucose in glucose tolerance test. Blood glucose level in every group in the 90th up to 150th minute decreased significantly when compared to the positive control group ($p < 0.05$). This shows that glucose tolerance does not occur in all treated groups althought they were treated with high glucose consentration. GLUT-4 expressions in mice cardiac-musce cells that treated with α-mangostin/xanthone/ glibenclamide/metformin significantly increased when compared to the positive control group, except in group treated with xanthone 5 mg/kgbw. GLUT-4 expressions also increase in adipocytes that treated with 3.125 mM; 6.25 mM and 25 mM *α*–mangostin, equivalent to pioglitazone. All treatment group results significantly different when compared with control. The effect of *α*–mangostin on GLUT-4 expression better than xanthone's. Conclusion: Alpha mangostin and xanthone are two substances that showed protective effect to glucose tolerance and also have potential effect to improve insulin resistance by increasing GLUT-4 on cardiac muscle and adipocyte.

Keywords: α-mangostin, xanthone, glucose tolerance, glucose transporter-4.

INTRODUCTION

The prevalence of diabetes mellitus (DM) risen dramatically in worldwide[1]. DM will affect 438 million people by 2030, with 70% of cases occur in low-middle income countries. Without proper treatments, DM may cause cardiovascular disease as the major cause of morbidity and mortality[2-4]. During the development of type-2 diabetes, insulin's ability to stimulate the cellular uptake of glucose from the blood was impaired[5-6].

Prolonged elevation of blood glucose concentration as in poorly controlled diabetes, may cause blindness, renal failure, cardiac and peripheral vascular disease, and neuropathy. Therefore, blood glucose concentrations need to be maintained within narrow limits. The rise in blood glucose levels after meal rapidly stimulates insulin secretion, which results within minute in increased glucose transport, metabolism, and storage by muscle and adipocytes[7].

Because the lipid bilayers that make up cell membranes are impermeable to carbohydrates, carbohydrate-transport systems are required. In recent years, two distinct molecular families of cellular transporters of glucose have been cloned, The sodium-linked glucose transporters are largely restricted to the intestine and kidney, where they actively transport glucose against a glucose-concentration gradient by using sodium co-transport as an energy sourcewhere they actively transport glucose against a glucose-concentration gradient by using sodium co-transport as an energy source[8].

The other group of transporters convey glucose by facilitated diffusion down glucose-concentration gradients. This group consists of five homologous transmembrane proteins, GLUT-1, 2, 3, 4, and 5, that are encoded by distinct genes. The GLUT proteins have distinct substrate specificities, kinetic properties, and tissue distributions that dictate their functional roles. Muscle is the principal site of insulin-stimulated glucose disposal in vivo; less glucose is transported into adipose tissue[9]. Previous studies have indicated that in muscle, glucose transport across the plasma membrane is the rate-

*Author for Correspondence: wellyratwita@gmail.com

limiting step for glucose metabolism in normal subjects[8].
Glucose transporter (GLUT)-4 is a high-affinity glucose transporter predominantly expressed in insulin-sensitive tissues such as muscle and adipocytes. Increasing GLUT-4 expression and plasma membrane translocation of GLUT-4 have been found in low blood glucose. This transporter enhance glucose transport and utilisation[12]. In normal muscle cells and adipocytes, GLUT-4 will be recycled between the plasma membrane and intracellular storage pools. GLUT-4 is different from others because 90% of them will be broken on the intracellular network when there is no stimulation of insulin, physical activity or other stimuli. The presence of insulin or other stimuli will trigger the translocation of GLUT-4 from the plasma membrane to intracellular network. At cardiac muscle, GLUT-4 translocation will lead to the transverse tubules[7].
Insulin stimulates translocation of GLUT-4 by initiating insulin binding to receptors on the plasma membrane. This bond will activate tyrosine phosphorylation of the receptor intracellular kinase. Stimulation of glucose transport in muscle cells and adipocytes need the phosphoinositide-3 kinase. Phosphoinositide-3 kinase will activate the protein kinase B (serine-threonine kinase). Stimulation of glucose transport in diabetic subjects impaired by the change of insulin levels, while activation of protein kinase remained normal[7].
One alternative way that can be used to overcome the disease of diabetes is to utilize native medicinal plants[13]. It is correlate with the program "Back to Nature Used Indonesian Traditional Medicine" launched by Indonesian Ministry of Health in 1998 with establish the Centers for development and application of traditional medicine (SP3T)[14]. One of Asia native plant is Mangosteen (*Garcinia mangostana* L.).
Phytochemical studies show that mangosteen contains oxygenated and prenylated xanthones. Xanthone is believed to have anti-cancer effects, anti-inflammatory, anti-viral and cardiovascular protection with the antioxidant effects. Xanthone most researched are alpha, beta and gamma mangostin, garcinone E, 8-deoksigartanin and gartanin. Xanthone could be found on the skin of fruit, fruit, bark and leaves of mangosteen[15-18].

MATERIAL AND METHODS

Glucose Tolerance Test

Glucose tolerance test were conducted using male wistar rat divided into 9 groups, which were normal, control (D-Glucose induced only), glibenclamide, various doses of α-mangostin and xanthone (5, 10, 20 mg/kgbw). All group induced by D-glucose 3 g/kg orally. Blood glucose levels changes were observed at 90th and 150th minute.
Blood from vein were collected and checked as the initial blood glucose levels. This process repeated 6 times every 30 minutes for 150 minutes. Blood glucose were checked using glucose oxidase method with a glucometer (One Touch Ultra, Life Scan Inc., Milpitas, CA)

GLUT-4 Expression in Cardiac Cell Muscle

GLUT-4 expression on cardiac cell muscle were detected using immunohistochemistry method. The tissue was taken from diabetic mice induced by alloxan monohydrate. Diabetic mice than treated with glibenclamide/metformin and various doses of α-mangostin and xanthone (5, 10, 20 mg/kgbw) for 21 days. Then the animals were sacrificed and cardiac muscle were taken. Cardiac muscle paraffin blocks then cutted using microtome (5 µm) and placed on an object glass coated with poly-L lysine 10%. The slides then rehydrated with down-graded ethanol (100, 95 and 80%), washed with water Deion before soaked in hydrogen peroxide 1% to inhibit endogenous peroxidase activity for 10 minutes. Slides then washed two times with PBS, before returning soaked in a solution of 5% BSA to reduce non-specific binding. The preparat then washed using Phosphate Buffer Saline (PBS), followed by hatching primary antibody (E-EL-M0564). Slides were washed using PBS four times, continued by provision of Biotinylated Goat Anti-Mouse, and then incubated for 10 minute at room temperature. Slides then washed again four times in PBS. 30 mL Diaminobenzidine (DAB) chromogen added to 1.5 mL of the substrate, and then homogenized. The slides was incubated for 1-10 minutes, and washed four times in PBS. Slides then stained with hematoxylin, dehydrated in graded ethanol (80, 95, 100%), cleaned with xylol 1 and 2, before closing with a glass cover[19].*GLUT-4 Expression on Adipocytes*

Preadipocytes were isolated from rat retroperitoneal tissue. Rat were 4 weeks old[20]. Fibrous tissue and blood vessels were removed first. Tissue was washed and chopped. Tissue suspension incubated with 0.2% collagenase type 1 (Sigma) for 45 minutes, at 37 ^{0}C on shaking condition. Incubation was stopped by the addition of culture medium, Dulbecco's Modified Eagle Media (DMEM/F12 (1:1) supplemented with 15 mmol/l HEPES, 14 mmol/NaHCO3, 33 µmol/l of biotin, 17 µmol l D-panthotenate and 10% Fetal Bovine Serum (FBS). The filtration using a nylon mesh (250 µm). Cell suspension then rotated 1500 rpm for 7 minutes. Fat layers (mature adipocyte and fat droplets) in the supernatant was discarded. Pellet containing fibroblast-like preadipocyte then resuspended in cell culture media, rotated 1500 rpm for 7 minutes and then resuspended again using the culture medium.

Cell Culture

Preadipocytes were grown in Dulbecco's Modified EagleMedia (DMEM) containing 10% Fetal Bovine Serum (FBS), 1% penicillin (10.000 U/mL), and 1% streptomycin (10.000 μg/mL supplemented in 37^{0}C incubator in a humidified atmosphere of 5% CO_2). Cells were subcultured every 3 to 4 days at approximately 80% confluence.
Mature adipocytes were seeded in 96-well plates and grown until confluence. α-mangostin/xanthone/pioglitazone was dissolved in dimethyl sulfoxide (DMSO) and treated for 48 h. Cells were then washed two times with PBS[21].

Induction of Adipocytes Differentiation

Before and after incubated in adipogenic media (DMEM/F12) 100 U/ml penicillin and 100 U/ml streptomycin, 66 nM insulin, 100 nM dexamethasone, 0.5 mM Methyl Iso buthyl Xantine (IBMX) and 10 µg ml transferrin were added for adipocytes differentiation.

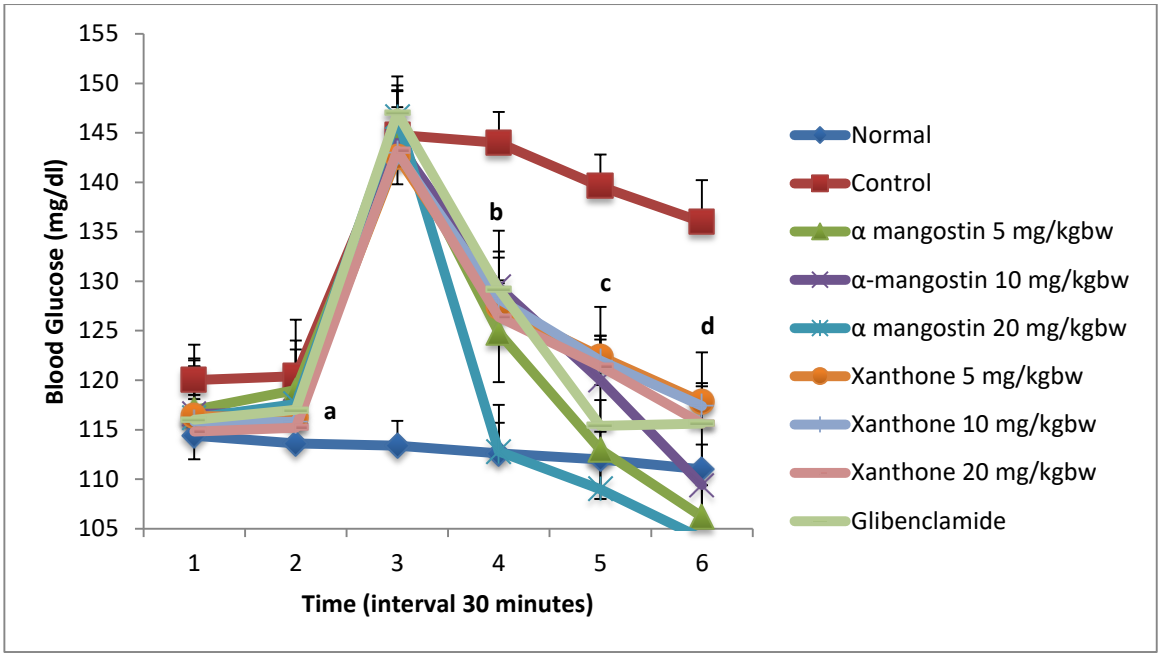

Figure 1: Effect of α-mangostin, xanthone in different concentration (5, 10, 20 mg/kgbw), glibenclamide on blood glucose level. Data is represented as mean±SD, with n=5 per group.
(a) $p < 0.05$ on xanthone 5, 10, 20 mg/kgbw compared to control group in 30th minutes. (b-d) $p < 0.05$ on α-mangostin-xanthone 5,10,20 mg/kgbw, and glibenclamide compared to control group in 90, 120 and 150th minutes.

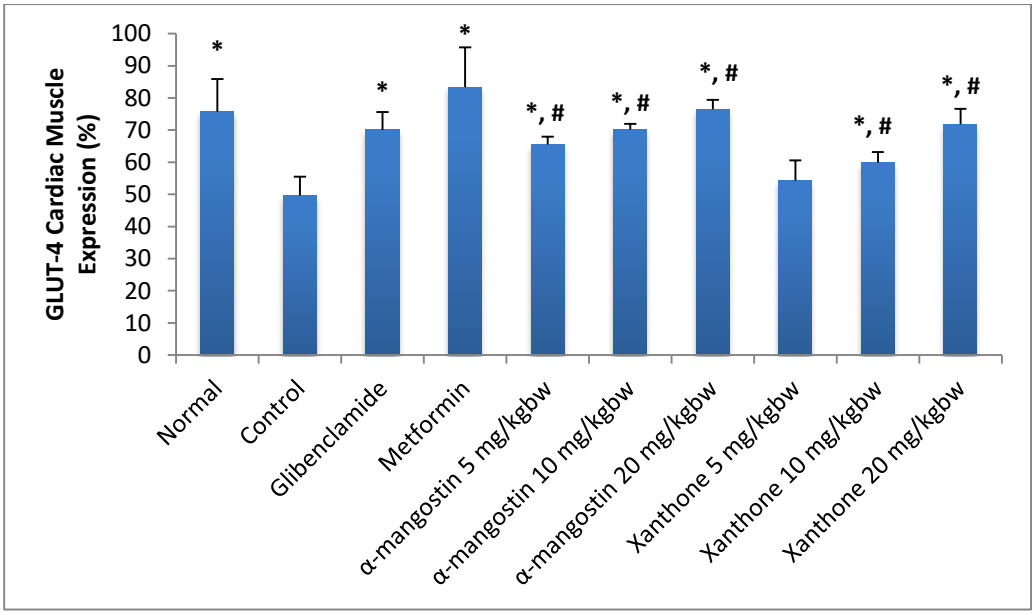

Figure 2: Effect of α-mangostin, xanthone in different concentration (5, 10, 20 mg/bw), glibenclamide on GLUT-4 Cardiac Muscle Expression (%). Data is represented as mean±SD, with n=5 per group. (*) $p < 0.05$ compared to control group, (#) $p < 0.05$ between doses in alpha mangostin/xanthon group.

Suspension cells grew in culture plates, incubated at 37 ^{0}C, 5 % CO_2 and 95 % humidity for 24 hours. Cells were washed once every 3 days.

Adipocyte Differentiation

Cells were seeded into 22-well plates at a density of 2 × 10^4 cells/well. Two days after confluence (defined as day-0), cells were stimulated to differentiate with differentiation medium containing DMEM with 10% FBS and MDI [0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.25 μM dexamethasone, and 1 μg/mL insulin] for 2 days.

In the course of screening adipocyte differentiation inhibitory activity, preadipocytes were treated with differentiation medium in the presence of various concentrations of test compound (0.78 µM, 1.56 µM, 3.125 µM, 6.25 µM, 12.5 µM, 25 and 50 µM of α-mangostin/xanthone/ pioglitazone) at day-0. At day-2, differentiating medium was replaced with 10% FBS/DMEM medium containing 1 μg/mL insulin and incubated for another two days (day-4). Thereafter, the cells were maintained in 10% FBS/DMEM medium for an

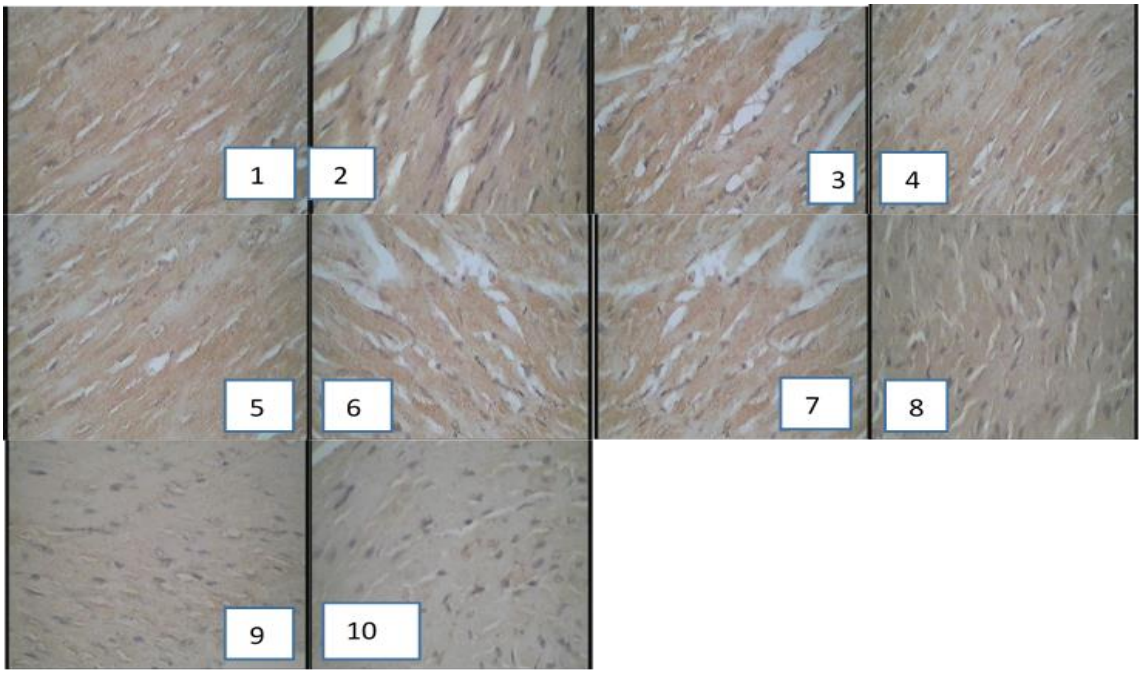

Figure 3: Stained GLUT-4 expression in cardiac muscle cell (magnification 400x). (1) Metformin. (2) Normal. (3) AM 20 mg/bw. (4) Xa 20 mg/bw. (5) AM 10 mg/bw. (6) Glibenklamid. (7) AM 5 mg/bw. (8) Xa 10 mg/bw. (9) Xa 5 mg/bw (10) Control, respectively. AM: α-mangostin; Xa: Xanthone.

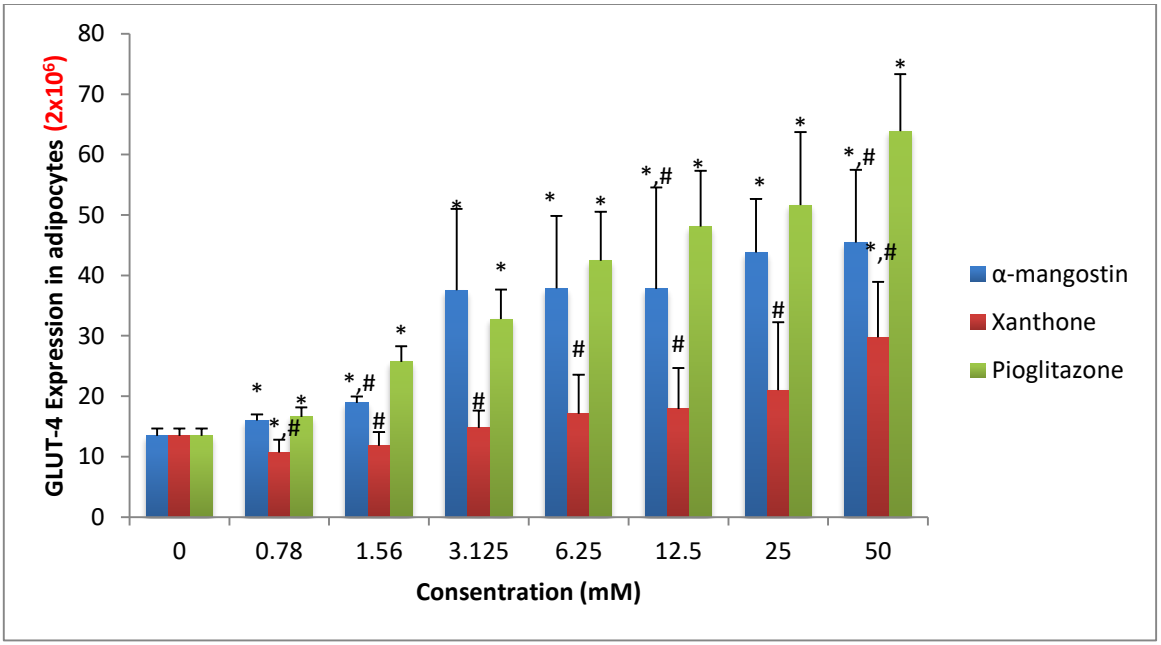

Figure 4: GLUT-4 expression on adipocytes cell culture, *) p <0.05 compared to control group, #) p < 0.05 compared to pioglitazone.

additional 4 days (day-8) with medium changes every 2 days. Before and after incubated in adipogenic media, adipocytes were calculated. Calculating the number of cells in Neubauer Improved counting chamber was performed using a light microscope. The data were calculated by the formula k = n x p x 2500, with k = cell density (cells/ml), n = the total number of cells in the four counting chamber, and p is the dilution rate used.[22]

Immunocytochemistry Staining

Adipocyte fixed in 10% formalin (v/v) in PBS (pH 7.4) for 20 minutes. Cells then washed with PBS (pH 7.4) three times, and treated by 0.02% (w/v) sodium azide. Cells then washed again with PBS (pH 7.4) three times for 5 minutes, then treated by H_2O_2 in PBS for 10 minutes. Reaction then stopped with 0.25% Triton-X blocking serum added in 5% FBS for 1 h, then washed with PBS. Adipocytes were treated with anti-GLUT-4 antibody diluted in serum 1:500 for 24 h, then incubated at 4^0C for 24 h. After incubated they were washed with PBS 3 times for 5 minutes each.

Cells were incubated in anti-rabbit secondary antibody 1:500 for 1 h at room temperature, then washed with PBS 3 times for 5 minutes each. Cells treated by SA-HRP for 40 minutes, then washed with PBS 3 times for 5 minutes each. Cells then treated with Diamino benzidine (DAB) in the DAB buffer. Cells treated with courstexin with Mayer hematoxilin for 10 minutes, then washed with tap water, followed by distilled water for 10 minutes. Oil Red O staining was used to confirm that cells that were differentiated were adipocytes.

Oil Red O Staining

Eight days after the differentiation induction, cells were washed three times with PBS and fixed with 10% formalin for 1 h at room temperature. After fixation, cells were washed once with PBS and stained with freshly diluted Oil Red O solution (3 parts of 0.6% Oil Red O in isopropanol and 2 parts of water) for 1 h. Cells were then washed twice with distilled water and visualised under a microscope[23]. Images were collected on an Olympus microscope.

RESULTS AND DISCUSSION

Glucose Tolerance Test

Rats were fasted for 8 hours, then the initial blood glucose levels were measured. They were then treated with glibenclamide, various dose of α-mangostin/xanthone, followed by an oral D-glucose load 30 minutes later. Blood glucose levels were measured every 30 minutes for 150 minutes.

Glucose tolerance test result showed in the 60t^{h} minute, glucose levels in the whole group increased, and did not differ significantly from each other. In the 90th minute glucose levels began to decrease, in all groups, except for the positive control group, and this continues until the 150th minute. Normal group (non-diabetic) responds slightly to the administration of glucose. Blood glucose in every group in the 90th up to 150th minute decreased significantly when compared to the positive control group ($p < 0.05$). This shows that despite the given high glucose, glucose tolerance does not occur in all treated groups. `

GLUT-4 Cardiac Cell Muscle

GLUT-4 is a major transporter of glucose-responsive insulin. GLUT-4 can be found in sceletal muscle, heart and adipocyte tissue. GLUT-4 expression were observed to the cardiac cell muscle of diabetic mice treated with α-mangostin/xanthone/glibenclamide/metformin. Slides were stained using immunohistochemical methods, and then analyzed using a semi-quantitative immunoratio application.

GLUT-4 expressions in mice cardiac cells muscle that treated with α-mangostin/xanthone/ glibenclamide/metformin significantly increased when compared to the positive control group, except in group treated with xanthone 5 mg/kgbw. GLUT-4 expession also significantly increased between different dose of α-mangostin. While between group that treated with xanthone, it only increased significantly between dose 10 and 20 mg/kgbw.

The expression of GLUT-4 in mice cardiac cell muscle were seen as a brownish pigmentation in plasma membrane between the nucleus of cardiac cell muscles. Metformin group showed best result on expressing GLUT-4 than other treatment groups, even more than the normal group. But we can also see that cardiac cell muscles in group treated with α–mangostin 10, 20 mg/kgbw, and xanthone 20 mg/kgbw expressed GLUT-4 more than group treated with glibenclamide.

GLUT-4, which transports glucose from blood into tissue, is the principal glucose transporter among several isotypes of glucose transporters in insulin-sensitive tissues such as skeletal muscle and adipocytes[24]. Decrease in the translocation of GLUT4 to the plasma membrane has been found to be the major cause of insulin resistance,[25] and it is required to activate GLUT-4 in skeletal muscle to improve insulin resistance and to maintain blood glucose homeostasis.

In impaired glucose tolerance and obesity, the expression of GLUT-4 will decrease in adipocyte cells, but levels in the muscle will not impaired. Muscle is a primary backup storage of glucose. Decreasing expression of GLUT-4 in muscle more related to the age factor than insulin sensitivity. Although the increased levels of GLUT-4 in muscle and adipocytes will improve glucose tolerance and insulin sensitivity in diabetic subjects[7].

Metformin, which is one of the widely used antidiabetic drugs, can enhance the insulin-stimulated glucose uptake by increasing the cell surface GLUT4 content[26]. In this study, to assess whether the increased glucose uptake stimulated by α-mangostin was due to the translocation of GLUT-4, the amount of GLUT-4 expression present in the cells was measured by using the immunoratio software.

Glibenclamide will improve transport GLUT-4 to the cell surface. Metformin increases the transport of GLUT4 in adipocytes and muscle in vitro in the presence of the effects of metformin in the form of short-term insulin-like effects.[7]

GLUT-4 cells adipocytes

Afterward, we investigated the effects of α–mangostin and xanthone on the GLUT-4 expression. The glucose uptake activity in adipocytes is related to the expression of glucose transporters. In this study, it was observed that all the GLUT-4 expression in fully differentiated adipocytes increased significantly after treatment with α–mangostin, xanthone and pioglitazone.

GLUT-4 is the main insulin-responsive glucose transporter and is located primarily in muscle cells and adipocytes. In normal muscle cells and adipocytes, GLUT-4 is recycled between the plasma membrane and intracellular storage pools. GLUT-4 differs from other glucose transporters in that about 90 percent of it is sequestered intracellularly in the absence of insulin or other stimuli such as exercise[27-28].

Adipocytes that treated with 3.125 mM; 6.25 mM and 25 mM α–mangostin increased GLUT-4 expression, equivalent to pioglitazone. All treatment group results significantly different when compared with control. The effect of α–mangostin on GLUT-4 expression better than xanthone's. This result indicates that the α -mangostin accelerated basal and insulin mediated glucose uptake by upregulating the GLUT-4 expression. Decreased GLUT4 expression in adipose tissue is associated with obesity and type 2 diabetes in humans[29].

Thus, it is important to use a compound that could improve glucose uptake and stimulate GLUT-4 expression. GLUT-4 is a high-affinity glucose transporter predominantly expressed in insulin-sensitive tissues such as muscle and adipocytes[30]. Increased expression and plasma membrane translocation of GLUT-4 have been found to lower blood glucose and enhance glucose transport and utilization.

In the presence of insulin or another stimulus, the equilibrium of this recycling process is altered to favor the translocation (regulated movement) of GLUT-4 from intracellular storage vesicles to the plasma membrane and, in the case of muscle, to the transverse tubules as well. The net effect is a rise in the maximal velocity of glucose transport into the cell[27-28]. Insulin-stimulated intracellular movement of GLUT-4 is initiated by the binding of insulin to the extracellular portion of the transmembrane insulin receptor[7].

CONCLUSION

In summary, the present study has shown that α-mangostin and xanthone isolated from mangosteen (*G. Mangostana* L.) are two substance that showed protective effect to glucose tolerance and also potential to improve insulin resintency by increasing GLUT-4 on muscle and adipocyte.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

ACKNOWLEDGMENTS

This work was supported by Jenderal Achmad Yani University and School of Pharmacy, Bandung Institute of Technology, Indonesia

REFERENCES

1. Gorelick, J., Kitron, A., et al. "Anti-diabetic activity of Chiliadenus iphionoides" Journal of Ethnopharmacology, Vol. 137 No.3, 1245–1249, 2011.
2. Yu, Z., Yin, Y., Zhao, W., Liu, J., Chen, F. "Anti-diabetic Activity Peptides from Albumin Against α-Glucosidase and α-Amylase. Dalam *Journal of Food Chemistry.* Vol 135. pp.2078-2085, 2012.
3. Yu, Z., Yin, Y., et al. "Characterization of ACE-inhibitory peptide associated with antioxidant and anticoagulation properties. Journal of Food Science", Vol. 76 No.8, pp 1149-1155, 2011.
4. Yu, Z., Yin, Y., et al. "Novel peptides derived from egg white protein inhibiting alpha-glucosidase." Food Chemistry, Vol.129 No.4, pp1376–1382, 2011.
5. C.M.Orme, J.S.Bogan, Sorting out diabetes. Science, Vol.324, pp1155–1156, 2009.
6. Z.Yu, Y.Yin, W.Zhao, J.Liu, F.Chen. "Anti-diabetic activity peptides from albumin against □□-glucosidase and □ -amylase" Food Chemistry Vol. 135. pp.2078–2085, 2012.
7. P.R.Shepherd, B.B.Kahn, "Glucose Transporters and Insulin Action, Implication for Insulin Resistance and Diabetes Mellitus" The New England Journal of Medicine Vol.341.No 4. pp. 248-256, 1999.
8. Wright EM, Turk E, Zabel B, Mundlos S, Dyer J. Molecular genetics of intestinal glucose transport. J Clin Invest Vol.88. pp1435-40, 1991.
9. DeFronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. Diabetes Rev 1997;5:177-269.3. Fink RI, Wallace P, Brechtel G, Olefsky JM. Evidence that glucose transport is rate-limiting for in vivo glucose uptake. Metabolism. Vol.41. pp 897-902, 1992.
10. Katz A, Nyomba BL, Bogardus C. No accumulation of glucose in human skeletal muscle during euglycemic hyperinsulinemia. Am J Physiol. Vol.255. pp942-945, 1998.
11. Y.K.I Jarvinen, A.A.Young, C.Lamkin, JE Foley. Kinetics of glucose disposal in whole body and across the forearm in man. J Clin Invest Vol. 79. pp1713-9, 1987.
12. M. Taher, M.Z.A.M. Amiroudine, T.M.F.S.T.Zakaria, D.Susanti, S.J.A.Ichwan, , M.A.Kaderi, O.U.Ahmed, Z.A.Zakaria, "α-Mangostin Improves Glucose Uptake and Inhibits Adipocytes Differentiation in 3T3-L1 Cells via PPARγ, GLUT4, and Leptin Expressions" Evidence-Based Complementary and Alternative Medicine Volume 2015, p 1-9, 2015.
13. Sari LK. "Pemanfaatan Obat Tradisional Dengan Pertimbangan Manfaat dan Keamanannya. *Majalah Ilmu Kefarmasian*. Vol.3. pp 1-7, 2006.
14. Keputusan Menteri Kesehatan Republik Indonesia Nomor 0584/Menkes/SK/VI/1995 Tentang Sentra Pengembangan dan Penerapan Pengobatan Tradisional, 1995.
15. Aisha, A., Abu-Salah, K., Siddiqui, M., Ismail, Z., Majid, A. "Quantification of α-, β- dan γ-mangostin in Garcinia mangostana Fruit Rind Extracts by a Reverse Phase High Performance Liquid Chromatography". Journal of Medicinal Plants Research. Vol.6 No.29. pp 4526-4534, 2012.
16. J.Chaverri, N.Rodriguez, M.Ibarra, J.Rojas, "Medical Properties of Mangosteen (Garcinia mangostana)." *Food and Chemical Toxicology*. Vol.46. pp 3227-3239, 2008.
17. J.Reddy, N.Ravikumar, G.Gaddamanugu, K. Naresh, S.Rajan, K.Solomon, Synthesis, Crystal Structure, Spectral Characterization and Fluorescence Studies of Salts of α-mangostin With APIs. Dalam *Journal of Molecular Structure*. pp 137-143, 2013.
18. H.Ryu, J.Cho, M.Long, H.Yuk, Y.Kim, S.Jung, B.Lee, K.Park, "α-Glukosidase Inhibition and Antihyperglycemic Activity of Prenylated Xanthones from Garcinia mangostana." *Journal of Phytochemistry.* Vol.72. pp 2148-2154, 2011.
19. H.Kojima, M.Fujimiya, K.Matsumura, T.Nakahara, M.Hara, L.Chan, "Extrapancreatic Insulin-Producing Cells in Multiple Organs in Diabetes", *Proceeding of National Academy of Sciences*, Vol.101 No.8. pp 2458-2463, 2004.
20. F.M. Gregoire, C.M. Smas, H.S.Sul, "Understanding Adipocyte Differentiation" American Physiological Society Journal Vol. 78, No. 3, p.783-809, 1998.

21. T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," Journal of Immunological Methods, vol. 65, no. 1-2, pp. 55–63, 1983.
22. D. Susantia, M. Z. A. M. Amiroudineb, M. F. Rezalic, and M. Taherb, "Friedelin and lanosterol from Garcinia prainiana stimulated glucose uptake and adipocytes differentiation in 3T3-L1 adipocytes," Natural Product Research, vol. 27, no. 4-5, pp. 417–424, 2013.
23. E. Shin, K.-M. Choi, H.-S. Yoo, C.-K. Lee, B. Y. Hwang, and M. K. Lee, "Inhibitory effects of coumarins from the stem barks of Fraxinus rhynchophylla on adipocyte differentiation in 3T3-L1 cells," Biological and Pharmaceutical Bulletin, vol. 33, no. 9, pp. 1610–1614, 2010.
24. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR. "Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation". Diabetes. Vol.46. pp524-7, 1997.
25. D. T. Ha, T. N. Trung, T. T. Phuong, N. Yim, Q. C. Chen, and K. Bae, "The selected flavonol glycoside derived from Sophorae Flos improves glucose uptake and inhibits adipocyte differentiation via activation AMPK in 3T3-L1 cells," Bioorganic and Medicinal Chemistry Letters, vol. 20, no. 20, pp. 6076–6081, 2010.
26. S.-S. Choi, B.-Y. Cha, K. Iida et al., "Artepillin C, as a PPARγ ligand, enhances adipocyte differentiation and glucose uptake in 3T3-L1 cells," Biochemical Pharmacology, vol. 81, no. 7, pp. 925–933, 2011.
27. Kandror KV, Pilch PF. "Compartmentalization of protein traffic in insulin-sensitive cells." Am J Physiol. Vol.271. pp1-14, 1996.
28. Gould GW, Holman GD. "The glucose transporter family: structure, function, and tissue-specific expression." Biochem J Vol.295. pp329-41, 1995.
29. L.Granlund, J.I.Pedersen, and H.I.Nebb, "Impaired lipid accumulation by trans10, cis12 CLA during adipocyte differentiation is dependent on timing and length of treatment," Biochimica et Biophysica Acta—Molecular and Cell Biology of Lipids, vol. 1687, no. 1-3, pp. 11–22, 2005.
30. R.T.Watson and J.E.Pessin, "GLUT4 translocation: the last 200 nanometers," Cellular Signalling, vol. 19, no. 11, pp. 2209–2217, 2007.