Avocado Peel Polyphenolic-Rich Extract Attenuates Low-Grade Inflammation in Alloxan-Induced Diabetic Male Wistar Rats

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ABSTRACT

Aim: This study sought to assess the effects of polyphenolic-rich extract of the peel of *Persea americana* (avocado) (APPE) on low-grade inflammation in alloxan-induced diabetic rats. **Methodology**: A total of 35 alloxan-induced diabetic male rats were randomly divided into five groups (n = 7) and designated: diabetic control (DC), and groups receiving 25 ($APPE_{25}$), 50 ($APPE_{50}$), 100 ($APPE_{100}$), and 200 ($APPE_{200}$) mg/kg of APPE. In addition, a normal control (NC) treated with distilled water was maintained. Treatment was administered orally once daily for 21 days. **Results**: After 21 days, the APPE-treated rats, particularly at 200 mg/kg, exhibited significant ($p<0.05$) improvements in both body weight and blood glucose levels compared with DC group. Significant ($p<0.05$) reductions in glycated hemoglobin, interleukins (1 and 6), and tumor necrosis factor-α levels were observed in the APPE-treated rats compared with DC. In contrast, the APPE-treated rats exhibited significant ($p<0.05$) improvement in insulin and interleukin-10 levels compared with DC rats. Furthermore, DC and $APPE_{25}$ and $APPE_{50}$ showed varying degrees of interfacial hepatitis and glomerulosclerosis. Nevertheless, treatment with APPE200 exhibited protection against the detrimental impact of diabetes mellitus on the liver and kidney in alloxan-induced diabetic rats. **Conclusion**: Avocado peel polyphenolic-rich extract at 200 mg/kg body weight effectively inhibited low-grade inflammation induced by oxidative stress in the liver and kidneys of rats exposed to alloxan.

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

1. INTRODUCTION

Persistent high blood sugar level is a major predictive determinant in the development of diabetes and its associated complications. Metal-ion-catalysed glucose oxidation potentiates the production of free radicals including hydrogen peroxide and ketoaldehydes. Ketoaldehyde in turn enhances the generation of superoxide, which is spontaneously converted to H_2O_2 and subsequently into the remarkably powerful hydroxyl radical [1]. The overwhelming effects of the continuous production of these free radicals in diabetes on biological antioxidants capacity leads to the emergence of oxidative stress-induced complications in individuals with diabetes [2].

Consequently, there exists a correlation between oxidative stress and the emergence of macrovascular problems in individuals with type 2 diabetes [3]. One of the primary mechanisms implicated in the non-enzymatic glycosylation of hemoglobin is the autooxidation of glucose. Hemoglobin that has been glycated is a powerful generator of free radicals. Therefore, there exists a causal relationship between diabetic complications, hemoglobin glycosylation and oxidative stress. Nevertheless, the aforementioned processes may be mitigated subsequent to the administration of antioxidants.

The tropical fruit known as avocado (*Persea americana* Mill. Lauraceae), sometimes referred to as alligator pea or butter fruit, possesses flesh that can be consumed. The waste generated during the production of avocado pear ranges from 35% to 50%, with the peel accounting for around 20% to 25% of this waste [4]. Nevertheless, it has been documented that avocado peel possesses a substantial quantity of phenolic chemicals. The phenolic content of avocado peel waste has been found to be much higher in comparison to avocado pulp [5]. Research has demonstrated that the phenolic extract derived from avocado pear exhibits a wide range of pharmacological properties, encompassing anti-diabetic [6], anti-inflammatory, and antioxidant effects [7]. Avocado peel waste is regarded as a promising and cost-effective biomass for extracting phenolic compounds from agricultural waste, due to its numerous health benefits. Therefore, the utilization of avocado peel waste holds significant potential for enhancing the value of the avocado processing industry and effectively mitigating the environmental consequences associated with avocado waste [8]. Recent reports indicate that specific foods and plant extracts have the potential to hinder the activity of α-amylase. The inhibition observed in these foods and plant extracts can be attributed to the presence of polyphenols, as documented [9]. Considering the potential existence of a causal association between oxidative stress induced by hyperglycemia and the glycosylation of hemoglobin, as well as the subsequent induction of inflammatory cytokines in individuals with diabetes. We predicted that the potential inhibition of carbohydrate hydrolyzing enzymes and/or the probable suppression of glucose oxidation via antioxidant mechanisms by avocado peel polyphenolic-rich extract could offer a viable method in the prevention and management of diabetes complications.

2. MATERIAL AND METHODS

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.

Plant material

Matured *Persea americana* (avocado pear) fruits were collected from a vegetation area near Omu-Aran, Kwara State, Nigeria (8°8'18" N; 5°6'9" E) after obtaining permission from the farm owner to collect the fruits between July 2020 and August 2020. Mr. Bolu of the University of Ilorin's Department of Plant Science, Ilorin, Nigeria, where the voucher specimen (UILH/011/1803) was deposited, identified and authenticated the plant sample.

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 with five animals per cage with a 12 h

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light/dark cycle and fed *ad libitum* with unrestricted access to water. Prior authorization for animal handling was sought from the Landmark University, Omu-Aran, Nigeria Research and Ethics Committee (approval number LMU/EC/098/2019).

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Ethics declaration

The care and handling of animals complied with the National Institutes of Health Manual on the care and use of laboratory animals [10] and was approved by the Animal Use Ethics Committee of the Landmark University, Omu-Aran, Nigeria, with the approval number LMU/EC/098/2019. Furthermore, this study was conducted according to the ARRIVE guidelines [11].

Microwave assisted polyphenol extraction

The modified microwave-assisted method of Simi *et al*. [12] was used to extract total polyphenols with little modification. Briefly, in a 500-mL Erlenmayer flask containing 250 mL of ethanol (50%), powdered avocado pear peel (50 g) was placed and put in a BP090 microwave oven (Microwave Research & Applications, Inc., Illinois, USA). A vertical condenser was securely connected to the flask. For 5 minutes, the extraction process was carried out at 300 W, and the liquid extract was separated from the residue using vacuum filtration and vacuum evaporated at 40 ^{o}C to obtain the concentrated polyphenolic-rich extract.

Determination of total polyphenol

The total polyphenol content of avocado peel polyphenolic extract was calculated using Ozcan *et al*. colorimetric Folin-Ciocalteu method [13].

GC-MS analysis

The preparation of the avocado peel polyphenolic extract was carried out according to the Oluba *et al*. method [14]. A weighed amount of the avocado peel polyphenolic extract was dissolved in GC-grade methanol (1:5 $^{w}/_{v}$). The mixture was shaken in an orbital shaker for 48 hours at 252 °C. Thereafter, the mixture was filtered through a muslin cloth to obtain the crude extract, which was concentrated using a rotary evaporator and stored at -4 ^{o}C until required for further analysis. The methanol extract was then subjected to GC-MS analysis in order to identify its constituent bioactive metabolites. The detection was carried out with an ionisation energy of 70 eV and 60 kPa using helium as the carrier gas. The oven temperature was initially set at 100 ^{o}C for 2 min with a ramp rate of 4 ^{o}C per min to 225 ^{o}C and then 1 ^{o}C per min to 245 ^{oC,} followed by 40 ^{o}C to 280 ^{o}C with a 30 min hold. Thereafter, a 2 µL were injected at 280 °C with a split ratio of 1:50. The detected spectra were identified from the NIST database using the ChemStation software.

Induction of diabetes

A single intraperitoneal administration of 150 mg/kg alloxan monohydrate in normal saline was administered to the rats [15]. Seventy-two hours after alloxan administration, rats were tested for fasting blood glucose levels from tail vein blood using a One-Touch Ultramini blood glucose meter (LifeScan, Milpitas, USA). Animals with 200 mg/dL and above fasting blood glucose were considered diabetic and included in the study.

Experimental design

The experimental design consisted of 35 alloxan-induced diabetic rats and a separate set of 7 non-diabetic rats. The animals were grouped as follows:
Normal control (NC): Normal rats (*n* = 7) treated with distilled water,
Diabetic control (DC): Alloxan-diabetic (*n* = 7) treated with distilled water,
$APPE_{25}$: Alloxan-diabetic rats (*n* = 7) treated with APPE (25 mg/kg *bw*),
$APPE_{50}$: Alloxan-diabetic rats (*n* = 7) treated with APPE (50 mg/kg *bw*),
$APPE_{100}$: Alloxan-diabetic rats (*n* = 7) treated with APPE (100 mg/kg *bw*),
$APPE_{200}$: Alloxan-diabetic rats (*n* = 7) treated with APPE (200 mg/kg *bw*).
Treatment was given orally once daily for 21 consecutive days, after which they were euthanized with the inhalation of isoflurane after which blood collected by cardiac puncture. 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, and 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.

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.

Determination of glycated hemoglobin

Hemoglobin glycosylation level was evaluated following Nayak and Pattabiraman method [16]. Briefly, whole blood sample was combined with a lysing reagent that consisted of a detergent and borate ions. This ensured the removal of the unstable Schiff's base during the process of hemolysis. The hemolysate was thoroughly mixed for a minimum of 5 minutes with a cation exchange resin that has a low affinity for binding. At this moment, HbA_0, which is the form of hemoglobin that is not glycated, is attached to the resin. A specialized resin separator was employed to extract the resin from the supernatant fluid, which included the HbA_1 (glycated HbA). The percentage of glycated hemoglobin in relation to total hemoglobin was calculated by measuring the absorbance of the glycohemoglobin and total hemoglobin at 415 nm and comparing it to a reference glycohemoglobin preparation used in the test.

Biochemical analysis

Whole blood was allowed to stand at room temperature (25 ± 2 ^{o}C) for 30 min before being centrifuged at 1500 x*g* for ten minutes in a refrigerated centrifuge, and serum was carefully pipetted into dry, clean, sterile bottles and stored at -4 ^{o}C for further biochemical analyses. Serum interleukins (1β, 6, and 10) and TNF-α levels, were determined using their respective diagnostic kits (R&D System, Minneapolis, USA) in strict accordance with the manufacturer's instructions.

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Histological evaluation

A small portion of the liver sample was cut and fixed in 10% neutral buffered formalin. For light microscopy, 5 µm thin sections were cut with a rotary microtome and stained with haematoxylin and eosin. The slides were viewed with an appropriate objective to check for the architecture of the tissues. The images were shown in photomicrographs, and the histology was examined.

Statistical analysis

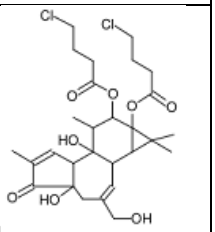
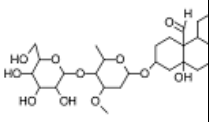
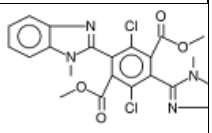
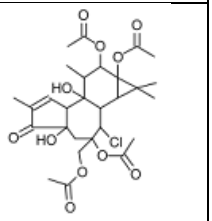
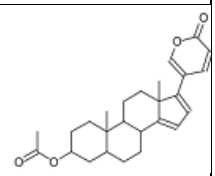
Data were expressed as means ± SEM. Statistical analysis and graphical representation were carried out using GraphPad Prism software (version 8.0). The mean comparison was done by one-way analysis of variance (ANOVA), followed by Least Significant Difference (LSD) test. *P* values less than 0.05 were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

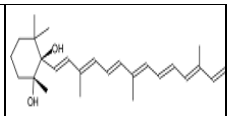
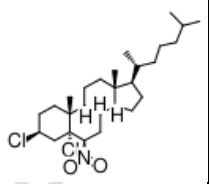
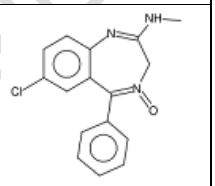
3. RESULTS AND DISCUSSION

Phytochemical composition

The total phenolic content of avocado peel polyphenolic extract was 127.1 mg garlic acid equivalent^{-1} sample. The GC-MS fingerprint of avocado peel polyphenolic extract is shown in Figure 1. According to the GC-MS profile, the following phytochemicals were identified, butanoic acid, 4-chloro-, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8 -tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1ar-(1aα,1b*β*,4aβ,7aα,7b*α*,8α,9β,9aα)]-; Card-20(22)-enolide, 3-[(2,6-dideoxy-4-O-*β*-D-glucopyranosyl-3-O-methyl-*β*-D-ribo-hexopyranosyl)oxy]-5,14 -dihydroxy-19-oxo-, (3*β*,5*β*)-; 2,5-Dichloro-3,6-bis-(1-methyl-1H-benzoimidazol-2-yl)-terephthalic acid, dimethyl ester; 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3, 4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1a*α*,1b*β*,2*α*,3*β*,4a*β*,7a*α*,7b*α*,8*α*,9*β*,9a*α*)]-; Bufa-14,16,20,22-tetraenolide, 3-(acetyloxy)-, (3*β*,5*β*)-; Azafrin; Cholestane, 3,5-dichloro-6-nitro-, (3*β*,5*α*,6*β*)-; and Chlordiazepoxide (Table 1). These identified molecules are either are either steroids, polyphenolics, terpenes or fatty esters, etc. The results show that quercetin was the major phenolic compound present in avocado pear peel. This observation is consistent with the report of Rosero *et al*. [17] From previous studies, quercetin has been proven to inhibit tissue oxidative damage via its augmenting effect on oxidative status. Moreover, it has been established that it enhances the regeneration of pancreatic islets [18]. In addition, plant phenolics have been indicated to inhibit the action of α-amylase in the digestion of carbohydrates in diabetes, while others have been proven to lower the risk of complications arising from type 2 diabetes [19]. Flavonoids, phenolic acids, and tannins have been shown to inhibit the actions of the two main enzymes involved in the breakdown of dietary carbohydrates, α-glucosidase and α-amylase [18].

Table 1: Phytochemical components of avocado pear peel polyphenolic extract obtained using GC-MS

s/n	Retention time (min)	Peak height	% of total	Molecular formula	Exact mass	Name	Structure
1	5.956	725385	1.203	$C_{28}H_{38}Cl_2O_8$	572.19	Butanoic acid, 4-chloro-, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1ar-(1aα,1bβ,4aβ,7aα,7bα,8α,9β,9aα)]-	
2	6.881	858344	1.628	$C_{36}H_{54}O_{14}$	710.35	Card-20(22)-enolide, 3-[(2,6-dideoxy-4-O-β-D-glucopyranosyl-3-O-methyl-β-D-ribo-hexopyranosyl)oxy]-5,14-dihydroxy-19-oxo-, (3β,5β)-	
3	7.288	157847	1.617	$C_{26}H_{20}Cl_2N_4O_4$	522.09	2,5-Dichloro-3,6-bis-(1-methyl-1H-benzoimidazol-2-yl)-terephthalic acid, dimethyl ester	
4	8.289	197419	1.737	$C_{28}H_{37}ClO_{11}$	584.20	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3, 4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1aα,1bβ,2α,3β,4aβ,7aα,7bα,8α,9β,9aα)]-	
5	9.506	26.83217	3.637	$C_{26}H_{32}O_4$	408.23	Bufa-14,16,20,22-tetraenolide, 3-(acetyloxy)-, (3β,5β)-	

6	11.782	46.82903	2.664	$C_{27}H_{38}O_4$	426.28	Azafrin	
7	12.561	8473565	6.024	$C_{27}H_{45}Cl_2NO_2$	485.28	Cholestane, 3,5-dichloro-6-nitro-, (3β,5α,6β)-	
8	12.882	3424344	1.808	$C_{16}H_{14}ClN_3O$	299.08	Chlordiazepoxide	

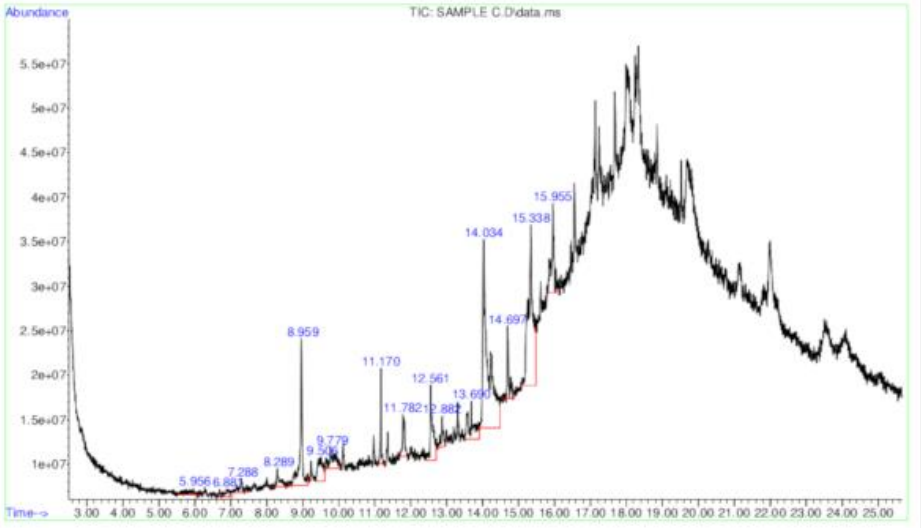


Figure 1: GC-MS fingerprint of avocado peel polyphenolic extract

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 the normal control group. Interestingly, treatment of diabetic animals with avocado peel polyphenolic-rich extract at 50, 100, and 200 mg/kg body weight (i.e. $APPE_{50}$, $APPE_{100}$, and $APPE_{200}$) led to a significant (P = .05) increased in body weight compared to diabetic control (DC). $APPE_{200}$ gave the most significant (P = .05) increase 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 difference in body weight was observed between $APPE_{100}$ and $APPE_{50}$ at the end of the treatment period (Fig. 2). Alloxan selectively destroys pancreatic islet Langerhans -cells, resulting in a massive reduction in insulin production, hyperglycemia, and a subsequent derangement in energy metabolism [20,21], 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 enhancing 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 a diabetic state could be attributed to reduced cellular uptake and utilisation of blood glucose, which also influence the rate of lipid dispensation and gluconeogenesis [19]. The increase in body weight in alloxan-induced diabetic rats administered avocado peel polyphenolic-rich extract could have been a resultant anabolic effect of its phenolic phytochemicals, which are capable of abrogating the physiological consequences of alloxan and hyperglycemia-induced oxidative stress through their antioxidant activity. Dietary plant polyphenols have been shown to improve hyperglycemia, dyslipidemia, and insulin resistance by regulating carbohydrate and lipid metabolism [22].

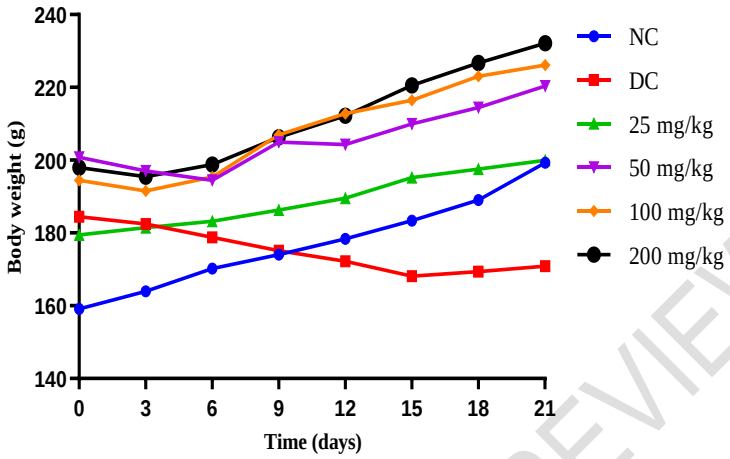


Figure 2: Effect of avocado pear (*Persea americana*) polyphenolic peel extract (APPE) on body weights in alloxan-induced diabetic rats. Results are means ± SEM of five replicates. Note: NC, normal control; DC, diabetic control; 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg, alloxan-induced diabetic rats treated with 25, 50, 100 and 200 mg/kg APPE, respectively.

Effects on blood glucose, insulin and glycated hemoglobin concentrations

In comparison to normal control, diabetic control had a significant (P = .05) increase in serum glucose level (241.4%). $APPE_{50}$, $APPE_{100}$, and $APPE_{200}$-treated diabetic rats had significantly lower serum glucose concentrations (P = .05) when compared to DC. At the end of the treatment period, $APPE_{200}$ provided the most significant (P = .05) reduction in blood glucose, followed by $APPE_{100}$ and $APPE_{50}$, which provided a similar reduction in glucose level. At the end of the treatment period, there was no significant (P = .05) difference in blood glucose levels between $APPE_{200}$ and NC (Fig. 3a). When comparing DC to NC, there was a significant drop in serum insulin concentration (69.1%). However, APPE-treated rats showed a dose-dependently significant (P = .05) increase in serum insulin levels when compared to control rats (Fig. 3b). When comparing DC to NC, there was a significant (P = .05) increase (96.4%) in glycated haemoglobin concentration. However, APPE-treated rats had a dose-dependently significant (P = .05) decrease in glycated haemoglobin concentration when compared to DC (Fig. 3c). The hallmark of diabetic management is the restoration of blood glucose levels to normal. As observed in the present study, avocado peel polyphenolic-rich extract at 200 mg/kg body weight proved to be an effective hypoglycemic agent in decreasing blood glucose concentrations to their normal value in alloxan-induced diabetic rats. As a result, avocado peel polyphenolics may be responsible for producing or amplifying a beneficial role in carbohydrate utilisation in alloxan-induced diabetic rats.

The results in this study showed a significantly higher level of glycosylated haemoglobin in diabetic but untreated rats (DC) compared to both the normal control and diabetic groups

treated with avocado peel polyphenolic-rich extract. This shows that haemoglobin becomes glycosylated in a condition of high blood glucose concentration. The reduction in the level of glycated hemoglobin in the avocado peel polyphenolic-rich extract-treated diabetic rats clearly suggests that avocado peel polyphenolic treatment reduces protein glycosylation in diabetic rats. The inhibitory action of avocado peel polyphenolics on protein glycosylation could be attributed to the antioxidative effects of polyphenolics through Millard reaction. Several antioxidants, including ascorbic acid and β-carotene, have been shown *in vivo* and *in vitro* to inhibit protein glycosylation [23,24]. Dietary antioxidants have also been reported to be capable of scavenging free radicals produced by protein glycosylation [25].

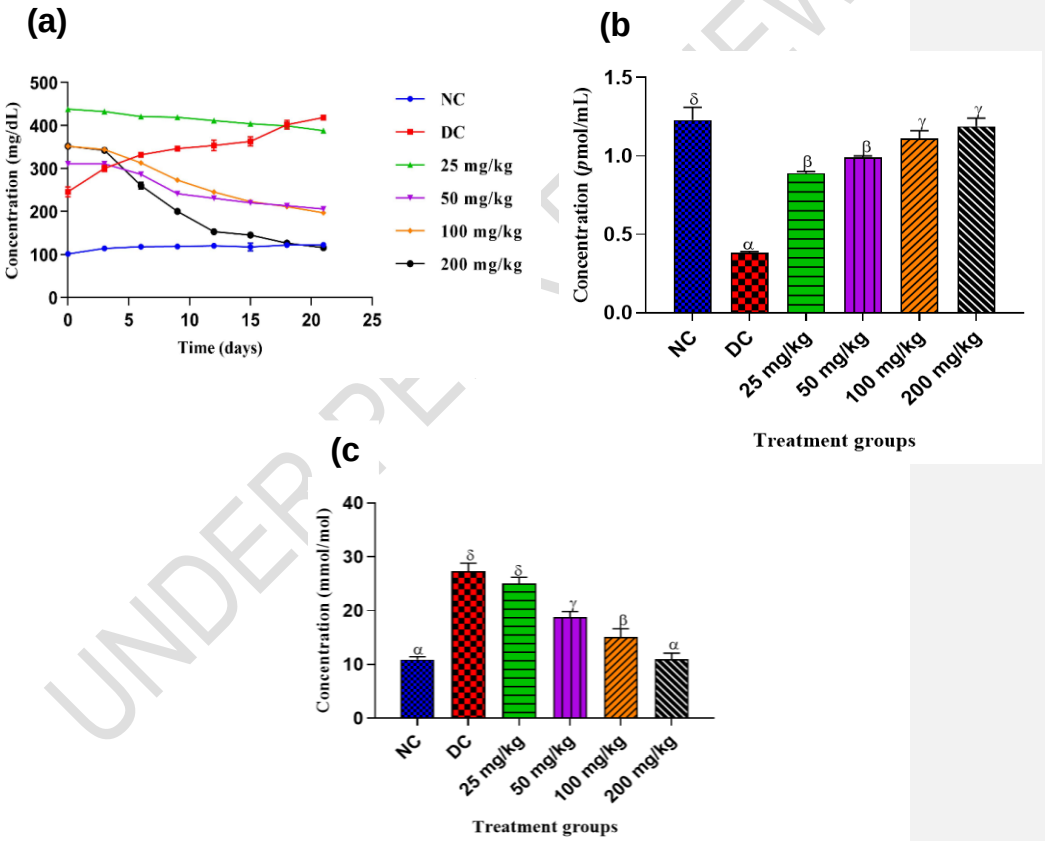


Figure 3: Effect of avocado pear (*Persea americana*) peel polyphenolic extract (APPE) on (a)blood glucose, (b) insulin and (c) glycated hemoglobin concentrations in alloxan-induced diabetic rats. Results are means ± SEM of five replicates. Note: NC, normal control; DC, diabetic control; 25 mg/kg, 50 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 = .05).

Effects of serum cytokine concentration

A significant (P = .05) increase (96.4%) in serum interleukin-1β concentration was observed in DC compared to NC. However, a dose-dependently significant (P = .05) decrease in serum IL-1β concentration was observed in the APPE-treated animals compared to DC (Fig. 4a). A significant (P = .05) increase (96.4%) in serum IL-6 concentration was observed in DC compared to NC. In contrast, a dose-dependently significant (P = .05) decrease in serum IL-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). When comparing DC to NC, there was a significant (P = .05) increase (96.4%) in serum TNF-α concentration. However, APPE-treated rats had a dose-dependently significant (P = .05) decrease in serum TNF-α concentration when compared to DC. The difference in TNF-α concentrations between $APPE_{25}$ and $APPE_{50}$ was not statistically significant (Fig. 4c). A marked drop in serum IL-10 concentration was observed in DC compared to NC. However, a dose-dependently significant (P = .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). Oxidative stress has been postulated to be capable of activating several pathological changes in almost every type of kidney cell, 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 closely linked to inflammation [26]. In accordance with previous postulations, the serum level of pro-inflammatory cytokines was observed to be elevated in alloxan-induced diabetic rats in this study [27,28]. This study discovered that serum pro-inflammatory molecules like 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 the anti-inflammatory cytokine IL-10.

Recently, the concentrations of these pro-inflammatory molecules were observed to increase and correlate positively with the progression of diabetic nephropathy [29]. These observations give an indication of a possible direct link between these inflammatory markers and glomerular damage. According to Akdis *et al* [30]. IL-10, also known as "cytokine synthesis inhibitory factor," is produced by $CD4^+$ Th2 cells and inhibits the synthesis of pro-inflammatory cytokines such as IL-4 and IL-5 (from Th2 cells) as well as the production of IL-2 and IFN-γ by Th1 cells. Thus, IL-10's anti-inflammatory role includes inhibitory actions on both adaptive and innate immune cells [31]. It is believed that the synthesis and secretion of these fibrogenic cytokines in the local microenvironment may be responsible for the observed damage in renal architecture [32]. Furthermore, the ability of these molecules to recruit circulating red blood cells and direct their migration into kidney tissue has been identified as a possible risk factor for diabetic nephropathy.

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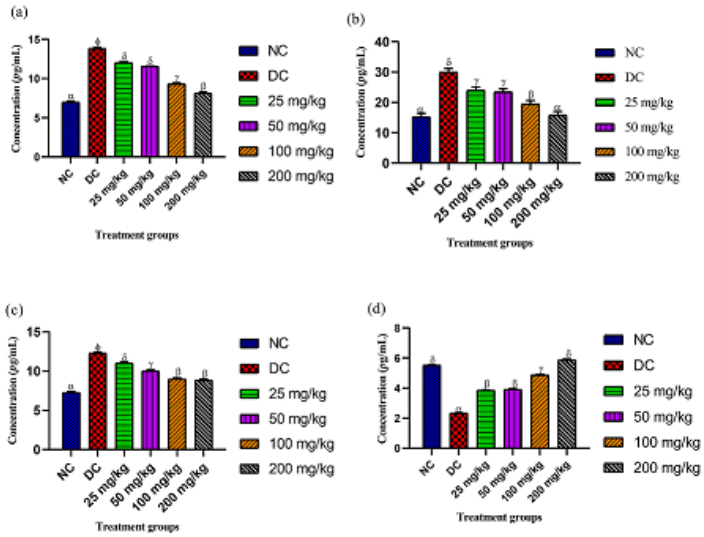

Figure 4: Effect of avocado pear (*Persea americana*) peel polyphenolic extract (APPE) on serum (a) interleukins-1β, (b) interleukin-6, (c) tumour necrosis factor-α, and (d) interleukin-10 concentrations in alloxan-induced diabetic rats. Results are means ± SEM of five replicates. Note: NC, normal control; 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 = .05).

Effect on cellular architecture

The cytological architecture of the intestine and heart across the different groups 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 avocado peel polyphenolic-rich extract at varying concentrations showed a dose-dependent improvement in the liver and kidney. Avocado peel polyphenolic-rich extract at 200 mg/kg restored the liver and kidney architecture to normal at the end of the treatment period (Fig. 5). 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 degrees of interface hepatitis and glomerulosclerosis were observed in the alloxan-induced diabetic rats. However, avocado peel polyphenolic-rich extract at 100 and 200 mg/kg body weight abrogated these abnormalities and restored the kidney architecture. These findings further reinforced the claim that diabetic nephropathy is the most prominent feature of diabetic complications.

Commented [HN10]: What the definition of this term?

Based on the findings of this study, it is hypothesised that the induction of diabetes in rats using alloxan, resulting in sustained hyperglycemia due to pancreatic beta cell destruction and the subsequent non-enzymatic glycosylation of hemoglobin, could have been responsible for the stimulation, recruitment, and release of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) thus potentiating kidney damage. IL-1β, IL-6 and TNF-α have been shown to activate the NF-B pathway, thereby mediating the phosphorylation of serine molecules in the insulin receptor substrate [33]. Insulin resistance is thought to be exacerbated by phosphorylation of the insulin receptor substrate [34]. However, avocado peel polyphenolic extract significantly reduced these effects, particularly at 200 mg/kg body weight, probably via its inhibitory effects on carbohydrate hydrolyzing enzymes [19] as well as its antioxidant activity in preventing the oxidation of glucose and the generation of reactive glycated proteins.

Commented [HN11]: What the abbreviation of this term?

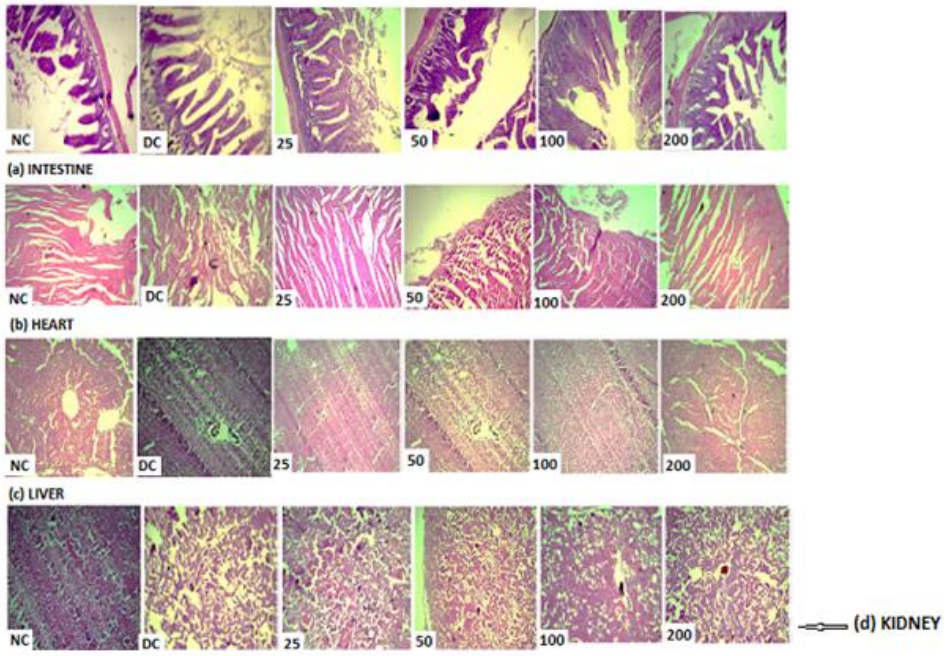

Figure 5: Haematoxylin and eosin stained photomicrographs of the (a) intestine, (b) heart, (c) liver, and (d) kidney. Note: NC, normal control, non-diabetic rats administered distilled water; DC, diabetic control, alloxan-induced diabetic rats administered distilled water; 25, 50, 100, and 200, alloxan-induced diabetic rats administered 25, 50, 100, and 200 mg/kg body weight, respectively avocado pear peel polyphenolic extract. Magnification X400.

4. CONCLUSION

This study found that a polyphenol-rich extract of *Persea americana* peel reduced the sustained hyperglycemia caused by alloxan administration in rats and its attendant oxidative stress-induced-low-grade inflammation by decreasing serum levels of glycated hemoglobin and pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α and increasing serum concentrations of serum IL-10, an anti-inflammatory cytokine. The restoration in renal cellular architecture observed in diabetic rats treated with the extract provided further justification for this claim.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

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