Saudi Journal of Biological Sciences 29 (2022) 324–330

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Effect of kolaviron on islet dynamics in diabetic rats

Omolola R. Oyenihi [a], Marlon E. Cerf [b,c], Motlalepula G. Matsabisa [d], Nicole L. Brooks [e], Oluwafemi O. Oguntibeju [a,*]

[a] Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa
[b] Grants, Innovation and Product Development, South African Medical Research Council, Tygerberg, South Africa
[c] Biomedical Research and Innovation Platform, South African Medical Research Council, Tygerberg, South Africa
[d] Pharmacology Department, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa
[e] Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Cape Town, South Africa

ARTICLE INFO

Article history:
Received 31 May 2021
Revised 23 August 2021
Accepted 29 August 2021
Available online 6 September 2021

Keywords:
Diabetes
Garcinia kola
Kolaviron
Islets
β-cell
α-cell

ABSTRACT

Kolaviron, a biflavonoid isolated from the edible seeds of *Garcinia kola*, lowers blood glucose in experimental models of diabetes; however, the underlying mechanisms are not yet fully elucidated. The objective of the current study was to assess the effects of kolaviron on islet dynamics in streptozotocin-induced diabetic rats. Using double immunolabeling of glucagon and insulin, we identified insulin-producing β- and glucagon-producing α-cells in the islets of diabetic and control rats and determined the fractional β-cell area, α-cell area and islet number. STZ challenged rats presented with islet hypoplasia and reduced β-cell area concomitant with an increase in α-cell area. Kolaviron restored some islet architecture in diabetic rats through the increased β-cell area. Overall, kolaviron-treated diabetic rats presented a significant ($p < 0.05$) increase in the number of large and very large islets compared to diabetic control but no difference in islet number and α-cell area. The β-cell replenishment potential of kolaviron and its overall positive effects on glycemic control suggest that it may be a viable target for diabetes treatment.

1. Introduction

Diabetes, a disease characterized by hyperglycaemia, increases the risk of developing micro-and macro-vascular complications including atherosclerosis, cardiovascular disease, neuropathy, nephropathy, and retinopathy (Forbes and Cooper, 2013). Blood glucose homeostasis is regulated primarily by two antagonistic hormones: insulin and glucagon produced by beta (β) - and alpha (α)- cells in the pancreatic islets. Insulin is secreted in the fed-state to normalize blood glucose and, glucagon is secreted during a fasted state to raise blood glucose concentrations (Aronoff et al., 2004). Absolute or relative insulin deficiency and/or impaired insulin function contributes to the hyperglycaemic state in type 1 and 2 diabetes. The insulin deficiency in type 1 diabetes is caused primarily by autoimmune responses leading to the infiltration and destruction of pancreatic cells by mononuclear cells (Rothe et al., 1999). In type 2 diabetes, a progressive loss of β-cells is caused by increased gluco-, lipo- or glucolipotoxicity, endoplasmic reticulum-induced stress, oxidative stress, inflammation (systemic and islet), and β-cell death (e.g. apoptosis) (Cerf, 2013; Galicia-Garcia et al., 2020). These events converge and contribute and/or exacerbate insulin resistance, β-cell dysfunction, and failure (Oh, 2015, Lankatillake et al., 2019).

The two main mechanisms for β-cell replenishment (β-cell regeneration) are replication (proliferation) of existing β-cells (β-cell self-replenishment or β-cell replication) and differentiation of new β-cells from non-β islet cells, pancreatic and extra-pancreatic cells including stem/progenitor cells (i.e. β-cell neogenesis from non-β-cells) (Xia et al., 2009, Demeterco et al., 2009, Lysy et al., 2012). Self-replenishment (self-renewal or self-duplication) is the ability of a cell to repeatedly divide without loss of identity or functional potential (Chambers and Smith, 2004). In rodents,

* Corresponding author.
E-mail address: oguntibejuo@cput.ac.za (O.O. Oguntibeju).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.08.095
1319-562X/

new β-cells are derived mainly from existing β- cells; i.e., β-cell self-replenishment, which is the dominant mechanism for normal β-cell turnover under physiological conditions (Tavana and Zhu, 2011). β-cell populations comprise the individual β-cell (i.e., β-cell numbers) that constitute the β-cell mass in organisms and respond to variable insulin demand governed by physiological and pathological states (Cerf, 2013). β-cell populations are balanced, to a large extent, by β-cell replenishment and death (Cerf, 2013).

Type 1 diabetes is mainly treated with exogenous insulin injection to improve glycemic control (American Diabetes Association, 2011). Management strategies for type 2 diabetes involve administration of insulin alone or with oral or injectable hypoglycemic agents such as; biguanide, thiazolidinediones, SGLT2 inhibitors, GLP-1 agonists and DDP-4 inhibitors. These drugs are also under investigation as adjunctive to insulin therapies for T1D patients (Bacha and Klinepeter Bartz, 2016). Although these drugs have demonstrated benefits in diabetes management, a robust, sustained glycemic control over time has not been achieved and, some associated adverse effects remain unresolved (Borse et al., 2021). Also, non-adherence to the insulin treatment regimen (Doggrell and Chan, 2015), risk of hypoglycemia, low insulin availability and affordability (Li et al., 2019), failure of insulin to achieve glycemic targets (Cohen et al., 2016; Harris et al., 2017), and recent findings of insulin resistance following intensive insulin treatment (Okamoto et al., 2011; Karras et al., 2019) limits the benefits of intensive insulin therapy. Despite the progress in diabetes therapy, maintaining near-normal metabolic control remains a challenge, and the rate of morbidity and mortality from vascular complications is still high (Bertoni et al., 2002; Groop et al., 2018; Lee et al., 2019). Therefore, an imperative need for better glycemic control persists.

The crucial role of the pancreas in glucose homeostasis has prompted investigations targeting the pancreatic β-cell as a promising strategy for treating diabetes. Also, hyperglucagonemia contributes to hyperglycemia by increasing hepatic glucose output. Therefore, α-cells are also potential targets in diabetes (Gaisano et al., 2012, Marroqui et al., 2014). Several natural compounds, including those derived from plants, have received research attention as potential adjuvant or as alternative agents for diabetes management (Yonamine et al., 2016; Shirpoor, 2017; Borse et al., 2021). Some natural compounds and herbs exert regenerating and protective effects on β-cells, thus improving β-cell function and glycemic control. Among these are plant-derived flavonoids (e.g. resveratrol, quercetin, rutin, fisetin and epicatechin), single herbs (*Nigella sativa*, *Artemisia dracunculus* L and *Vernonia amygdalina*) and polyherbal formulations (e.g. Diabecon®, a well-marketed formulation containing herbs and naturally occurring minerals) (Modak et al., 2007; Oh, 2015; Choudhury et al., 2018, Ghorbani et al., 2019; Wickramasinghe et al., 2021).

Garcinia kola Heckel (family; Guttiferae) is a highly valued tree largely cultivated in West and Central Africa for its edible nuts. In traditional medicine, regular consumption of Garcinia kola nuts (commonly called bitter kola) is believed to lower blood glucose levels and is used in the treatment of inflammation and viral infections (Adaramoye and Adeyemi, 2006). Kolaviron is a bi-flavonoid complex (Fig. 1) extracted from *Garcinia kola* (Ayepola et al., 2014) and is reportedly known as the most active phytochemical present in these nuts (Iwu, 2014). Previous studies from our laboratory revealed a reduction in blood glucose concentrations and an increase in insulin concentrations in diabetic rats treated with kolaviron, suggesting an ameliorative effect on β-cells (Ayepola et al., 2013). However, in the present study, using immunohistochemical and morphometric analysis, we sought to determine whether kolaviron (i) affects new islet formation (islet number), (ii) alters β-cell area, and (iii) alters α-cell area.

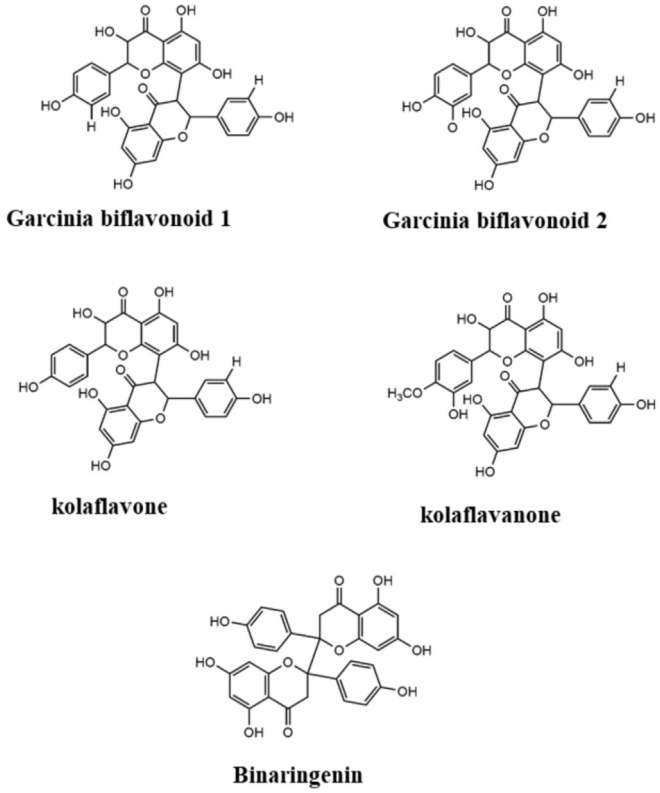

Fig. 1. Chemical structure of the bioactive compounds in Kolaviron – a Garcinia biflavonoid complex.

2. Material and methods

2.1. Plant materials

Fresh seeds of *Garcinia kola* were purchased from a local market in Ibadan, Oyo State, Nigeria and authenticated by Professor E. A Ayodele at the Department of Botany, University of Ibadan. A voucher specimen (FHI-109777) is available at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan.

2.2. Extraction of kolaviron from Garcinia kola seeds

Fresh seeds of *Garcinia kola* were peeled, sliced and air-dried. Kolaviron was isolated from these seeds according to the method of Iwu and colleagues (Iwu et al., 1990). Briefly, *Garcinia kola* seeds were grounded to powdered form and extracted with light petroleum ether (bp 40–60 °C) in a soxhlet for 24 hr. The defatted dried product was repacked and extracted with acetone. The concentrated extract was then diluted twice its volume with water and extracted with ethyl acetate. The resulting concentrate yielded kolaviron, a well-characterized bioflavonoid complex (Ayepola et al., 2013).

2.3. Animals

Forty healthy male Wistar rats (11–12 weeks) weighing 270 ± 25 g (g) were used for the study. The animals were bred at the animal facility of the South African Medical Research Council (SAMRC), with strict adherence to all standard operating procedures. The rats were housed in individual plastic cages at the animal facility of the SAMRC at room temperature (22 ± 2 °C) with 55 ± 5% humidity and an automatically controlled light–dark cycle (12 h/12 h). Standard rat diet (supplied by the SAMRC) and water

were provided ad-libitum, and rats were acclimatized to the experimental conditions one week before experimentation. The animal study was approved by the Research Ethics Committee of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology (Ethics number: CPUT/HW-REC 2012/AO4).

2.4. Experimental design and sample collection

At the start of the experiment (before STZ administration), 40 healthy Wistar rats free, from specific pathogens were randomly distributed into four experimental groups (n = 10 per group) using a stratified randomization procedure considering the body weights (~270 ± 25 g) and fasting (~18 h) blood glucose levels (~5.6 ± 1. 14 mmol/L). The groups were:

Group 1: Non-diabetic control (C)
Group 2: Kolaviron-treated non-diabetic control (C + KV),
Group 3: Untreated diabetic (D), and
Group 4: Kolaviron-treated diabetic (D + KV).

Afterwards, diabetes was induced in overnight fasted rats in groups 3 and 4 by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ; 50 mg kg^{-1} body weight) in citrate buffer (0.1 M, pH 4.5). Control rats in Groups 1 and 2 were injected with citrate buffer vehicle only. Random blood glucose was measured weekly throughout the study period with a glucometer (Accu-Chek, Roche, Germany) using blood obtained from the caudal vein. Kolaviron, at a dose of 100 mg/kg/day, was dissolved in a vehicle [dimethylsulphoxide (DMSO)] and administered orally (5 days/week; Monday - Friday) for six weeks. Control (C) rats also received vehicles five times a week for six weeks. At the end of the study (6 weeks after confirmation of diabetes in groups D and D + KV), the rats were euthanized with sodium pentobarbital (60 mg/kg). Pancreatic tissues were immediately excised and fixed in 10% (v;v) neutral buffered formalin and embedded in paraffin wax.

2.5. Immunodetection of insulin and glucagon in the pancreas

Fixed pancreatic tissues were cut into 5 μm sections for double immunolabeling of glucagon and insulin. Briefly, each section was dewaxed and immunolabeled for α-cells using a polyclonal glucagon antibody (Dako, Carpinteria, CA) and incubated for 30 min at room temperature. A secondary biotinylated anti-rabbit link antibody (Vector Laboratories, Burlingame, CA, USA) was applied at a 1:1000 dilution, and positive immunolabeling was visualized using the peroxidase diaminobenzidine and substrate chromagen system (Dako Corporation, Carpinteria, CA, USA). Thereafter, β-cells were immunolabeled with a monoclonal insulin antibody (1:10000; Sigma Immunochemicals St. Louis, MO, USA) using the alkaline phosphatase method. This was followed by a separate incubation with a rabbit/mouse link, AP Enzyme Enhancer, and substrate working solution (Envision G/2 System/AP, Rabbit/Mouse Kit). The light microscope was interfaced with a computer *via* Leica Qwin image analysis software (Leica, Wetzlar, Germany). Stained pancreatic sections were viewed with an X20 objective and images were analysed with an Olympus BX60 light microscope comprised of a mounted Nikon DS-Fi1 digital camera.

2.6. Measurement of α-cell, β-cell area and islet size distribution

The whole section area was measured, and the total islet areas were estimated by adding the tissue area measured in each field of view using the interactive measurement option of the Leica software. The total islet area and areas of α-cell and β-cells were determined with the aid of colour segmentation and thresholding on immunofluorescence-stained pancreatic sections. Afterwards, the ratio (%) of the immunoreactive α-cell area and β-cell area to the whole area of islet cells were calculated. The islets were counted and classified as small, medium, large and very large according to the different sizes and numbers of islets in each size group was expressed as a percentage of the total number of islets. All morphometry studies were conducted in a blinded fashion.

2.7. Statistical analysis

The data were expressed as mean values (±SD). Significant differences between glucose levels were determined by two-way repeated analysis of variance (ANOVA) (Treatment × Time as repeated measures) followed by post-hoc Dunnett test for multiple comparisons. Each islet measurement, i.e., total islet number, β-cell and α- cell area, were separately analysed with one-way ANOVA to compare mean differences between groups. Differences were considered significant at $p < 0.05$.

3. Results

Fig. 2 shows the baseline blood glucose levels in non-diabetic and diabetic rats and glucose levels after treatment with kolaviron. Injection of streptozotocin stimulated a diabetogenic response evident by a significant increase ($p < 0.05$; about 300 % higher than non-diabetic controls) in blood glucose level by day 5 of STZ injection. The elevation in glucose level was maintained through the study duration. By the 6th week of treatment, kolaviron (100 mg/kg) significantly ($p < 0.05$) lowered blood glucose level in diabetic rats by ~ 40 % compared to diabetic controls.

Immunohistostaining of the pancreas of normal control rats (C) revealed the peripheral location of α-cells (glucagon) while β-cells (insulin), which are numerous, were centrally located (Fig. 3). Also, the insulin-positive islet area comprised about 90% of the whole islet. However, untreated diabetic rats (D) showed distorted islet architecture (Fig. 3) and a depleted number of islets (Fig. 4a). Islet degeneration post STZ induction was evident by irregularly shaped islets and depleted immunopositive β-cells. Also, most of the β-cells in diabetic rats (Fig. 3; D and D + KV) were very lightly stained compared to non-diabetic rats. On the other hand, the glucagon-

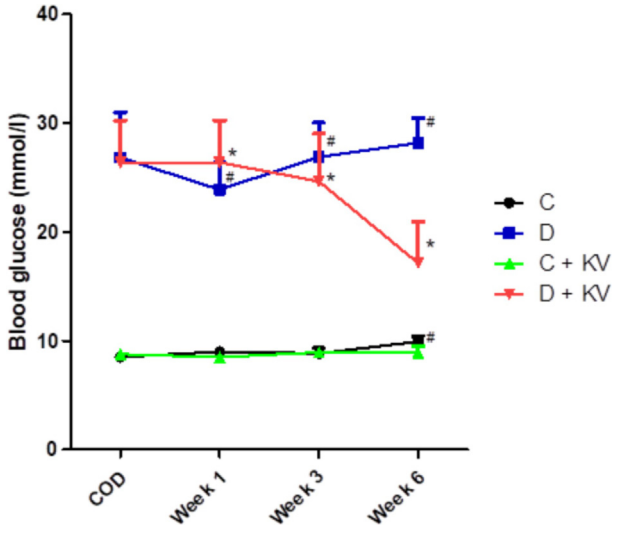

Fig. 2. The effect of kolaviron on glucose level in diabetic and non-diabetic rats over a 6-week treatment period. Data are presented as means ± S.D. Confirmation of diabetes (COD); Non-diabetic control (C), control treated with kolaviron (C + KV), untreated diabetic rats (D), diabetic rats treated with kolaviron (D + KV). * $p < 0.05$ compared to non-diabetic control rats, # $p < 0.05$ compared to diabetic control rats.

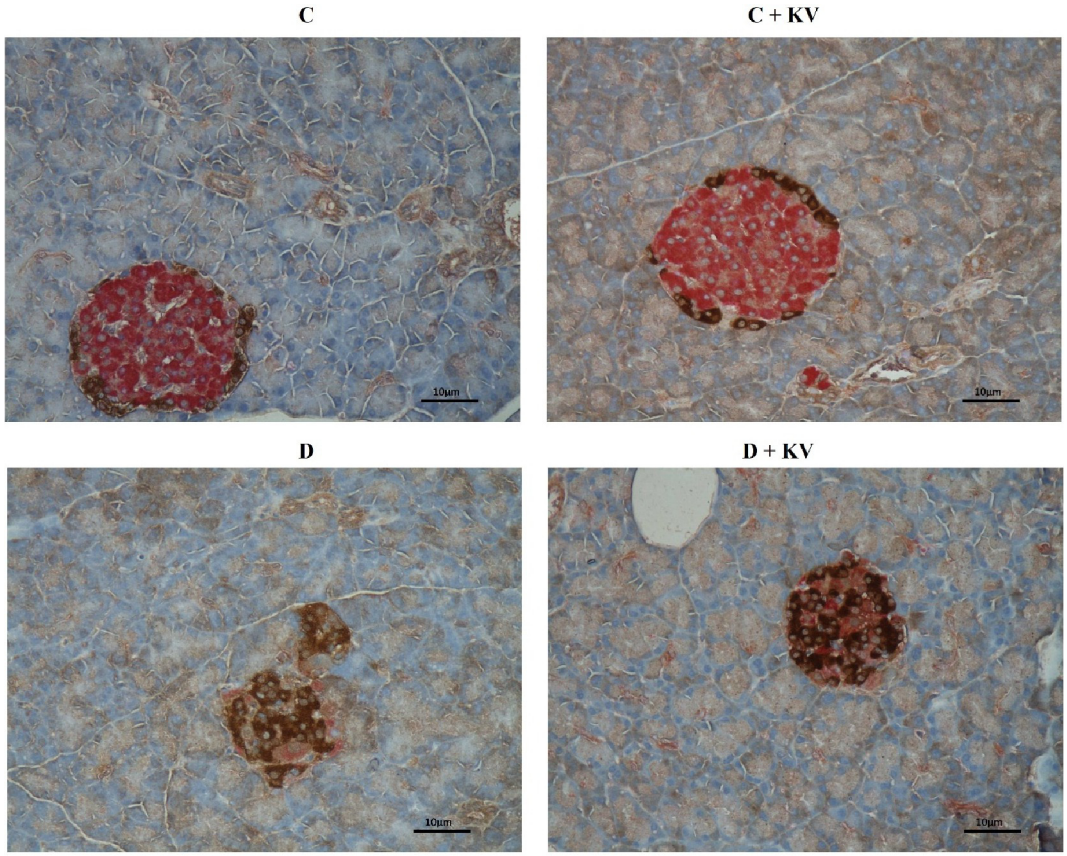

Fig. 3. Pancreata immunostained for glucagon-positive α-cells (brown staining) and insulin-positive β-cells (pink staining). Non-diabetic control (C), control treated with kolaviron (C + KV), untreated diabetic rats (D), diabetic rats treated with kolaviron (D + KV). Magnification X20.

producing α-cells were strongly expressed and centrally localized in the islets. A decrease in β-cell area and a reciprocally increased α-cell area were observed in diabetic rats (Fig. 4b and c). In addition, a lower number of large and very large-sized islets in diabetic rats *versus* control suggest beta cell deterioration (Fig. 5).

The staining intensity of immunoreactive β-cells also increased in kolaviron-treated diabetic rats (Fig. 3). As shown in Fig. 4b, injection of STZ caused a ~43% decrease in β-cell/islet area (35.9 1 ± 2.68) compared with non-diabetic control rats (61.44 ± 3.18). Kolaviron treatment of diabetic rats resulted in an increased β-cell area (45.83 ± 1.12) compared to control rats (35.91 ± 2.68). Although treatment of diabetic rats with kolaviron did not affect islet number and α-cell area, the pancreata of kolaviron-treated diabetic rats contained more large islets (in the range of 12,500–20,000 μm^2) and very large islets (>20,000 μm^2) and lower numbers of small and medium islets compared to diabetic control rats (Fig. 5).

4. Discussion

Kolaviron administration to diabetic rats lowered blood glucose and showed a potent effect on the islets, as demonstrated by immunohistochemical observations and morphometric results of the islet area. The glucose-lowering effect of kolaviron has been previously reported (Adaramoye and Adeyemi, 2006, Adaramoye, 2012, Ayepola et al., 2013, Tchimene et al., 2016). Furthermore, several mechanisms of the antidiabetic effect of kolaviron have been proposed, including glucose utilization in extrahepatic tissues, direct reduction of macrophage infiltration, the improvement of β-cell function (Ayepola et al., 2013), and increased functional activity of glucose transporters. Previous investigations including studies from our laboratory clarified some mechanistic aspects of the kolaviron beyond its glucose-lowering effect such as antiapoptotic action (Ayepola et al., 2014), the inhibitory effect of liver microsomal glucose-6-phosphatase (Adaramoye and Adeyemi, 2006), anti-inflammatory activity (Ayepola et al., 2013), and antioxidant effect. (Oyenihi et al., 2015).

The findings presented herein support the hypothesis on the stimulating action of kolaviron on β cells. Many studies have documented the direct benefits of phytotherapy on the pancreas through different mechanisms, which include: increased islet (i.e. islet hyperplasia) *via* regeneration of new islets, increased β-cell number and density, reduced lymphocyte infiltration in the islets and reduced oxidative stress indices (Hosseini et al., 2015; Wickramasingh et al., 2021). A strong correlation exists between the β-cell area and established indexes of β-cell function and glucose control (Meier et al., 2009, Meier et al., 2012). The present findings showed an increase in β-cell area and marked insulin staining in kolaviron-treated diabetic rats despite no change in islet number. The higher number of large islets in kolaviron-treated diabetic rats suggest that the extract could stimulate regeneration or exert protective effects on residual β-cells in STZ challenged rats, thereby improving β-cell function (Hafizur et al., 2015). In kolaviron-treated diabetic rats, there were no effects on α-cell area or islet number, which suggest that kolaviron is β-cell selective.

Most of the β-cells in diabetic rats were very lightly stained compared to non-diabetic rats - this may reflect low insulin content (Brereton et al., 2014). The increased α-cell area relative to the islet area suggests an increase in the secretory activity of α-cells to maintain islet size, a compensatory response to the loss

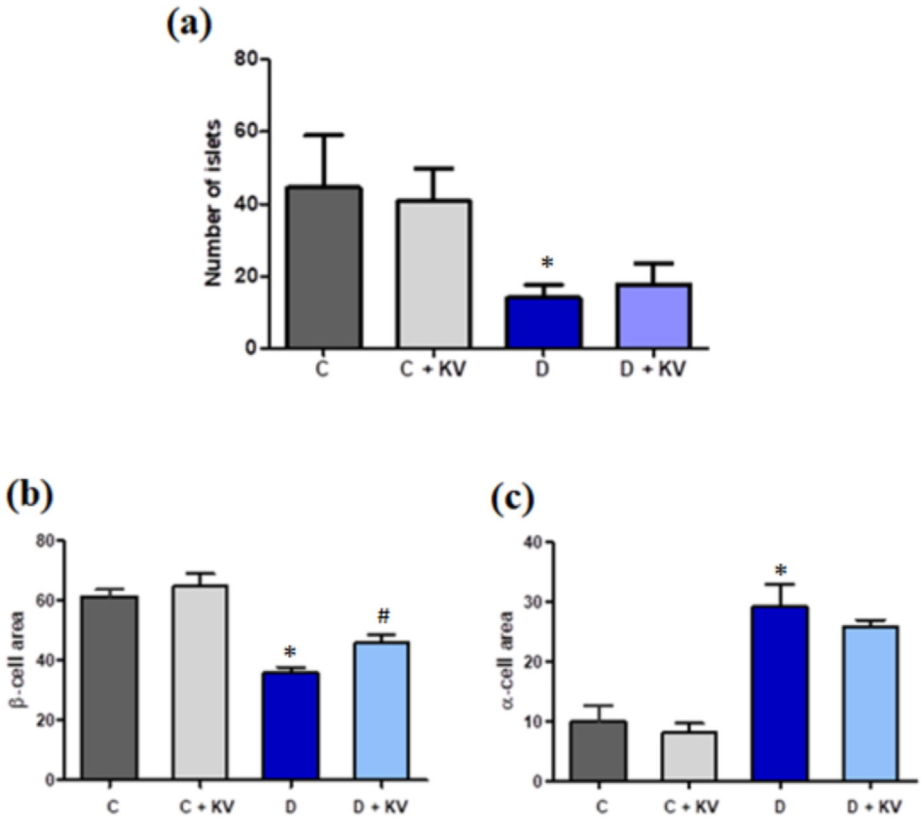

Fig. 4. Total islet number, β-cell and α- cell area. Data are presented as means ± S.D. * $p < 0.05$ compared to non-diabetic control rats, #$p < 0.05$ compared to diabetic control rats. Non-diabetic control (C), control treated with kolaviron (C + KV), untreated diabetic rats (D), diabetic rats treated with kolaviron (D + KV).

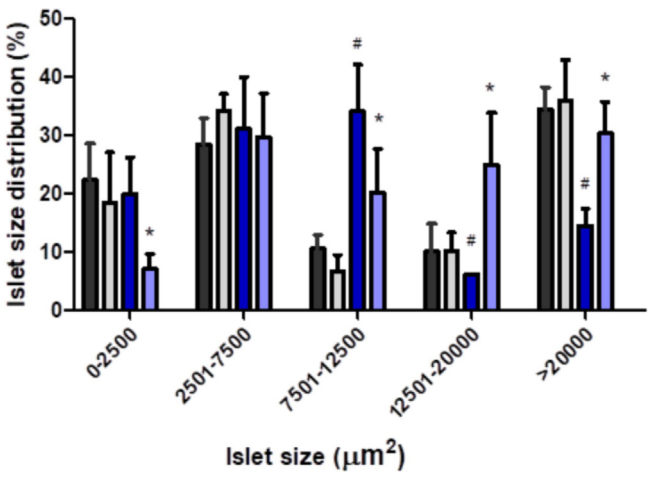

Fig. 5. Size distribution of islets in rats. Size distribution of small-medium islets (0–12,500 μm) and large-very large islets (12,501 – >20,000 μm) in normal and diabetic rats. Data are presented as means ± S.D. * $p < 0.05$ compared to non-diabetic control rats, # $p < 0.05$ compared to diabetic control rats. Non-diabetic control rats (Black), non-diabetic control rats treated with kolaviron (Grey), untreated diabetic rats (Deep blue), diabetic rats treated with kolaviron (Light blue).

of β-cells (Bru-Tari et al., 2019, Zhang et al., 2019). A reduction in β-cell mass would subject β-cells to an increased functional load, which may eventually exhaust insulin release (Leahy, 1990). The ability of kolaviron to improve β-cell degeneration may be due to its stimulating and rejuvenating effects on residual β-cells and other extra-pancreatic action such as modulation of hepatic glucose output and reduced glucotoxicity, modulation of altered cellular redox status, and its anti-inflammatory action (Adaramoye and Adeyemi, 2006, Ayepola et al., 2014, Oyenihi et al., 2015). DNA alkylation and hyperglycemia-mediated oxidative damage by free radicals (reactive oxygen or reactive nitrogen species) have been implicated in β-cell toxicity by STZ (Wu and Yan, 2015). Also, there are reports that hyperglycemia can cause β-cell degeneration by inducing apoptosis (Chang-Chen et al., 2008, Anuradha et al., 2014). The survival of β-cells in kolaviron-treated rats may be partly due to its antioxidant effect (Olayinka et al., 2014), its modulatory effect on the altered inflammatory state (Ayepola et al., 2013, Abarikwu, 2014), and a reduction of β-cell death.

5. Conclusion

The synthesis and release of insulin by the β-cells maintains glucose homeostasis and prevents metabolic diseases. Stressed and inflamed β-cells are functionally compromised and do not effectively respond to increased insulin demand which aggravates β -cell dysfunction resulting in β-cell failure and diabetes (Cerf, 2020). In the present study, we showed that treatment with kolaviron has an ameliorative effect by replenishing the β-cell area. The findings from the present study suggest that kolaviron elicits a direct action on β-cells and enhances β-cell survival. The β-cell replenishment potential of kolaviron and its overall positive effects on glycemic control indicates that it may be a viable target for diabetes treatment.

Funding

This study was supported by the University Research Fund (URF) of the Cape Peninsula University of Technology (CPUT) (grant recipient; (Prof. OO Oguntibeju; Dr Omolola Oyenihi) and the National Research Foundation (NRF) South Africa awarded to OO

Oguntibeju. Funding and support were also received from CPUT and the South African Medical Research Council (SAMRC) by Dr NL Brooks and Dr ME Cerf respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are grateful for the assistance provided by Mrs Joritha van Heerden, Charna Chapman and Candice Roux of the SAMRC.

References

Abarikwu, S.O., 2014. Kolaviron, a natural flavonoid from the seeds of *Garcinia kola*, reduces LPS-induced inflammation in macrophages by combined inhibition of IL-6 secretion, and inflammatory transcription factors, ERK1/2, NF-kappaB, p38, Akt, p-c-JUN and JNK. Biochim. Biophys. Acta 1840 (7), 2373–2381. https://doi.org/10.1016/j.bbagen.2014.03.006.

Adaramoye, O.A., 2012. Antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, in Wistar rats. Afr. Health Sci. 12 (4), 498–506. https://doi.org/10.4314/ahs.v12i4.16.

Adaramoye, O.A., Adeyemi, E.O., 2006. Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in streptozotocin-induced diabetes mellitus rats. J Pharm. Pharmaco.l 58 (1), 121–128. https://doi.org/10.1211/jpp.58.1.0015.

American Diabetes Association, 2011. Standards of medical care in diabetes. Diabetes Care 34 (1), S11–S61. https://doi.org/10.2337/dc11-S011.

Anuradha, R., Saraswati, M., Kumar, K.G., Rani, S.H., 2014. Apoptosis of beta cells in diabetes mellitus. DNA Cell Biol. 33 (11), 743–748. https://doi.org/10.1089/dna.2014.2352.

Aronoff, S.L., Berkowitz, K., Shreiner, B., Want, L., 2004. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectr. 17 (3), 183–190. https://doi.org/10.2337/diaspect.17.3.183.

Ayepola, O.R., Cerf, M.E., Brooks, N.L., Oguntibeju, O.O., 2014. Kolaviron, a biflavonoid complex of *Garcinia kola* seeds modulates apoptosis by suppressing oxidative stress and inflammation in diabetes-induced nephrotoxic rats. Phytomedicine 21 (14), 1785–1793.

Ayepola, O.R., Chegou, N.N., Brooks, N.L., Oguntibeju, O.O., 2013. Kolaviron, a Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. BMC Complement. Altern. Med. 13, 363. https://doi.org/10.2337/diaspect.17.3.183.

Bacha, F., Klinepeter Bartz, S., 2016. Insulin resistance, role of metformin and other non-insulin therapies in pediatric type 1 diabetes. Pediatr. Diabetes 17 (8), 545–558. https://doi.org/10.1111/pedi.12337.

Bertoni, A.G., Krop, J.S., Anderson, G.F., Brancati, F.L., 2002. Diabetes-related morbidity and mortality in a national sample of US elders. Diabetes care 25 (3), 471–475. https://doi.org/10.2337/diacare.25.3.471.

Borse, S.P., Chhipa, A.S., Sharma, V., Singh, D.P., Nivsarkar, M., 2021. Management of type 2 diabetes: current strategies, unfocussed aspects, challenges, and alternatives. Med. Princ. Pract. 30 (2), 109–121. https://doi.org/10.1159/000511002.

Brereton, M.F., Iberl, M., Shimomura, K., Zhang, Q., Adriaenssens, A.E., Proks, P., Ashcroft, F.M., 2014. Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. Nat. Commun. 5 (1), 1–11. https://doi.org/10.1038/ncomms5639.

Bru-Tari, E., Cobo-Vuilleumier, N., Alonso-Magdalena, P., Dos Santos, R.S., Marroqui, L., Nadal, A., Quesada, I., 2019. Pancreatic alpha-cell mass in the early-onset and advanced stage of a mouse model of experimental autoimmune diabetes. Sci. Rep. 9 (1), 9515. https://doi.org/10.1038/s41598-019-45853-1.

Cerf, M.E., 2020. Beta cell physiological dynamics and dysfunctional transitions in response to islet inflammation in obesity and diabetes. Metabolites 10 (11), 452. https://doi.org/10.3390/metabo10110452.

Cerf, M.E., 2013. Beta cell dysfunction and insulin resistance. Front. Endocrinol. 4 (37), 37. https://doi.org/10.3389/fendo.2013.00037.

Chang-Chen, K., Mullur, R., Bernal-Mizrachi, E., 2008. β-cell failure as a complication of diabetes. Rev. Endocr. Metab. Disord. 9 (4), 329–343. https://doi.org/10.1007/s11154-008-9101-5.

Chambers, I., Smith, A., 2004. Self-renewal of teratocarcinoma and embryonic stem cells. Oncogene. 23 (43), 7150–7160.

Choudhury, H., Pandey, M., Hua, C.K., et al., Jul 2018. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. J Tradit Complement Med. 8 (3), 361–376.

Cohen, O., Filetti, S., Castañeda, J., Maranghi, M., Glandt, M., 2016. When intensive insulin therapy (MDI) fails in patients with Type 2 diabetes: switching to GLP-1 receptor agonist versus insulin pump. Diabetes Care 39 (2), S180–S186. https://doi.org/10.2337/dcS15-3029.

Doggrell, S.A., Chan, V., 2015. Adherence to insulin treatment in diabetes: can it be improved? J. Diabetes 7, 315–321. https://doi.org/10.1111/1753-0407.12212.

Demeterco, C., Hao, E., Lee, S.H., Itkin-Ansari, P., Levine, F., Nov 2009. Adult human beta-cell neogenesis? Diabetes Obes Metab. 11 (Suppl 4), 46–53.

Forbes, J.M., Cooper, M.E., 2013. Mechanisms of diabetic complications. Physiol. Rev. 93 (1), 137–188. https://doi.org/10.1152/physrev.00045.2011.

Gaisano, H.Y., Macdonald, P.E., Vranic, M., 2012. Glucagon secretion and signaling in the development of diabetes. Front Physiol. 3, 349.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Martín, C., 2020. Pathophysiology of type 2 diabetes mellitus. Int. J. Mol. Sci. 21 (17), 6275. https://doi.org/10.3390/ijms21176275.

Ghorbani, A., Rashidi, R., Shafiee-Nick, R., 2019. Flavonoids for preserving pancreatic beta cell survival and function: A mechanistic review. Biomed Pharmacother. 111, 947–957.

Groop, P.H., Thomas, M., Feodoroff, M., Forsblom, C., Harjutsalo, V., 2018. Excess mortality in patients with type 1 diabetes without albuminuria—separating the contribution of early and late risks. Diabetes Care 41 (4), 748–754. https://doi.org/10.2337/dc17-1618.

Hafizur, R., Fatima, N., Shaukat, S., 2015. Immunohistochemical evidence of pancreatic [Beta]-cell regeneration in streptozotocin-induced type 2 diabetic rats treated with Gymnema sylvestre extract. J. ytol. histol. 6 (4), 1. https://doi.org/10.4172/2157-7099.1000342.

Harris, S.B., Mequanint, S., Miller, K., Reichert, S.M., Spaic, T., 2017. When insulin therapy fails: the impact of SGLT2 inhibitors in patients with type 2 diabetes e141–e142 Diabetes Care 40 (10). https://doi.org/10.2337/dc17-0744.

Hosseini, A., Shafiee-Nick, R., Ghorbani, A., 2015. Pancreatic beta cell protection/regeneration with phytotherapy. Braz. J. Pharm. Sci. 51 (1), 1–16. https://doi.org/10.1590/S1984-82502015000100001.

Iwu, M.M., Igboko, O.A., Okunji, C.O., Tempesta, M.S., 1990. Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*. J. Pharm. Pharmacol. 42 (4), 290–292. https://doi.org/10.1111/j.2042-7158.1990.tb05412.x.

Karras, S.N., Koufakis, T., Zebekakis, P., Kotsa, K., 2019. Pharmacologic adjunctive to insulin therapies in type 1 diabetes: the journey has just begun. World J. Diabetes 10 (4), 234. https://doi.org/10.4239/wjd.v10.i4.234.

Lankatillake, C., Huynh, T., Dias, D.A., 2019. Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. Plant Methods 15, 105. https://doi.org/10.1186/s13007-019-0487-8.

Leahy, J.L., 1990. Natural history of beta-cell dysfunction in NIDDM. Diabetes Care 13 (9), 992–1010. https://doi.org/10.2337/diacare.13.9.992.

Lee, Y.B., Han, K., Kim, B., Lee, S.E., Jun, J.E., Ahn, J., Kim, J.H., 2019. Risk of early mortality and cardiovascular disease in type 1 diabetes: a comparison with type 2 diabetes, a nationwide study. Cardiovasc. Diabetol. 18 (1), 1–17. https://doi.org/10.1186/s12933-019-0953-7.

Li, Z., Feng, Q., Kabba, J.A., Yang, C., Chang, J., Jiang, M., Fang, Y., 2019. Prices Availability and affordability of insulin products: a cross-sectional survey in Shaanxi Province, western China. Trop. Med. Int. Health 24 (1), 43–52. https://doi.org/10.1111/tmi.13167.

Lysy, P.A., Weir, G.C., Bonner-Weir, S., 2012. Concise review: pancreas regeneration: recent advances and perspectives. Stem cells translational medicine. 1 (2), 150–159.

Marroqui, L., Alonso-Magdalena, P., Merino, B., Fuentes, E., Nadal, A., Quesada, I., Jun 2014. Nutrient regulation of glucagon secretion: involvement in metabolism and diabetes. Nutr Res Rev. 27 (1), 48–62.

Meier, J., Breuer, T., Bonadonna, R., Tannapfel, A., Uhl, W., Schmidt, W., Menge, B., 2012. Pancreatic diabetes manifests when beta cell area declines by approximately 65% in humans. Diabetologia 55 (5), 1346–1354. https://doi.org/10.1007/s00125-012-2466-8.

Meier, J.J., Menge, B.A., Breuer, T.G., Muller, C.A., Tannapfel, A., Uhl, W., Schrader, H., 2009. Functional assessment of pancreatic beta-cell area in humans. Diabetes 58 (7), 1595–1603. https://doi.org/10.2337/db08-1611.

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Paul, T., Devasagayam, A., 2007. Serial review indian herbs and herbal drugs used for the treatment of diabetes. J. Clin. Biochem. Nutr. 40, 163–173. https://doi.org/10.3164/jcbn.40.163.

Oh, Yoon Sin, 2015. Plant-derived compounds targeting pancreatic beta cells for the treatment of diabetes. Evid. Based Complement. Alternat. Med. 2015, 1–12. https://doi.org/10.1155/2015/629863.

Okamoto, M.M., Anhê, G.F., Sabino-Silva, R., Ferreira Marques, M., Freitas, H.S., Mori, R.C.T., Machado, U.F., 2011. Intensive insulin treatment induces insulin resistance in diabetic rats by impairing glucose metabolism-related mechanisms in muscle and liver. J. Endocrinol. 211 (1), 55. https://doi.org/10.1530/JOE-11-0105.

Olayinka, E., Ore, A., Fashiku, K.A., 2014. Kolaviron and L-ascorbic acid ameliorates chlorambucil—induced hepatic and renal toxicity in rat. Int. J. Toxicol. Appl. Pharmacol. 4 (1), 23–32. https://doi.org/10.1155/2014/587015.

Oyenihi, O.R., Brooks, N.L., Oguntibeju, O., 2015. Effects of kolaviron on hepatic oxidative stress in streptozotocin induced diabetes. BMC Complement. Altern. Med. 15, 236. https://doi.org/10.1186/s12906-015-0760-y.

Rothe, H., Hausmann, A., Casteels, K., Okamura, H., Kurimoto, M., Burkart, V., Kolb, H., 1999. IL-18 inhibits diabetes development in nonobese diabetic mice by counterregulation of Th1-dependent destructive insulitis. J. Immunol. 163 (3), 1230–1236.

Shirpoor, A., 2017. Medicinal plants for management of diabetes: alternative or adjuvant? Anatol. J. Cardiol. 17 (6), 460. 10.14744/AnatolJCardiol.2017.24122.

Tavana, O., Zhu, C., 2011. Too many breaks (brakes): pancreatic β-cell senescence leads to diabetes. Cell Cycle. 10 (15), 2471–2484.

Tchimene, M., Anaga, A., Ugwoke, C., Onoja, O., Ezugwu, C., Okunji, C., Iwu, M., 2016. Anti-diabetic profile of extract, kolaviron, biflavonoids and garcinoic acid from Garcinia kola seeds. Int. J. Curr. Microbiol. Appl. Sci. 5 (2), 317–322. https://doi.org/10.20546/ijcmas.2016.501.036.

Wickramasinghe, A.S.D., Kalansuriya, P., Attanayake, A.P., 2021. Herbal Medicines Targeting the Improved β-Cell Functions and β-Cell Regeneration for the Management of Diabetes Mellitus. Evid. Based. Complement. Alternat. Med. https://doi.org/10.1155/2021/2920530.

Wu, J., Yan, L.-J., 2015. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes Metab. Syndr. Obes. 8, 181. https://doi.org/10.2147/DMSO.S82272.

Xia, B., Zhan, X.-R., Yi, R., Yang, B., 2009. Can pancreatic duct-derived progenitors be a source of islet regeneration? Biochemical and biophysical research communications. 383 (4), 383–385.

Yonamine, C.Y., Pinheiro-Machado, E., Michalani, M.L., Freitas, H.S., Okamoto, M.M., Corrêa-Giannella, M.L., Machado, U.F., 2016. Resveratrol improves glycemic control in insulin-treated diabetic rats: participation of the hepatic territory. Nutr. Metab. 13 (1), 1–10. https://doi.org/10.1186/s12986-016-0103-0.

Zhang, Z., Hu, Y., Xu, N., Zhou, W., Yang, L., Chen, R., Chen, H., 2019. A new way for beta cell neogenesis: transdifferentiation from alpha cells induced by glucagon-like peptide 1. J Diabetes Res., 2583047 https://doi.org/10.1155/2019/2583047.