Attenuation of Alloxan-induced Low-grade Inflammation by Persea Americana Polyphenolic Peel Extract in Male Wistar Rats

OLAREWAJU MICHAEL oluba (oluba.olarewaju@lmu.edu.ng)
Landmark University

Daniel O. Abasiri
Landmark University

Babatunde Ibitoye
Ekiti State University

Samuel Ojeaburu
University of Benin

George Eidangbe
Ambrose Alli University

Ayokunmi Akinduko
Federal University of Technology

Olayinka Alabi
Landmark University

Research Article

Keywords: Avocado peel extract, polyphenolic extract, cytokines, inflammation, diabetic complications

Posted Date: September 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-910440/v1

Abstract

Background: Based on the conceived causal relationship that exist between oxidative stress, hyperglycemia and low-grade inflammation, this study was hypothesized to evaluate how treatment with polyphenolic peel extract of *avocado pear* (APPE), with established antioxidant activity, may be deployed in the management of complications arising from diabetes.

Methods: Twenty adult male alloxan-induced diabetic Wistar rats randomly assigned to five groups (n = 5) designated: diabetic control (DC), treated with distilled water; and 25, 50, 100 and 200 mg/kg APPE, respectively. A sixth group comprising of five normal rats administered distilled water served as normal control (NC).

Results: Following a 21-day single daily oral administration of APPE, remarkable improvements in body weight and blood glucose concentration were noted in diabetic rats especially at 200 mg/kg compared to DC. A dose-related significant reductions in glycated hemoglobin, interleukins (1β and 6), tumour necrosis factor-α (TNF-α) levels in diabetic rats treated with APPE compared to DC. Conversely, serum insulin and interleukin-10 concentrations were significantly elevated in a dose-related fashion in APPE administered diabetic rats compared to DC. Unlike the intestine and heart which were spared severe morphological treatment due to alloxan intoxication, diabetic control rat and those on low dose of the extract showed varying degree of interface hepatitis and glomerulosclerosis. However, diabetic rats on 200 mg/kg APPE were protected from the damaging effects of diabetes mellitus on the liver and kidney.

Conclusion: APPE abrogated the oxidative stress-induced hepatic and nephrotic damage in diabetic rats.

1. Introduction

Modern lifestyle and excessive intake of carbohydrates have contributed significantly to the incidence rate of diabetes and obesity. The high level of circulating lipids in obese most often result in adipose tissue lipotoxicity thus interfering with glucose metabolism and consequently potentiate systemic insulin resistance. Lipodystrophy-induced insulin resistance has been demonstrated in both animals and humans [1]. Deranged lipid metabolism in adipocytes has been shown to be associated with adipokines production [2]. In addition, mitochondrial-derived reactive oxygen species (ROS) instigated as a consequence of protracted hyperglycemia has also been shown to aggravate β-cell dysfunction and ultimately, glucose intolerance [3].

Sustained hyperglycemia has been implicated as a major prognosis in the pathogenesis of diabetic complications. Through a series of reactions aided by transition metals, the oxidation of glucose gives rise to free radicals such as hydrogen peroxide and keto-aldehydes. The generation of keto-aldehydes is complemented with the release of superoxide (O_2^{-}.) which is spontaneously transmutated to H_2O_2, and then the highly potent hydroxyl radical [4]. The overwhelming generation of these radicals over the threshold of biological antioxidants culminate in the development of oxidative stress in diabetes.

Therefore, oxidative stress is a risk factor in the potentiation and development of macrovascular complications in diabetes mellitus [5]. The auto oxidation of glucose is a major process involved in non-enzymatic glycosylation of hemoglobin. Glycated hemoglobin is potent source of free radicals. Hence, diabetic complications are coordinately linked to both oxidative stress and protein glycosylation. However, the two processes could be abated following treatment with antioxidants.

Alloxan has been reportedly used to model compound in the elucidation and understanding of reactive oxygen species-mediated beta cell toxicity in type 1 and type 2 diabetes mellitus. The suppression of the beta cells of the pancreas to glucose is usually preceded by a sudden surge in insulin secretion following alloxan treatment [6].

Avocado (*Persea americana* Mill. Lauraceae) pear also being referred to as alligator pea or butter fruit, is a tropical fruit with edible flesh. The process involved in the processing of avocado pear generate as much as 35–50% waste with the peel constituting an estimated 20–25% of this waste [7]. However, avocado peel has been reported to be a rich source of phenolic compounds, thus, it is a sustainable biomass with promising applications in many industries including pharmaceuticals, cosmetics and beauty, food preservation [8]. Avocado peel waste has been established to be much higher phenolic content compared to avocado pulp [9]. Phenolic extract of avocado pear has been shown to display a broad spectrum of pharmacological activities including anti-diabetic [10], anti-inflammatory, and antioxidant [11] effects. Given its numerous health benefits, avocado peel waste is considered a promising, low cost biomass for salvaging phenolic compounds from agro-wastes. Hence, the exploitation of avocado peel waste will go a long way in adding value to avocado processing industry as well served as a veritable strategy for reducing the environmental impacts of avocado wastes [12]. The potential inhibition of α-amylase by certain food and plant extracts was reported recently. It was also documented that the presence of polyphenols in these foods and plant extracts accounts for this inhibition [13]. Given the possibility of a causal relationship between hyperglycemia-induced oxidative stress and hemoglobin glycosylation and the induction of inflammatory cytokines in diabetes. We hypothesized that the potential inhibition of carbohydrate hydrolyzing enzymes and/or the possible suppression of glucose oxidation via antioxidant mechanism could offer a promising strategy in the prevention and management of diabetic complications.

2. Materials And Methods

2.1. Chemicals and reagents

Chemicals and reagents used in this study were AnalaR grades and were products of Merck Life Science UK Ltd. (Gillingham, United Kingdom) except as stated otherwise.

2.2. Plant material

Matured *Persea americana* fruits were collected from a vegetation area around Omu-Aran, Kwara State, Nigeria within the months of July. The plant sample was identified and authenticated at the department of Plant Science, University of Ilorin, Ilorin, Nigeria where specimen was deposited.

2.3. Animals

Male Wistar rats weighing 180–200 g procured from the animal holding unit of the Department of Biochemistry, Landmark University, Omu-Aran, Nigeria were used for the study. The animals were housed in standard rat cages of five animals per cage under a regulated environment with a 12 h light/dark cycle and fed *ad libitum* with unrestricted access to water. Prior authorization for animal handling was sought from Landmark University, Omu-Aran, Nigeria Research and Ethics Committee (approval number LMU/EC/098/2019).

2.4. Ethics declaration

The care and handling of animals complied strictly with National Institutes of Health Manual on care and use of laboratory animals [14] and approved by the Animal Use Ethics Committee of the Landmark University, Omu-Aran, Nigeria, under the approval number LMU/EC/098/2019. Furthermore, this study was conducted according to ARRIVE guidelines[15].

2.5. Microwave assisted polyphenol extraction

Total polyphenol extract was carried out using the modified microwave assisted method of Simić *et al*. [16] with little modifications. Powdered avocado pear peel (50 g) was put in 500 mL Erlenmayer flask containing 250 mL ethanol (50%). The Erlenmayer flask with its content was then put inside a BP090 microwave oven (**Microwave Research & Applications, Inc.** Illinois, USA). The flask was securely connected to a vertical condenser. The extraction process was performed at 300 W for 5 min. Thereafter, the liquid extract was separated from the residue through vacuum filtration. The liquid extract was vacuum evaporated at 40 ^{o}C to obtain the concentrated polyphenol extract.

2.6. Determination of total polyphenol

The content of total polyphenol in avocado peel polyphenolic extract was estimated using the colorimetric Folin-Ciocalteu method as described by Ozcan *et al*. [17].

2.7. HPLC analysis

The preparation of the avocado peel polyphenolic extract was carried out according to Šeruga *et al*. [18] method.

2.8. Induction of diabetes

The rats were made diabetic by a single intraperitoneal administration of 150 mg/kg alloxan monohydrate in normal saline [19]. Forty-eight post-alloxan induction, diabetes was confirmed in the animals and rats having fasting blood glucose level above 250 mg/dL were included in the study.

2.9. Experimental design

The experimental design consisted of 25 alloxan-diabetic rats and a separate set of 5 non-diabetic rats. The grouping of animals is as shown below:

Normal control (NC): Normal rats (*n* = 5) treated with distilled water,

Diabetic control (DC): Alloxan-diabetic rats (*n* = 5) treated with distilled water,

$APPE_{25}$: Alloxan-diabetic rats (*n* = 5) treated with APPE (25 mg/kg *bw*),

$APPE_{50}$: Alloxan-diabetic rats (*n* = 5) treated with APPE (50 mg/kg *bw*),

$APPE_{100}$: Alloxan-diabetic rats (*n* = 5) treated with APPE (100 mg/kg *bw*),

$APPE_{200}$: Alloxan-diabetic rats (*n* = 5) treated with APPE (200 mg/kg *bw*).

Treatment was given orally once daily for 21 consecutive days after which they were sacrificed by cardiac puncture. Records of body weight and fasting blood glucose level were taken every three days. After the 21st-day treatment, rats in each group were sacrificed under anesthesia, blood was collected into clean sterile tubes while intestine, liver, heart and kidney samples were quickly excised, blotted with tissue paper, freed of fats and stored in formalin.

2.10. Blood sugar determination

Blood was taken from cut tip of the respective rat tail and fasting blood sugar level was estimated using One Touch Ultramini glucometer.

2.11. Determination of glycated hemoglobin

Hemoglobin glycosylation level was evaluated following Nayak and Pattabiraman [20] method.

2.12. Biochemical analysis

Whole blood was allowed to stand for 30 min before being centrifuged at 3000 rpm for ten minutes and serum carefully pipetted into dry clean, sterile bottles and stored at -4 ^{o}C for further biochemical analyses. Serum interleukins 1β, 6 and 10 as well as tumor necrosis factor alpha (TNF-α) were estimated using their respective diagnostic kit (R&D system, Minneapolis, USA) with strict compliance to manufacturer's instructions.

2.13. Histological evaluation

A small portion of liver sample was cut and fixed in 10% neutral buffered formalin. Thin sections of 5μm were taken using a rotary microtone, stained with haematoxylin and eosin for light microscopy. The slides

were viewed at an appropriate objective to check for the architecture of the tissues. The pictures were displayed in photomicrographs and the histology was analyzed.

2.14. Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis and graphical representation were carried out using GraphPad Prism software (Version 8.0). Mean comparison was by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test. *P* values less than 0.05 were considered significant.

3. Results

3.1. Phytochemical composition

The HPLC fingerprint of APPE revealed the presence of quercetin (41.58 ppm), kampferol (1.23 ppm) and a negligible amount of p-coumaric acid (8.80×10^{-2} ppm) (Fig. 1).

3.2. Effect on body weight

At the end of the treatment period, a significant decrease in body weight (-32.3%) was observed in alloxan-induced diabetic rats (DC) in comparison with that of normal control group. Interestingly, treatment of diabetic animals with avocado peel polyphenolic extract at 50, 100 and 200 mg/kg body weight doses (($APPE_{50}$, $APPE_{100}$, and $APPE_{200}$) led to significant ($p < 0.05$) improvement in body weight compared to diabetic control (DC). $APPE_{200}$ gave the most significant ($p < 0.05$) improvement in body weight followed by $APPE_{50}$ and $APPE_{100}$ which gave a similar effect in body weight at the end of the treatment period. No significant ($p > 0.05$) difference was observed in body weight between $APPE_{100}$ and $APPE_{50}$ at the expiration of the treatment period (Fig. 2).

3.3. Effects on blood glucose, insulin and glycated hemoglobin concentrations

A remarkable increase in serum glucose level (241.4%) was observed in DC in comparison with NC. Interestingly, significant ($p < 0.05$) reduction in serum glucose concentration were noted in $APPE_{50}$, $APPE_{100}$ and $APPE_{200}$ compared to DC. $APPE_{200}$ gave the most significant ($p < 0.05$) reduction in blood glucose followed by $APPE_{100}$ and $APPE_{50}$ which gave a similar reduction in glucose level at the end of the treatment period. No significant ($p > 0.05$) difference was observed in blood glucose level between $APPE_{200}$ and NC at the expiration of the treatment period (Fig. 3a). A marked drop in serum insulin (69.1%) concentration was observed in DC compared to NC. However, a dose-dependent significant ($p < 0.05$) improvement in serum insulin level was observed in APPE-treated rats compared to DC (Fig. 3b). A significant ($p < 0.05$) increase (96.4%) in glycated hemoglobin concentration was observed in DC

compared to NC. However, a dose-dependent significant ($p < 0.05$) decrease in glycated hemoglobin concentration was observed in the APPE-treated rats compared to DC (Fig. 3c).

3.4. Effects of serum cytokines concentration

A significant ($p < 0.05$) increase (96.4%) in serum interleukin-1β concentration was observed in DC compared to NC. However, a dose-dependent significant ($p < 0.05$) decrease in serum interleukin-1β concentration was observed in the APPE-treated animals compared to DC (Fig. 4a). A significant ($p < 0.05$) increase (96.4%) in serum interleukin-6 concentration was observed in DC compared to NC. However, a dose-dependent significant ($p < 0.05$) decrease in serum interleukin-6 concentration was observed in the APPE-treated rats up to 100 mg/kg compared to DC. IL-6 level was not significantly different in $APPE_{100}$ and $APPE_{200}$ (Fig. 4b). A significant ($p < 0.05$) increase (96.4%) in serum tumour necrosis factor alpha (TNF-α) concentration was observed in DC compared to NC. However, a dose-dependent significant ($p < 0.05$) decrease in serum TNF-α concentration was observed in APPE-treated rats compared to DC. The observed difference in TNF-α concentrations in $APPE_{25}$ and $APPE_{50}$ was not significant (Fig. 4c). A marked drop in serum interleukin-10 (IL-10) concentration was observed in DC compared to NC. However, a dose-dependent significant ($p < 0.05$) decrease in serum IL-10 concentration was observed in $APPE_{50}$ to $APPE_{200}$ compared to DC. The observed difference in IL-10 concentrations in $APPE_{25}$ and $APPE_{50}$ was not significant (Fig. 4d).

3.5. Effect on cellular architecture

The cytological architecture of the intestine and heart were observed to be normal at the end of the treatment period. However, photomicrographs of the liver and kidney showed interface hepatitis and glomerulosclerosis, respectively in alloxan-induced but untreated diabetic rats. Treatment with APPE at varying concentrations showed a dose-dependent improvement in the liver and kidney. APPE at 200 mg/kg restored the liver and kidney architecture to normal at the end of the treatment period (Fig. 5).

4. Discussion

From this study, quercetin appears to be the major phenolics present in APPE. From previous studies, quercetin has been proven to inhibit tissue oxidative damage via its augmentation effect on oxidative status. Moreover, it has been established to promote insulin release by enhancing the regeneration of pancreatic islets [21]. In addition, plants phenolics has been indicated to inhibit the action of amylase in the digestion of carbohydrate in diabetes while others have been proven to lower the risk of complications arising from type 2 diabetes [22]. The two principal enzymes involved in the breakdown of dietary carbohydrates, α-glucosidase and α-amylase, have been shown to be under the influence of flavonoids, phenolic acid and tannins which inhibit their actions [21].

Alloxan exhibits a selective destruction of islet Langerhans β-cells of the pancreas which usually result in massive reduction in insulin production culminating in hyperglycemia and the subsequent derangement

in energy metabolism [23, 24] as vividly observed in this study. In compliance with previous studies, the present study showed that alloxan intoxication in rats led to reduced body weight while leading to enhanced food consumption. Though data on food intake is not reported in this study alloxan-induced rats were observed to consume more food than non-diabetic control rats. The usual decrease in body weight in diabetic state could be attributed to reduce cellular uptake and utilization of blood glucose which as well impact the rate of lipid dispensation and gluconeogenesis [22]. The improvement in body weight in alloxan-induced diabetic rats administered APPE could have been a resultant anabolic effect of its phenolic phytochemicals which are capable of abrogating the physiological consequences of both alloxan- and hyperglycemia-induced oxidative stress through their antioxidant activity. Dietary plant polyphenols have been reported to control carbohydrate and lipid metabolism thus improving hyperglycemia, dyslipidemia and insulin resistance through improved β-cell function [25].

The hallmark of diabetic management is the restoration of blood glucose level to normal. As observed in the present study, APPE at 200 mg/kg body weight proofs to be an effective hypoglycemic agent in decreasing blood glucose concentrations to normal value in alloxan-induced diabetic rats. Hence, APPE could be attributed to produce or potentiate a beneficial role on carbohydrate utilization in alloxan-induced diabetic rats.

Data obtained in this study showed significantly higher level of glycosylated haemoglobin in diabetic but untreated rats (DC) compared to both normal control and diabetic groups treated with APPE. This shows that haemoglobin becomes glycosylated in a condition of high blood glucose concentration. The reduction in the level of glycated hemoglobin in the APPE-treated diabetic rats clearly suggests that APPE treatment reduces protein glycosylation in diabetic rats. The inhibitory action of APPE on protein glycosylation could be attributed to the antioxidative effects of polyphenolics on Millard reaction. Several antioxidants including ascorbic acid and β-carotene have been demonstrated to inhibit protein glycosylation both in vivo and in vitro [26, 27]. Dietary antioxidants have also been reported to be capable of scavenging free radicals produced by protein glycosylation [28].

Oxidative stress has been postulated to be capable of activating several pathological changes in almost every type of kidney cells including endothelial cells, mesangial cells, tubular cells etc. This process in turn results in the development of diabetic neuropathy. It has also been pointed out that fibrosis which appears to be the most prominent feature of diabetic neuropathy is coordinately linked to inflammation [29]. In accordance with previous postulations, serum level of pro- inflammatory cytokines were observed to be elevated in alloxan-induced diabetic rats in this study [30, 31]. Data provided in this study revealed that serum pro-inflammatory molecules including IL-1β, IL-6 and TNF-α were upregulated in alloxan-induced diabetic rats. On the contrary, there was a corresponding reduction in serum concentrations of anti-inflammatory cytokines, IL-10.

Recently, the concentrations of these pro-inflammatory molecules were observed to increase correlate positively with the progression of neuropathy [32]. These observations give an indication of a possible

direct link between these inflammatory markers and glomerular damage. According to Akdis *et al*. [33]. IL-10 produced from $CD4^+$ Th2 cells otherwise known as cytokine synthesis inhibitory factor, inhibits the synthesis of pro-inflammatory cytokines including IL-4 and IL-5 (from Th2 cells) and the production of IL-2 and IFN-γ by Th1 cells. Thus, the anti-inflammatory role of IL-10 involves its inhibitory actions on both adaptive and innate immune cells [34]. It is viewed that the synthesis and secretion of these fibrogenic cytokines in the local microenvironment may be responsible for the observed damage in renal architecture [35]. In addition, the potentials of these molecules in recruiting circulating while blood cells and their migration into the kidney tissue have also be pinpointed as possible risk factor in diabetic neuropathy.

The histological examinations of the intestine, heart, liver and kidney revealed intact architecture in all the tissues across the various treatment groups except in the kidney where varying degree of interface hepatitis and glomerulosclerosis was observed in the alloxan-induced diabetic rats. However, APPE at 100 and 200 mg/kg body weight abrogated these abnormalities and restored the kidney architecture. These findings further reinforced the claim that diabetic neuropathy is the most prominent feature of diabetic complications.

Based on findings from this study, it is posited that the concerted effects of sustained hyperglycemia (as a consequence of alloxan-destruction of pancreatic beta cells) and the eventual generation of keto-aldehydes leading to the production of the highly potent free radical, H_2O_2 as well as glycosylation of blood protein in hemoglobin could have been responsible for the stimulation, recruitment and release of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) thus leading to the observed kidney damage. Pro-inflammatory cytokines such as TNF-α and IL-1β have been reported to trigger the NF-κB pathway thus mediating the phosphorylation of serine molecules in insulin receptor substrate [36]. The phosphorylation of insulin receptor substrate is thought to potentiate insulin resistance [37]. However, these negative effects were remarkably abrogated by avocado peel polyphenolic extract especially at high doses (especially at 200 mg/kg body weight) via its inhibitory effects on carbohydrate hydrolyzing enzymes [22] as well as its antioxidant activity in preventing the oxidation of glucose to ketoaldehydes and generation of reactive glycated proteins.

5. Conclusion

Thus, the sustained hyperglycemia, possibly resulting from insulin resistance, and the consequent glycated hemoglobin could have aggravated the process of free radical generation culminating in oxidative stress and the consequential upgrade in pro-inflammatory cytokine release. The consternation of all these processes must have worked in concert to bring about the observed damaged in kidney cytoskeletal architecture. However, the polyphenolic extract of avocado peel when administered orally for a period of 21 days to alloxan-induced diabetic rats was observed not only to produce a remarkable drop in fasting blood glucose concentration but in addition resulted in significant decrease in hemoglobin glycosylation, as well as reduced serum levels of pro-inflammatory molecules (IL-1β, IL-6 and TNF-α).

More so, serum IL-10, an anti-inflammatory cytokine was significantly augmented. The totality of these effects contributed to the abrogation of the oxidative stress-induced nephrotic damage generally observed in diabetic complications. The intake of polyphenolic-rich plant and plant products by diabetes is highly encouraged in the preventing and management of diabetic complications.

Declarations

Acknowledgements

The authors express their appreciation to Mr. James at the Federal Medical Center, Lokoja, Kogi state, Nigeria for assistance in the histological analysis.

Author contributions

O. M. O. conceptualized the study, supervised the study and wrote the manuscript; D. O. A., A. A. A. and G. O. E. carried out the experiment, analyzed the data; B. O. I. and O. O. A. carried out the histopathological analysis and interpreted the results; S. I. O. assisted in data analysis and interpretation, and drafted the manuscript; All authors read and approved the final manuscript.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Availability of data and materials

All data generated and analyzed during the current study are available with the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Competing interest

The authors declare that there is no competing interest.

References

1. Boutari, C., Polyzos, S. A. & Mantzoros, C. S. Of mice and men: why progress in the pharmacological management of obesity is slower than anticipated and what could be done about it? *Metab: Clin. Exp*, **96**, 6–11 (2019).
2. Medina-Urrutia, A. *et al.* Role of adiponectin and free fatty acids on the association between abdominal visceral fat and insulin resistance. *Cardiovasc. Diabetol*, **14**, 20 (2015).

3. Jeyaraman, M. M. *et al.* Resveratrol for adults with type 2 diabetes mellitus.*Cochrane Database Syst. Rev.***1** (2020).
4. Pamplona, R. Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. *Biochim. Biophy. Acta-Bioenergetics*, **1777**, 1249–1262 (2008).
5. Bigagli, E. & Lodovici, M. Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications.*Oxid. Med. Cell Longev.*(2019).
6. Danilova, T. & Lindahl, M. Emerging roles for Mesencephalic Astrocyte-Derived Neurotrophic Factor (MANF) in pancreatic beta cells and diabetes. *Front. Physiol*, **9**, 1457 (2018).
7. Figueroa, J. G., Borrás-Linares, I., Lozano-Sánchez, J. & Segura-Carretero, A. Comprehensive characterization of phenolic and other polar compounds in the seed and seed coat of avocado by HPLC-DAD-ESI-QTOF-MS. *Food Res. Int*, **105**, 752–763 (2018).
8. Melgar, B. *et al.* Bioactive characterization of Persea americana Mill. by-products: A rich source of inherent antioxidants. *Ind. Crops Prod*, **111**, 212–218 (2018).
9. Ferreira da Vinha, A., Sousa, C., Soares, M. D. & Barreira, S. Avocado and its by-products: natural sources of nutrients, phytochemical compounds and functional properties. *Curr. Res. Agric. Food Sci*, **1**, 82–96 (2020).
10. Oguntibeju, O. O. Hypoglycaemic and anti-diabetic activity of selected African medicinal plants. *Int. J. Physiol. Pathophysiol. Pharmacol*, **11**, 224 (2019).
11. Alkhalaf, M. I., Alansari, W. S., Ibrahim, E. A. & ELhalwagy, M. E. Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (Persea americana) fruit and seed extract. *J. King Saud. Univ. Sci*, **31**, 1358–1362 (2019).
12. Oluba, O. M. *et al.* Effects of Talinum triangulare leaf flavonoid extract on streptozotocin-induced hyperglycemia and associated complications in rats. *Food Sci. Nutr*, **7**, 385–394 (2019).
13. Colombo, R. & Papetti, A. Avocado (Persea americana Mill.) by-products and their impact: from bioactive compounds to biomass energy and sorbent material for removing contaminants. A review. *Int. J. Food Sci. Technol*, **54**, 943–951 (2019).
14. National Research Council. *Guide for the care and use of laboratory animals* (National Academies Press, 2010).
15. du Sert, P. N. *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol*, **18** (7), e3000410 https://doi.org/10.1371/journal.pbio.3000410 (2020).
16. Simić, V. M. *et al.* Optimization of microwave-assisted extraction of total polyphenolic compounds from chokeberries by response surface methodology and artificial neural network. *Sep. Purif. Technol*, **160**, 89–97 (2016).
17. Ozcan, M. M. *et al.* Effect of microwave and oven drying processes on antioxidant activity, total phenol and phenolic compounds of kiwi and pepino fruits. *J. Food Sci. Technol*, **57**, 233–242 (2020).
18. Šeruga, M., Novak, I. & Jakobek, L. Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. *Food*

Chem, **124**, 1208–1216 (2011).

19. Macdonald Ighodaro, O., Mohammed Adeosun, A. & Adeboye Akinloye, O. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, **53**, 365–374 (2017).
20. Nayak, S. S. & Pattabiraman, T. N. A new colorimetric method for the estimation of glycosylated hemoglobin. *Clin Chim Acta*, **109**, 267–274 (1981).
21. Shetty, K., Randhir, R. & Sarkar, D. *Bioprocessing Strategies to Enhance L-DOPA and Phenolic Bioactives in the Fava Bean (Vicia Faba)* (Sources of Functional Foods and Ingredients, Functional Foods and Biotechnology, 2019).
22. Yang, D. K. & Kang, H. S. Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. *Biomol. Ther*, **26**, 130 (2018).
23. Lawal, R. T., Oyeleke, G. O., Ishola, A. D. & Akinsuroju, M. O. Determination of Physiochemical Properties and Lactic Acid Bacteria Presence in Ackee (*Blighia sapida*) Fruit.*Int. J. Environ. Agric. Biotechnol.***3**, (2018).
24. Ruikar, A. *et al.* Antioxidant screening of a medicinal Plant Artocarpus heterophyllus. *Int. J. Pharm. Sci. Rev. Res*, **33**, 35–43 (2015).
25. Cao, H. *et al.* Dietary polyphenols and type 2 diabetes: human study and clinical trial. *Crit. Rev. Food Sci. Nutr*, **59**, 3371–3379 (2019).
26. Berryman, A. M., Maritim, A. C., Sanders, R. A. & Watkins, I. I. I. J. B. Influence of treatment of diabetic rats with combinations of pycnogenol, β-carotene, and α-lipoic acid on parameters of oxidative stress. *J. Biochem. Mol. Toxicol*, **18**, 345–352 (2005).
27. Zafar, H., Sheikh, M. A., Hussain, F. & Maan, M. A. Inhibition of protein glycation and advanced glycation end products by ascorbic acid. *Afr. J. Biotechnol*, **11**, 11309–11314 (2012).
28. Perez, G. R., Flores, C. L. & Gonzalez, A. M. Evaluation of the antioxidant and anti-glication effects of the hexane extract from Piper auritum leaves in vitro and beneficial activity on oxidative stress and advanced glycation end-product-mediated renal injury in streptozotocin-treated diabetic rats., **17**, 11897–11919 (2012).
29. Wada, J. & Makino, H. Inflammation and the pathogenesis of diabetic nephropathy. *Clin. Sci*, **124**, 139–152 (2013).
30. Domingueti, C. P. *et al.* Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *J. Diabetes Complicat*, **30**, 738–745 (2016).
31. Domingueti, C. P. *et al.* Association of different biomarkers of renal function with D-dimer levels in patients with type 1 diabetes mellitus (renal biomarkers and D-dimer in diabetes). *Arch. Endocrin. Metab*, **62**, 27–33 (2018).
32. Yaribeygi, H., Butler, A. E., Barreto, G. E. & Sahebkar, A. Antioxidative potential of antidiabetic agents: A possible protective mechanism against vascular complications in diabetic patients. *J. Cell Physiol*, **234**, 2436–2446 (2019).

33. Akdis, C. A., Joss, A., Akdis, M., Faith, A. & Blaser, K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *The FASEB J*, **14**, 1666–1668 (2000).
34. Rouhi, Y., Naseri, F., Elahe, T. E., Tebyanian, H. & Moghaddam, M. M. Effect of Vitamin D on the Expression Level of the IL-10 and IL-4 in Asthmatic Mice. *Biointerface Res. Appl. Chem*, **10**, 7077–7084 https://doi.org/10.33263/BRIAC106.70777084 (2020).
35. Sindhughosa, D. A. & Pranamartha, A. G. The involvement of proinflammatory cytokines in diabetic nephropathy: Focus on interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α) signaling mechanism. *BMJ*, **6**, 299 (2017).
36. Ajiboye, B. O. *et al.* Anti-hyperglycemic and anti-inflammatory activities of polyphenolic-rich extract of Syzygium cumini linn leaves in alloxan-induced diabetic rats. *J. Evid. Based Integr. Med*, **23**, 251569018770630 (2018).
37. Gratas-Delamarche, A., Derbré, F., Vincent, S. & Cillard, J. Physical inactivity, insulin resistance, and the oxidative-inflammatory loop. *Free Rad. Res*, **48**, 93–108 (2014).

Figures

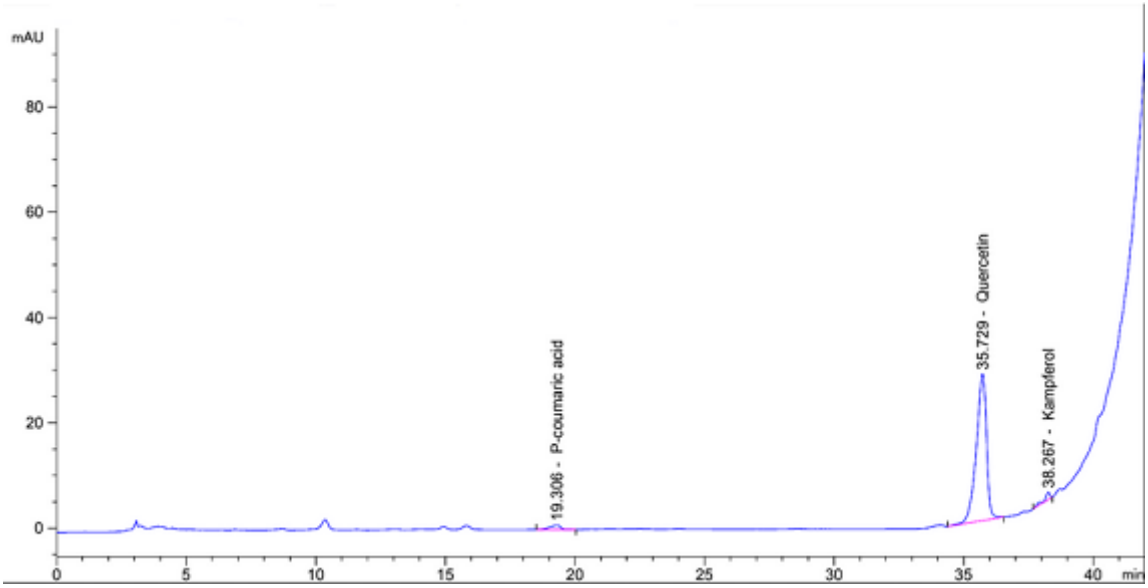

Figure 1

HPLC fingerprint of avocado peel polyphenolic extract

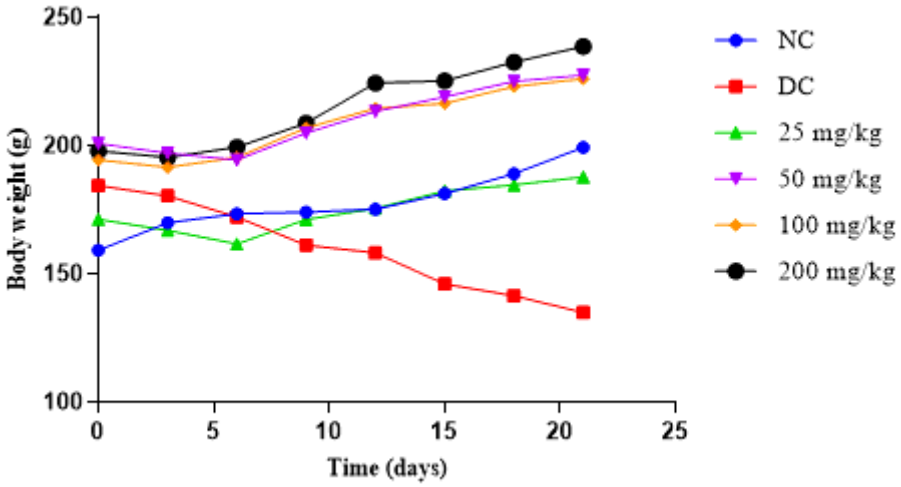

Figure 2

Effect of avocado pear (Persea americana) polyphenolic peel extract (APPE) on body weights in alloxan-induced diabetic rats. Results are means ± SEM of five replicates. Note: NC, normal control; DC, diabetic control; 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg, alloxan-induced diabetic rats treated with 25, 50, 100 and 200 mg/kg APPE, respectively.

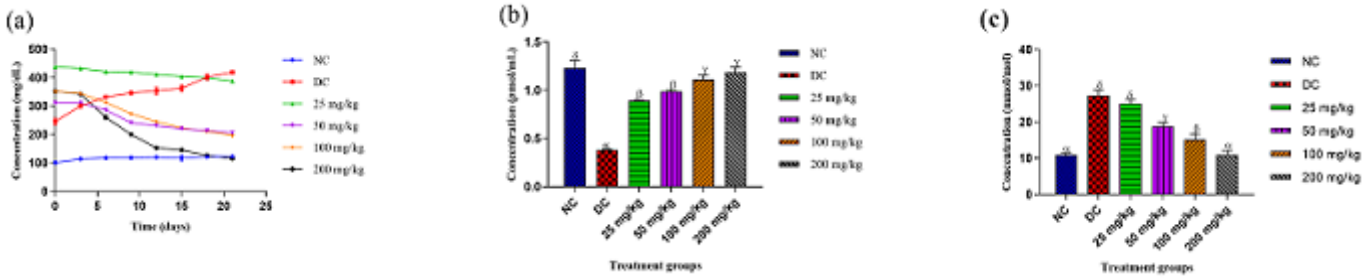

Figure 3

Effect of avocado pear (Persea americana) polyphenolic peel extract (APPE) on body weights in alloxan-induced diabetic rats. Results are means ± SEM of five replicates. Note: NC, normal control; DC, diabetic control; 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg, alloxan-induced diabetic rats treated with 25, 50, 100 and 200 mg/kg APPE, respectively.

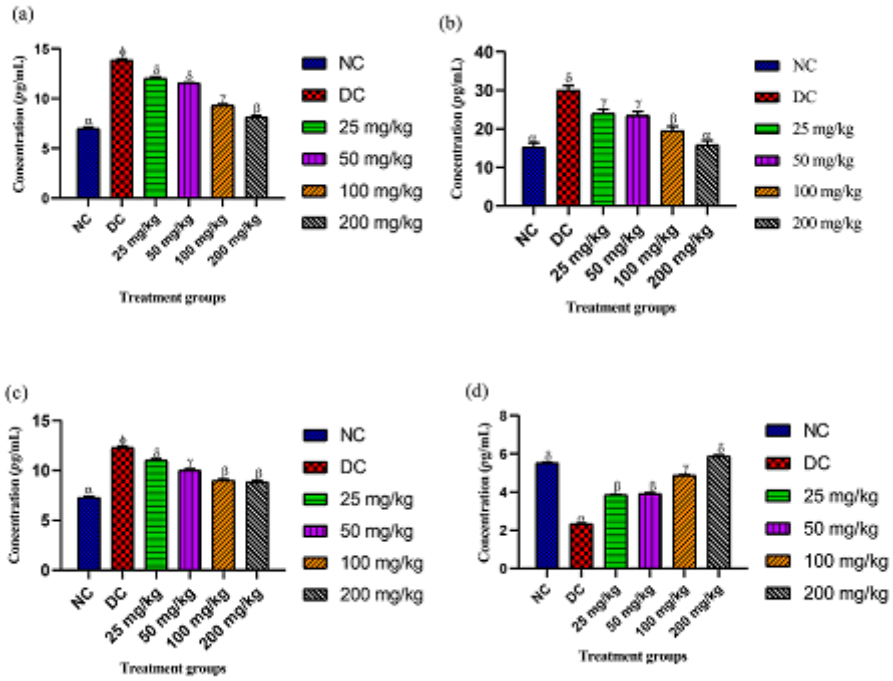

Figure 4

Effect of avocado pear (Persea americana) peel polyphenolic extract (APPE) on serum (a) interleukins-1β, (b) interleukin-6, (c) tumour necrosis factor-α, and (d) interleukin-10 concentrations in alloxan-induced diabetic rats. Results are means ± SEM of five replicates. Note: NC, normal control; DC, diabetic control; 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg, alloxan-induced diabetic rats treated with 25, 50, 100 and 200 mg/kg APPE, respectively. Bars carrying different Greek alphabets are significant ($p < 0.05$).

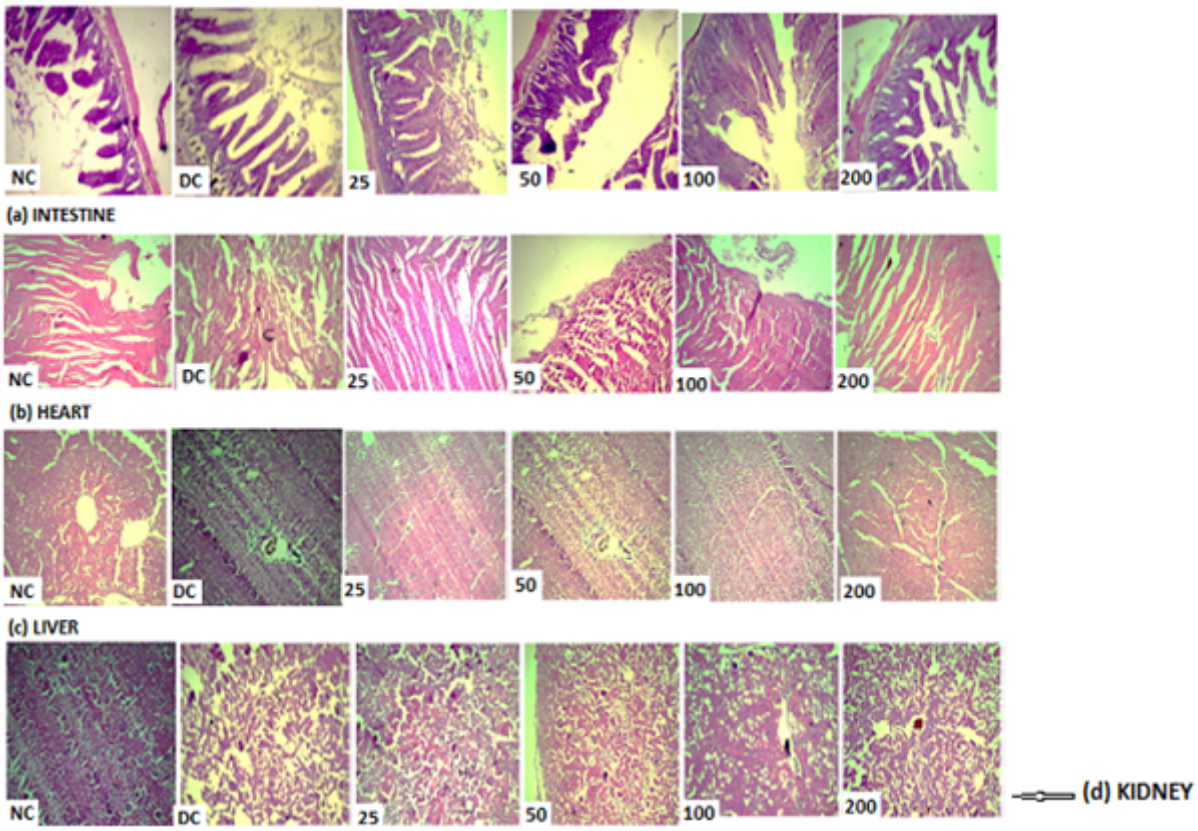

Figure 5

Photomicrographs of the (a) intestine, (b) heart, (c) liver, and (d) kidney. Note: NC, normal control, non-diabetic rats administered distilled water; DC, diabetic control, alloxan-induced diabetic rats administered distilled water; 25, 50, 100, and 200, alloxan-induced diabetic rats administered 25, 50, 100, and 200 mg/kg body weight, respectively avocado pear peel polyphenolic extract.