Improvement of Insulin Secretion and Pancreatic *β*-Cell Function in Streptozotocin-induced Diabetic Rats Treated with *Dioscorea esculenta* Extract

Suggested title:

Effects of Inulin from *Dioscorea esculenta* on Insulin Secretion and Pancreatic β-Cell Function in Streptozotocin-induced Diabetic Rats

Nilawati Uly[1], Ari Yuniastuti[2*], R Susanti[2], Yanuarita Tursinawati[3]

[1]*Institute of Higher Health Education, Mega Buana Palopo, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Central Java, Indonesia*
[3]*Faculty of Medicine, University of Muhammadiyah Semarang, Central Java, Indonesia*
[*]*Corresponding author. E-mail: ariyuniastuti@mail.unnes.ac.id*
Tel: +628156615624

ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia that can destroy pancreatic β-cells due to its toxic effect. A local Indonesian vegetable known as lesser yam (*Dioscorea esculenta*) possesses anti-diabetic properties. The present study evaluated the effect of inulin extract from lesser yam on pancreatic β-cell count and insulin expression in streptozotocin (STZ)-induced diabetic rats. Inulin was extracted from lesser yams. Streptozotocin was used to induce diabetes in Wistar rats. The rats were divided into six groups: I (control), II (DM), III (galvus drug-control), IV, and V (DM rats administered with inulin at 100 and 300 mg/kg body weight for 21 days, respectively), and VI (DM rats administered with inulin extract at 300 mg/kg body weight for 30 days). Pancreatic β-cell count and insulin expression were assessed using immunohistochemical and histopathological methods, respectively. The results showed that on the 21st and 28th days after treatment, there was a significant difference in the number of pancreatic β-cells between the groups. On day 14, the insulin expression of pancreatic β-cells between groups was significantly different. There was a significant difference in the insulin expression of pancreatic β-cells on the 21st and 28th-day treatments between the various groups. The findings of the study reveal an increase in the β-cell number and insulin expression of pancreatic β-cells in diabetic rats when inulin extract from lesser yam was administered. The inulin dose variation had no significant effect on the number and insulin expression of pancreatic β-cells in Wistar rats.

***Keywords**:* Diabetes, *Dioscorea esculenta*, Insulin, Inulin, Pancreatic β-cells, Streptozotocin

Introduction

Degenerative diseases like diabetes mellitus have replaced infectious diseases as the predominant types of illness in Indonesia as a result of the adaptation to Western lifestyles of the present generation. In the presence of pre-existing risk factors and the environment, diabetes mellitus (DM), both types 1 and 2, causes a decrease in pancreatic β-cell mass. This decrease in function manifests as hyperglycemia.[1] The report of Basic Health Research (RISKESDAS) in 2013 indicated that 6.9% (about 12,191,564) of Indonesians over the age of 15 have diabetes.[2] The management of type 2 diabetes has been based on drugs that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues), reduce hepatic glucose production (biguanides), delay digestion and absorption of intestinal carbohydrates (alpha-glucosidase inhibitors), or improve insulin action (thiazolidinediones).[2,3] Patients with type 2 DM have abnormalities in homeostasis of blood glucose, resulting in increasing fasting sugar levels. This is due to decreased function and mass of pancreatic β-cells and insulin secretion.[3] As blood glucose levels begin to increase, β-cells release insulin through the process of exocytosis. In the condition of hyperglycemia, β-cells of the pancreas are damaged due to the toxic effect of hyperglycemia, which results in insulin resistance.[4,5] Increased apoptosis and a significant reduction in the number of β-cells have been observed in type 2 DM as a result of glycation-mediated free radical production. It also has the impact of reducing insulin gene transcription, which results in less insulin release.[6]

Indonesia has many kinds of local vegetables, such as gembili (*Dioscorea esculenta* L) or lesser yam, which is a member of the Dioscoreceae family and a member of the tuber group. The lesser yam tuber has yellow or white flesh and a sweet and pleasant flavour similar to that of sweet potatoes. It contains bioactive compounds such as phenol,[7] diosgenin,[8] dioscorin, and inulin.[9] The results of the exploratory study indicate that it also contains antioxidant agents.[10] Other studies suggest that yam has antioxidant effects *in vitro*.[11-13] A study by Kyu *et al*., 2015

demonstrated that administering yam to diabetic control rats resulted in much less β-cell granulation and less pancreatic islet vacuolation.[14] Based on another study by Tursinawati *et al*., inulin in lesser yams has stronger α-glucosidase inhibitory activity *in vitro* than acarbose, which has an antidiabetic activity.[15] Inulin is a type of fructosan that is extracted from plants such as lesser yams. The compound is prebiotic, which cannot be hydrolyzed by digestive enzymes but can be utilized by the beneficial bifidobacteria in the large intestine.[16] A study showed that inulin had antioxidant capacity related to the non-enzymatic reactive oxygen species (ROS) defense system. Inulin improved the *in vivo* antioxidant status of laying hens.[17] Therefore, it is essential to create DM medications that not only prevent the destruction of β-cells and increase β-cell count through antioxidant activity, such as that observed in inulin, but also enhance insulin secretion.

The present study was conducted to investigate the protective effect of lesser yam inulin extract on pancreatic β-cell counts and expression of insulin in streptozotocin-induced diabetic Wistar rats.

Materials and Methods

Plant collection and preparation of extracts

The tubers of lesser yam were collected from Mekarsari Community (KSM), Kulon Progo, Yogyakarta. The collection was made specifically on Mount Gondang (RT 21/RW 11), Margosari, Pengasih, Kulonprogo, Yogyakarta from July 5 to August 5, 2019. The tubers were identified by Nilawaty Uli from the Institute of Higher Health Education, Mega Buana Palopo, Indonesia, and Ari Yuniastuti from the Department of Biology, Faculty of Mathematics and Nature Science, Semarang State University, Indonesia. The extraction of inulin from lesser yam was done by cleaning, washing, peeling, and cutting into small pieces. The lesser yam was blended using a blender. After mixing in hot water at a ratio of 10:1 (tuber: water), diffusion

was carried out in a water bath shaker at 90°C for an hour. The mixture was filtered, cooled, and frozen at -20° C for 24 hours. The frozen filtrate was defrosted and then centrifuged for 15 minutes at 1500 rpm to produce a white precipitate, which was subsequently separated. The resultant white precipitate was mashed and then sieved after being dried in a drier cabinet at 60°C for 5 hours.[18]

Source of experimental animals

Forty-two (42) male Wistar rats weighing 180–220 g at 5 months of age were used in this study. The rats were purchased from the xxxx. The rats were kept in plastic cages and fed standard laboratory food and water ad libitum.

Ethical approval

Ethical approval for this study was obtained from the Health Research Ethics Committee (KEPK) of the Universitas Negeri Semarang, Indonesia (Approval No. 035/KEPK/EC/2019).

Experimental design

This research used an experimental method with modifications of a pretest-posttest randomized controlled group design. The rats were divided into six groups with each group consisting of seven rats. Group I (the control group) consisted of normal rats that received 1% NaCMC; Group II (DM group) received one dosage of STZ at a dose of 40 mg/kg body weight (diabetic rats); Group III (galvus drug control group) consisted of normal rats that received 0.9 mg/200 g/body weight orally; the diabetic rats in Group IV received inulin extract at 100 mg/kg body weight orally over the course of 21 days; the diabetic rats in Group V received inulin extract at

300 mg/kg body weight orally over the course of 21 days; the diabetic rats in Group VI received inulin extract at 300 mg/kg body weight orally over the course of 30 days.

Induction of diabetes in Wistar rats

Streptozotocin (STZ) was used to induce diabetes in Wistar rats by causing pancreatic β-cell cytotoxicity.[19] The rats were fasted for 12 hours before the induction of diabetes. Streptozotocin was prepared in citrate buffer (0.1 M, pH 4.5) and injected intraperitoneally (i.p.) at a single dose of 45 mg/kg. Seventy-two hours later, blood glucose levels of STZ-treated fasted rats greater than 200 mg/dl were considered diabetic.

Histopathological and immunohistochemical examination of pancreatic β-cells

The quantitative count of pancreatic β-cells was determined through a histopathological analysis of pancreatic β-cells. The expression of insulin was examined using an immunohistochemical assay. The procedure was as previously described.[20] In each group, 2 rats were taken for histopathological and immunohistochemical analyses on the 1st, 7th, 14th, 21st, and 28th days. The rats were anesthetized with diethyl ether and then sacrificed by cervical dislocation. Histopathological and immunohistochemical examinations were conducted at the Stikes Mega Buana Palopo Pharmaceutical Laboratory.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS; version 21), and the values are expressed as means ± standard errors of the mean (SEM). Differences between groups were evaluated by analysis of variance (ANOVA) with the Bonferroni post hoc test. Statistical significance was set at $p < 0.05$.

Results and Discussion

Pancreatic β-cell *count*

The means and standard deviations of pancreatic β-cell count of Wistar rats in groups I-VI are presented in Table 1. Group VI had a higher mean number of pancreatic β-cells (89.40 ± 9.02) on the first day. On the seventh day, group I had the highest mean number of pancreatic β-cells (87.12 ± 5.57). On the 14th day, Group I (88.20 ± 5.19) had the highest mean number of pancreatic β-cells. The group with the highest mean number of pancreatic β-cells on the 21st day was group VI (90.62 ± 4.10). On day 28, group VI had the highest mean number of pancreatic β-cells (93.70 ± 3.98). There was no significant difference in the mean number of pancreatic β-cells among Wistar rat groups on day 1 because the rats did not receive any treatment. On days 7 and 14, there was no significant difference in the mean number of pancreatic β-cells between the groups of Wistar rats. On day 7, there was a little but not significant decrease in the number of pancreatic β-cells in the treatment groups (Groups IV, V, and VI). In contrast, the number of pancreatic β-cells increased, but the increase was not significant.

The results of the analysis on the 21st and 28th days showed that the number of pancreatic β-cells in STZ-induced rats (Group I) was significantly ($p < 0.05$) lower than in Group I, whereas there was no significant ($p < 0.05$) difference between Group I and Groups III, IV, V, and VI. The number of pancreatic β-cells in treated groups (Groups III, IV, V, and VI) was significantly ($p < 0.05$) higher than in the DM group (Group II). There was no significant difference ($p < 0.05$) between Group III and Groups IV, V, and VI, or between Group IV and Group V and VI, or between Group V and Group VI. This suggests that the administration of inulin extract on the 21st and 28th days affected the increase of pancreatic β-cell number induced by STZ. Also, the administration of inulin dose variation did not have a significant ($p < 0.05$) effect on the increasing number of pancreatic β-cells of Wistar rats.

Insulin expression of pancreatic β-cells *in STZ-induced diabetic rats*

The percentage of insulin expression of pancreatic β-cells in STZ-induced diabetic rats in Groups I, II, III, IV, V, and VI are presented in Table 2. On the first day, the group with a higher mean percentage of insulin expression of pancreatic β-cells was Group II with a value of 91.43±5.97. On the seventh day, Group I (91.41±5.90) had the highest mean proportion of pancreatic cells expressing insulin. On the 14th day, Group I (91.33±7.50) had the highest mean proportion of pancreatic cells expressing insulin. Group VI (94.06±4.38) had the highest mean proportion of pancreatic cells expressing insulin on day 21. The group with the highest mean percentage of pancreatic cells expressing insulin on day 28 was Group VI (94.44±3.12). There was no significant difference in the mean percentage of insulin expression of pancreatic β-cells among the groups of Wistar rats on day 1, indicating that the initial insulin expression of pancreatic β-cells of rats was almost the same because there was no treatment on the rats. The pancreatic β-cells in the treatment groups (Groups IV, V, and VI) have a little increased level of insulin expression, but not significantly. On days 14, 21, and 28 after treatment, the mean percentage of insulin expression of pancreatic β-cells of Group II was significantly lower than that of Group I. The mean percentage of insulin expression in pancreatic β-cells of Groups III, IV, V, and VI was significantly higher than in Group II. There were no statistically significant differences between Group I and Groups III, IV, V, and VI; between Group III and Groups IV, V, and VI; between Group IV and Groups V and VI; or between Group V and Group VI. Administration of inulin extract on day 14 influenced the increase of insulin expression in pancreatic β-cells in Wistar rats induced by STZ, and there was no significant difference in the effect of dosage variation (Figure 2).

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia caused by impaired insulin secretion, insulin resistance, or both.[21,22] Streptozocin, a toxic glucose analog, is used in laboratories to induce type 1 DM, which irreversibly damages pancreatic β-cells.[23] The loss

of pancreatic islets, a decrease in β-cell mass, and insulin secretion were all associated with the development of hyperglycemia in STZ-induced diabetic rats.[24] Because of its toxic effects, which lead to insulin resistance, hyperglycemia destroys pancreatic β-cells.[4,5] The degree of oxidative stress, which has been linked to diabetes-related tissue damage, is affected by hyperglycemia.[25] In the present study, a significant difference in the number of pancreatic β-cells between Group I (control group) and Group II (DM group) was observed on the 21st and 28th days. In Group II, the pancreatic β-cell number was significantly lower than in Group I. This observation is likely due to the destruction of pancreatic β-cells by STZ induction in Group II. Streptozocin has a cytotoxicity mechanism by inducing cellular oxidative stress and mitochondrial respiratory dysfunction that is mediated by ROS, reactive nitric oxide species (NO/RNS), and induction of inflammatory responses.[26-28] The cytotoxicity of STZ in pancreatic β-cells may take the form of necrotic cell deaths brought on by β-cell apoptosis, which leads to a reduction in the number of pancreatic cells.

The number of pancreatic β-cells in the treated groups (Groups III, IV, V, and VI) was significantly higher than in the DM group (Group II). This suggests that the administration of inulin extract from lesser yam affects the increase of pancreatic β-cell number induced by STZ. This result indicates that inulin could provide a protective effect on pancreatic β-cells. Inulin has an antioxidant ability that may scavenge ROS indirectly through the enhancement of the antioxidant enzymes and short-chain fatty acids (SCFAs).[29] This ability may prevent pancreatic cells from being destroyed. According to a 2018 study by Mei, supplementing laying hens with inulin can enhance their levels of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), while also lowering the serum concentration of MDA.[17] This result suggests that inulin could enhance antioxidant status. Another study by Hyeon *et al.*, in 2015, which investigated the therapeutic efficacies of another yam family member, such as crude yam (*Dioscorea batatas*) found that yam effectively

increases antioxidant, ameliorates the effect of chronic oxidative stress, and shows a significant decrease in malondialdehyde.[14]

Due to insulin resistance in peripheral tissues, type 2 diabetes is brought on by a high demand for insulin. Apoptosis causes a slow decrease of β-cell mass, and malfunction leads to a deficiency in insulin output. According to a 2012 study by Talchai *et al*., type 2 diabetes is caused by the mature insulin-producing cell de-differentiating to a naive state.[30] Regenerating the lost or damaged β-cell mass is thus a novel treatment for DM. The increased number of pancreatic β-cells in diabetic rats receiving treatment suggests that inulin extract may repair cell damage through cell regeneration. By conducting further research, inulin could be considered as a potential DM treatment.

The mean percentage of insulin expression in pancreatic cells from Group II was significantly lower than that of Group I on days 14, 21, and 28 of treatment. The lack of pancreatic cells in Group II suggests that these cells have been destroyed, which would impede insulin secretion. The pancreatic β-cells in Groups III, IV, V, and VI had a significantly higher mean percentage of insulin expression than Group II. The rats in Groups IV, V, and VI received varying doses of inulin. Glucagon-like peptide-1 (GLP-1), which is produced by the L cells of the gastrointestinal mucosa in response to nutritional stimulation, can be increased by inulin. By enhancing β-cell mass and function, GLP-1 encourages pancreatic β-cell proliferation and insulin release. This finding suggests that better pancreatic β-cell quantity and functionality are responsible for the higher expression of insulin in this study. Further research is required to identify the details of the histopathological features.

Conclusion

The findings of this study reveal that administration of inulin extract from lesser yam influenced the increase in β-cell number and insulin expression of pancreatic β-cells in STZ-

induced diabetic rats. The inulin dose variation did not have a significant effect on the number and insulin expression of pancreatic β-cells in Wistar rats.

Conflict of Interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors wish to thank the Directorate of Research and Community Service, Directorate General of Research and Development, Ministry of Research, Technology and Higher Education, Republic of Indonesia, for the Higher Education Excellence Research Scheme of 2019.

References

1. American Diabetes Association (ADA). Standards of Medical Care in Diabetes-2018. Diab Care. 2018; 41(1): S14-S28.
2. Ministry of Health. The report of Basic Health Research (RISKESDAS). Indon Center Health Res Dev. 2013; xx:xx-xx.
3. Meier JJ, Bonadonna RC. Role of reduced *β*-cell mass versus impaired *β*-cell function in the pathogenesis of type 2 diabetes. Diab Care. 2013; 2: S113– S119.
4. Robertson RP, Harmon J, Tran PO, Poitout V. *β*-cell glucose toxicity, lipotoxicity, and chronic oxidative street in type 2 diabetes. Diab. 2004; 53(1): 119-24.
5. Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diab. 2005; 54(2): S97–107.
6. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabs. 2003; 52: 102–110.
7. Shajeela PS, Mohan VR., Jesudas LL, and Soris PT. Nutritional and Antinutritional Evaluation of Wild Yam (*Dioscorea spp.)* TSA. 2011; 14: 723-30.
8. AY. Prabowo Karakteristik Fisiko Kimia, Bioaktif, dan Organoleptik Mie Berbasis Tepung Gembili (*Dioscorea esculenta L*.). Skripsi. Universitas Brawijaya, Malang, Indonesia. 2013; xxp.
9. GM Karunia. Pembuatan Inulin bubuk dari umbi Gembili (*Dioscorea esculanta*) dengan metode Foam Mat Drying. Skripi. Food Technology Studies Program. Industrial Technology Faculty UON "Veteran" Jawa Timur, Surabaya, Indonesia. 2013; xxp.
10. Ari Y, Retno SI, Wijayanti N. Pengembangan Pangan Fungsional Berbasis umbi-umbian sebagai sumber antioksidan dalam upaya meningkatkan derajat kesehatan masyarakat melalui pendekatan nutrigenomik. Research Report. Research institutions and community service. Universitas Negeri Semarang. 2015; xxp.
11. Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. Biosci. Biotechnol Biochem. 2007; 71: 3063–3071.
12. Kwon JE, Kwon JB, Kwun IS, Sohn HY. Antimicrobial and antioxidant activity of the *Dioscorea alata L*. J. Microbiol. Biotechnol. 2010; 38: 283–288.
13. Chang SJ, Lee YC, Liu SY, Chang TW. Chinese yam (*Dioscorea alata* cv. Tainung No. 2) feeding exhibited antioxidative effects in hyperhomocysteinemia rats. J Agric Food Chem. 2004; 52: 1720–1725.
14. Go HK, Md.Mahbubur R, Gi BK,Chong SN, Choon HS, Jin SK, Shang JK, Hyung SK. Antidiabetic effects of yam (*Dioscorea batatas*) and its active constituent, allantoin, in a rat model of streptozotocin-induced diabetes. Nutrients. 2015; 7(10): 8532-8544.
15. Tursinawati Y, Retno SI, Ari Y. Antidiabetic Activity of inulin from gembili (*Dioscorea esculenta*) through the inhibition of α glucosidase enzyme in vitro. Proceedings of the 11th International Conference on Preventive Medicine; 2018 May 2-3, Faculty of Medicine Universitas Brawijaya. 2018; xxp.
16. Mudannayake DC, Wimalasiri KMS, Silva KFST, Ajlouni S. Comparison of properties of new sources of partially purified inulin to those of commercially pure chicory inulin. J Food Sci. 2015; 80: C950–960.
17. Shang HM, Hai ZZ, Jun YY, Ran L, Hui SH XW. *In vitro* and in vivo antioxidant activities of inulin. PLoS ONE. 2018; 13(2): 1-12.

18. Winarti, S, Harmayani E, Marsono Y, Pranoto Y. Pengaruh Foaming Pada Pengeringan Inulin Umbi Gembili (*Dioscorea esculenta*) Terhadap Karakteristik Fisiko-Kimia dan Aktivitas Prebiotik. Agritech. 2013; 33: 4.
19. Manaer T, Yu L, Zhang Y, Xiao XJ, Nabi XH. Anti-diabetic effects of shubat in type 2 diabetic rats induced by combination of high-glucose-fat diet and low-dose streptozotocin. J Ethnopharmacol. 2015; 169: 269–274.
20. Karaca T, Kara A, Nejdet S, Uslu S, Tekįner D, Yörük M. Immunohistochemical distribution of glucagon, insulin, somatostatin, gastrin, and serotonin, containing cells in the pancreas of the Van cat. Turk J Vet Anim Sci. 2010; 38: 304-311.
21. De D, Chatterjee K, Ali KM, Bera TK, Ghosh D. antidiabetic potentiality of the aqueous methanolic extract of seed of *Swietenia mahagoni* (L.). Jacq. in streptozotocin-induced diabetic male albino rat: A correlative and evidence-based approach with antioxidative and antihyperlipidemic activities. Evid Based Comp Alternat Med. 2011; 2011: 892807.
22. Zhang T, Gao J Jin ZY, Xu XM, Chen HQ. Protective effects of polysaccharides *Lilium* l*ancifolium* on streptozotocin-induced diabetic mice. Int J Biol Macromol. 2014; 65: 436-40.
23. Gohari A, Ali NMA, Fahimeh ZG, Atefeh S. Urtica Dioica Distillate Regenerates Pancreatic Beta Cells in Streptozotocin-Induced Diabetic rats. Iran J Med Sci. 2018; 32(2): 174-183.
24. Pirmoradi L, Noorafshan A, Safaee A, Dehghani GA. Quantitative assessment of proliferative effects of oral vanadium on pancreatic islet volumes and beta cell numbers of diabetic rats. Iran Biomed J. 2016; 20: 18-25.
25. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. J Biomark. 2013; 2013: 378790.
26. Friederich M, Hansell P, and Palm F. Diabetes, oxidative stress, nitric oxide and mitochondria function. Curr Diab Rev. 2009; 5: 120–144.
27. Raza H and John A. Streptozotocin-induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma HepG2 cells. Int J Mol Sci. 2012; 13: 5751–5767.
28. Raza H, Prabu SK, John A, and Avadhani NG. Impaired mitochondrial respiratory functions and oxidative stress in streptozotocin-induced diabetic rats. Int J Mol Sci. 2011; 12: 3133–3147.
29. Gargari BP, Dehghan P, Aliasgharzadeh A, Jafar-abadi MA. Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with Type 2 diabetes. Diab Metab. 2013; 37: 140–148.
30. Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM. Involvement of endogenous glucagon-like peptide-1(7–36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrin. 2005; 185: 457–465.
31. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. Cell. 2012; 150(6): 1223–1234.
32. Egharevba, E., Chukwuemeke-Nwani, P., Eboh, U., & Okoye, E. Evaluation of the Antioxidant and Hypoglycaemic Potentials of the Leaf Extracts of Stachytarphyta jamaicensis (*Verbenaceae*). Trop J Nat Prod Res. 2023; 3(5): 170–174.

Table 1: The mean number of pancreatic β-cells of streptozotocin-induced diabetic Wistar rats after administration of inulin extracts.

Observation day	The mean number of pancreatic β-cells					
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	87.35±5.50	87.75±5.21	87.77±8.43	88.07±4.67	88.10±5.33	89.40±9.02
7	87.12±5.57	86.45±5.09	84.62±4.17	84.72±5.02	84.52±5.07	84.85±4.96
14	88.20±5.19	79.57±6.80	84.55±6.17	85.57±1.36	86.02±3.79	87.97±4.59
21	83.50±10.48^{a}	64.07±4.97^{b}	84.00±2.59^{a}	88.30±7.32^{a}	90.20±4.20^{a}	90.62±4.10^{a}
28	89.10±5.62^{a}	50.07±3.30^{b}	86.05±2.19^{a}	89.70±5.65^{a}	92.45±3.27^{a}	93.70±3.98^{a}

Group I: Control; Group II: Diabetic group; Group III: Galvus drug control group; Group IV: Diabetic group that received inulin extract at 100 mg/kg body weight over the course of 21 days; Group V: Diabetic group that received inulin at 300 mg/kg body weight over the course of 21 days; VI: Diabetic group that received inulin at 300 mg/kg body weight over the course of 30 days; Values with different superscripts ^{a,b} in a row differed significantly at $p < 0.05$.

Table 2: The mean percentage of insulin expression of pancreatic β-cells in streptozotocin-induced diabetic rats after administration of inulin extract examined with immunohistochemical stain.

Observation day	The mean percentage of insulin expression of pancreatic β-cells					
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	90.10±7.50	91.43±5.97	90.81±5.21	90.89±6.05	90.29±1.07	89.85±7.22
7	91.41±5.90	86.81±5.26	83.85±3.53	86.68±5.69	87.87±0.75	88.12±7.37
14	$91.33±7.50^{a}$	$68.22±4.10^{b}$	$86.78±4.44^{a}$	$88.64±5.54^{a}$	$90.44±0.93^{a}$	$90.76±6.26^{a}$
21	$92.87±5.49^{a}$	$59.85±1.89^{b}$	$91.53±4.21^{a}$	$91.99±2.72^{a}$	$92.05±1.55^{a}$	$94.06±4.38^{a}$
28	$92.64±8.45^{a}$	$47.28±7.00^{b}$	$93.93±3.43^{a}$	$93.21±1.61^{a}$	$93.18±4.80^{a}$	$94.44±3.12^{a}$

Group I: Control; Group II: Diabetic group; Group III: Galvus drug control group; Group IV: Diabetic group that received inulin extract at 100 mg/kg body weight over the course of 21 days; Group V: Diabetic group that received inulin at 300 mg/kg body weight over the course of 21 days; VI: Diabetic group that received inulin at 300 mg/kg body weight over the course of 30 days; Values with different superscripts ^{a,b} in a row differed significantly at $p < 0.05$.

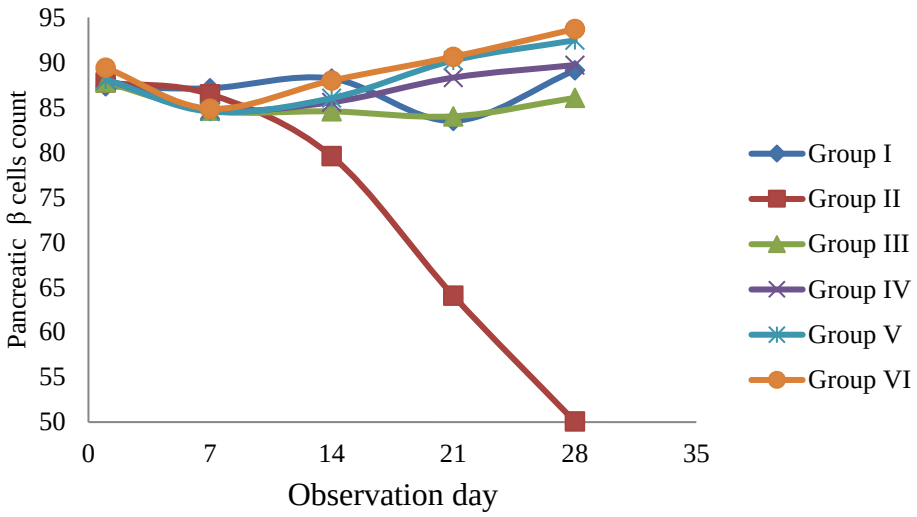

Figure 1: Pancreatic β-cell count in the experimental animal groups.

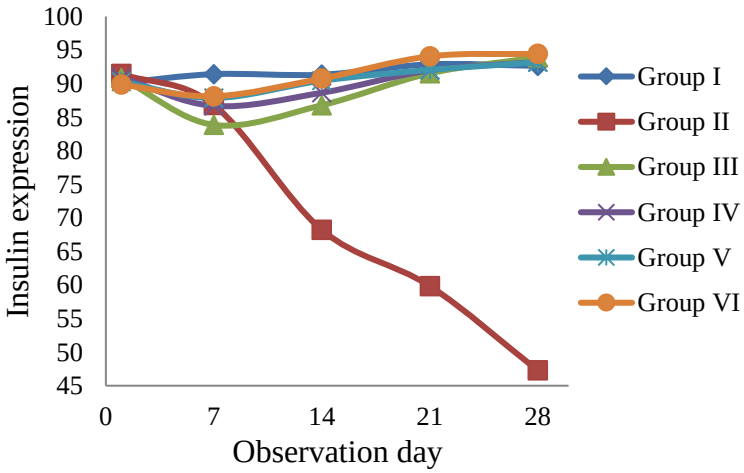

Figure 2: Insulin expression in pancreatic β-cells in the experimental animal groups.

Tropical Journal of Natural Product Research
Official Journal of the Natural Product Research Group
Faculty of Pharmacy, University of Benin 300001, Benin City, Nigeria
Phone: +2348073184488, Email: editor.tjnpr@gmail.com; editor.tjnpr@uniben.edu; Website: www.tjnpr.org

ISSN: 2616-0684 (Print); 2616-0692 (Online), DOI: 10.26538/tjnpr, ISI IF: 0.562 (2017)
Abstracted/Indexed: Index Copernicus ICV (2017): 59.83, Open-J-Gate, EMBASE, EBSCO Host, WorldCat, AJOL, CrossRef (USA), JIF, NCBI (PubMed), CAS

SCOPUS indexed, SCImago SjR 0.13

Ref. No. 7552499 DATE:9th July 2023

Biology Department, Faculty of Mathematic and Nature Science, Universitas Negeri Semarang, Indonesia

Dear Dr. Ari Yuniastuti,

Provisional Acceptance letter for Article Manuscript Number TJNPR MY230ARN

Title: Improvement of Insulin Secretion and Pancreatic β-cell Function in Streptozotocin-induced Diabetic Rats Treated with Dioscorea esculenta Extract

Authors: Nilawati Uly, Ari Yuniastuti, R Susanti, dan Yanuarita Tursinawati

I am pleased to inform you that your manuscript sent to the Tropical Journal of Natural Product Research has been reviewed and recommended for publication as a short communication article. However, before the issues raised by the Reviewers are forwarded, to enable you revise your manuscript accordingly, please pay a publication charge of **$ USD280**. The actual publication of the paper will be in the upcoming issue (**Vol 7 issue 7, 2023**).

Please, the manuscript number (**TJNPR MY230ARN**) should be included in the bank transfer.

TJNPR is Q3

Congratulations.

The money should be remitted in favour of:

Name of account:	**Abiodun FALODUN**
Bank Name:	Access Bank Plc
Account Number:	1456136521.
Sort Code:	044040699
Swift code:	ABNGNGLA
Address of Bank:	Ransome Kuti Road,University of Benin, Benin City, Edo State, Nigeria

Sincerely,

Professor A. Falodun, PhD, FAAS
Editor-in-Chief