Bioactive Peptides from African Yam (AVIAIMF and GPADPF) and Taro (NGDF and NGNW) Reveal Multifunctional Antidiabetic Effects Using Biochemical and Cellular Models

Mohammed Auwal Ibrahim [1,*], June Cheptoo Serem[2], Aliyu Dantani Abdullahi[1], Suleiman Aminu[1], Abubakar Babando Aliyu[3], Aliyu Muhammad Musa[4]; Bashir Musa[5], Megan J. Bester[2], Anabella R. M. Gaspar[6]

[1] Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

[2] Department of Anatomy, University of Pretoria, Pretoria 0002, South Africa

[3] Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

[4] Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria

[5] Center for Dryland Agriculture, Bayero University, Kano, Nigeria

[6] Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria 0002, South Africa

*Correspondence to Mohammed Auwal Ibrahim. Email: mauwalibrahim@gmail.com; maibrahim@abu.edu.ng

Abstract

The multifactorial nature of type 2 diabetes mellitus (T2DM) has driven a need to discover multifunctional antidiabetic peptides preferably from functional food sources for possible application as antidiabetic supplements. Herein, the antidiabetic effects of bioactive peptides previously identified in yam (AVIAIMF & GPADPF) and taro (NGDF & NGNW) were investigated. The peptides showed significantly ($p < 0.05$) higher dipeptidyl peptidase IV inhibitory activities than vildagliptin with NGDF having the best activity. AVIAIMF, GPADPF, and NGNW significantly inhibited the formation of methylglyoxal-induced advanced glycosylated end products (AGEs) while AVIAIMF and NGNW showed oxygen radical scavenging (ORAC) activities. The peptides also showed significant ($p < 0.05$) nitric oxide (NO) scavenging activities in murine macrophage (RAW 264.7) cells and were not cytotoxic to the RAW 264.7 cells in the presence and absence of lipopolysaccharide. The peptides did not show a biologically significant inhibition of lipid formation in 3T3-LI adipocytes and were not cytotoxic to human colon adenocarcinoma (Caco-2) cells, suggesting safety. The ORAC negatively correlated (− 0.40) with % AGEs formed and positively correlated (0.53 and 0.41) with the viability of LPS + and LPS- RAW 264.7 cells respectively. AVIAIMF, GPADPF and NGNW have shown promising multifunctional anti-T2DM activities that could be considered as potential antidiabetic peptides for application in functional antidiabetic foods.

Keywords: African tuber; Dioscorin ; DPP-IV inhibition; Type 2 diabetes Mellitus; Antidiabetic peptide; Tarin

Introduction

The incidence of type 2 diabetes mellitus (T2DM) is increasing worldwide with a devastating socioeconomic effect especially in the developing countries (Cho et al. 2018; Guariguata et al. 2014). The hallmark of T2DM is defective insulin action which leads to hyperglycemia and the impairment of metabolic processes (Brownlee 2001). The pathogenesis of T2DM is associated with increased blood glucose levels and consequently inhibitors of digestive enzymes (α-amylase and α-glucosidase) are the first line of treatment (Bashary et al. 2020; van de Laar et al. 2005). A further enzymatic target is dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) that rapidly hydrolyses incretin hormones thereby modulating glucose homeostasis by reducing glucose dependent insulin secretion (Lacroix and Li-Chan 2016; Mulvihill 2018). Consequently, this leads to a reduction in the blood glucose level of diabetic patients leading to antidiabetic effects. DPP IV is a 110 kDa plasma membrane glycoprotein that belongs to a family of prolyl oligopeptidases with a preference for cleaving dipeptides, especially those containing alanine or proline at position 2 of the parent oligopeptides (Omar and Ahrén 2014; Zhong et al. 2015). The pharmaceutical industry is currently investing in the exploration of promising DPP IV inhibitors in a bid to provide access to efficient antidiabetic drugs (Ahrén 2019).

A characteristic feature of T2DM is increased oxidative stress that causes significant damage to tissues and organs due to the accumulation of methylglyoxal (MGO) and formation of advanced glycation end-products (AGEs) (Desai et al. 2010). AGEs bind to a class of receptors (RAGE) to exacerbate T2DM through promotion of oxidative stress and inflammation via NADPH oxidase to cause reactive oxygen species (ROS) generation (Nakamura and Kawaharada 2022; Chang et al. 2008). In addition, associated with T2DM is endothelial dysfunction due to metabolic alterations and hyperglycemia which in turn adversely impacts nitric oxide (NO) production (Avogaro et al. 2011). Elevated levels of NO further contributes to cellular damage, oxidative stress and macrovascular complications by forming highly reactive peroxynitrite radicals that reacts with cellular proteins (Pacher et al. 2007; Guo et al. 2020) established a probable link between NO-mediated inflammation in T2DM to peroxisome proliferator activated receptor gamma/endothelial NO synthase (PPARγ/eNOS) cascades. Glucose and lipid homeostasis is disrupted in adipocytes with dysfunctional differentiation resulting in lipodystrophy (Arimochi et al. 2016). In addition, glucose transporter 4 (GLUT 4) and PPARγ are highly expressed in adipocytes due to hyperglycemia which increases the chances of obesity and metabolic syndrome associated with T2DM (Hasan et al. 2017). The multifactorial nature of this disease highlights the need for a multitargeted approach that includes the inhibition of specific enzymes (in)directly involved in carbohydrate metabolism, targeting oxidative stress markers as well as mitigating the lipid-associated complications (Nong and Hsu 2021).

Bioactive peptides from food sources have garnered much interest due to their multifunctionality and potential simultaneous targeting of several pathological mechanisms, in addition to low side effects and toxicity, efficiency and tissue specificity (Nong and Hsu 2021; Lammi et al. 2019). The peptide based therapies are increasingly being developed due to advancements in screening, computational biology, biotechnology and genetic engineering especially in areas of peptide production, formulation and delivery of peptide-based drugs (Antony and Vijayan 2021).

Bioactive peptides (2–20 amino acid residues) from different food sources including dairy, meat, and plants have been reported to possess antidiabetic, antioxidant, anti-inflammatory, antithrombotic and anticancer activities (Antony and Vijayan 2021; Ibrahim et al. 2019a, 2020a). Previously, we used network and computational peptidomics to explore tuber storage proteins from potato (patatins), sweet potato (sporamins), yam (dioscorins) and taro (tarin) for potential bioactive peptides (Ibrahim et al. 2019b). Overall, 387 peptide fragments were released from *in silico* mimicry of gastrointestinal digestion using a combination of pepsin, chymotrypsin and trypsin where bioactive peptides with pronounced biological activities such as anticancer, antimicrobial, antidiabetic and antioxidant were identified (Ibrahim et al. 2019b). However, for some of these peptides, the biological activity could not be predicted but the PeptideRanker bioactivity score with a range of 0.7–0.93 indicates a highly probable and novel bioactivity for these peptide fragments. The dearth of information about these peptides in relation to their bioactivities in relevant biological databases also alludes to probable novelty. Therefore, four of these peptide fragments GPADPF (dioscorin A), AVIAIMF (dioscorin B), NGDF (tarin) and NGNW (tarin) were explored in this study to investigate their ability to inhibit DPP-IV, modulate oxidative stress and inflammation in addition to attenuating effects on lipid formation in the 3T3-L1 adipocyte cell model.

Materials and Methods

Chemicals and Reagents

The peptides GPADPF, AVIAIMF, NGDF and NGNW were obtained from DGpeptides Co., Ltd., (Hangzhou, China). The purity, amino acid analysis and molecular mass of these peptides were determined by the manufacturer through mass spectrometry and high performance liquid chromatography. Kidney DPP-IV was obtained from EMD Millipore, Germany. Dexamethasone, Dulbecco's modified Eagle's medium (DMEM), Oil Red O (ORO), rosiglitazone, methylglyoxal (MGO), bovine serum albumin (BSA), isobutylmethylxanthine (IBMX), antibiotic solution (10,000 units penicillin, 10 mg streptomycin, 25 µg amphotericin B per mL), reduced glutathione (GSH), fluorescein, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and Gly-Pro-p-nitroanilide were purchased from Sigma-Aldrich Chemical Company. Crystal violet (CV) N-(1-naphthyl) ethylenediaminedihydrochloride (NED) and dimethylsulfoxide (DMSO) were procured from Merck (Johannesburg, South Africa) while fetal calf serum (FCS) was obtained from Capricorn Scientific (GmBH, Germany) supplied via BIOCOM, South Africa.

DPP-IV Inhibitory Activity

The DPP-IV inhibitory activity was determined according to the protocol described by Konrad et al. (2014). The peptides, at final concentrations of 62.5–500 µM, were incubated with 0.02 U/mL DPP-IV (in 100 mM Tris-HCl buffer (pH 8.0)) at 37 °C for 1 h. A volume of 25 µL of a 6 mM Gly-Pro-p-nitroanilide solution was then added and the reaction mixture was kept for 60 min at 37 °C after which the absorbance was measured at 405 nm using Multiskan microplate spectrophotometer (ThermoFisher Scientific, Norway). The positive control was vildagliptin and DPP-IV inhibitory activity was calculated using the formula:

$$DPP-IV\ inhibitory\ activity\ (\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100 \quad (1)$$

where A_s and A_c represents the absorbance of the sample and control respectively. Peptide concentrations corresponding to 50% enzyme activity inhibition (IC_{50}) were determined using plots of % DPP-IV inhibition against logarithm of peptide concentrations. The vildagliptin was selected as a positive control because it is an excellent competitive inhibitor of DPP-IV with profound glucose lowering effect.

Interaction of Methylglyoxal and Peptides to Generate Advanced Glycosylated end Products Formation

The method described by Siddiqui et al. (2016) with some modifications was used to measure the ability of the peptides to interact with MGO and reduce the formation of AGE. A volume of 50 µL of peptides (62.5–500 µM, final concentration) was transferred into a 96 well fluorescence plate, followed by 50 µL of MGO (56 mM). The plate was incubated for 1 week under 5% CO_2 at 37 °C. Glutathione at a concentration range of 62.5–500 µM was used. The positive control contained 50 µL BSA (40 mg/mL, final concentration of 20 mg/mL) and MGO (56 mM, final concentration of 28 mM) and the experimental blank was the peptides-buffer solution. Florescence was measured at an excitation and emission wavelengths of 330 and 420 nm, respectively using a FLUOstar OPTIMA plate reader (BMGLab technologies, Offenburg, Germany). The %AGEs formed was expressed as percentage of florescence in the presence of the peptides and MGO relative to the positive control set up MGO (100% AGEs formation) as shown below:

$$\backslash\%\ \text{AGEs formed} = \left(1 - \frac{F_c}{F_s}\right) \times 100 \quad (2)$$

where F_s and F_c represents florescence intensity of sample and control respectively.

Oxygen Radical Absorbance Capacity (ORAC) Antioxidant Assay

For the determination of antioxidant activity with the ORAC assay according to the method of Ou et al. (2002), 165 µL of 0.139 nM fluorescein was added to 10 µL of the peptide samples (125–1000 µM) and GSH (peptide positive control). Then, 25 µL of AAPH (0.24 mM) was added followed by immediate measurement of the fluorescence every 1 min for 120 min at excitation and emission wavelengths of 485 and 520 nm to generate 120 cycles using a FLUOstar OPTIMA plate reader (BMG Labtechnologies, Offenburg, Germany). Fluorescein-AAPH solution was used as a positive control while vehicle control was fluorescein-ddH_2O solution. Trolox was used in a concentration range of 0–50 µM. The area under the curve was determined, expressed as µM TE and % ORAC was calculated.

Cellular Anti-Inflammatory (RAW 264.7 Cells + LPS) Activity

The LPS stimulated RAW 264.7 macrophages were used as an in vitro model of inflammation. RAW 264.7 cells were purchased from CELLONEX Separations, RSA at passage 10 and used between passages 20–33. A 70 µL aliquot of RAW 264.7 cells were plated at cell density of 1.25×10^6 cells/mL and immediately treated with 10 µL of 1 µg/mL *E. coli* LPS and 10 µL of each peptide was added, yielding final concentrations of 1×10^6 cells, 0.1 µg/mL LPS and 12.5–100 µM peptides. The cells were then incubated for 24 h at 37 °C, 5% CO_2. Thereafter, 50 µL

of the supernatant was collected and 50 µL of the Griess reagent (0.1% sulfanilamide with 0.01% N-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid) added to determine NO levels. To ensure that the changes in NO production were not due to cell death, the % cell number was determined with the CV assay and was reported as % relative to the control, no peptide added.

Cell Cultures and Differentiation of 3T3-L1 Cells

The 3T3-L1 cells were purchased from CELLONEX Separations, RSA at 39th passage while passages 45–47 were used for these experiments. The media, DMEM was supplemented with 10% (v/v) FCS and 1% antibiotics (DMEM/FCS) and cells were cultured at 37 °C under 5% CO_2. Differentiation was induced by seeding pre-adipocyte cells at a density of 1×10^3 per 100 µL in a 96 well plate followed by culturing for 3 days until confluent. On the fourth day, the media was changed to DMEM/FCS supplemented with 10 µg/mL insulin, 50 µM dexamethasone, 25 mM IBMX and 100 µM rosiglitazone. It was used twice over 6 days. On the tenth day, the media was replaced with DMEM/FCS for a further 3 days. On Day 14, the medium was changed to DMEM/FCS and with phase contrast microscopy, the presence of distinct lipid droplets confirmed differentiation.

Effects of the Peptides on Lipid Accumulationin 3T3-L1 Differentiated Adipocytes

Two different experimental approaches were used to assess the effects of the peptides on lipid formation in differentiated 3T3-L1 adipocytes. To evaluate the ability of the peptides to inhibit lipid droplet formation, 100 µM peptide (final concentration) was added to the differentiation media at days 4, 7 and 10. Secondly, to evaluate the effect on formed lipid droplets, 100 µM peptide (final concentration) was added to fully differentiated 3T3-L1 adipocytes for 24 h.

Oil Red O Staining Assay

Lipid droplet accumulation was determined with ORO staining where the adipocytes were fixed with 2% formaldehyde, stained with 100 µL ORO (ORO – 0.5% in 60% isopropanol, then further diluted 1.7X and filtered) and then extracted with 60% isopropanol. The absorbance was determined at 405 nm using a BioTek plate reader. The percentage (%) lipid accumulation was calculated relative to the unexposed differentiated 3T3-L1 adipocyte cells.

Effects on Cell Number and Crystal Violet Assay

The human colon adenocarcinoma (Caco-2) cell line (American Type Culture Collection, ATCC) was used between passages 9–16. The Caco-2 cells were exposed to 12.5–100 µM peptide for 30 min. Thereafter, both the RAW 264.7 macrophages and Caco-2 cells were fixed for 30 min with 2% formaldehyde, followed by staining with CV for a further 30 min. The plate was then washed, dried before the dye was extracted with 100 µL of a 10% acetic acid solution. The absorbance was measured at 570 nm and the cell number was reported as a percentage relative to the control, no peptide added.

Statistical Analysis

Python 3.6 was used with pandas, numpy, matplotlib.pyplot, seaborn and scikit-learn packages to analyze data relationships by way of heatmaps. Data are expressed as mean ± SEM of three independent experiments performed in triplicate The data were analyzed using Tukey's-HSD multiple range post-hoc test in SPSS statistical software package on Windows, version 18, IBM Corporation, NY, USA. Values were considered significantly different at $p < 0.05$.

Results

The in vitro capacity of peptides to inhibit DPP-IV was investigated (Table 1). All peptides showed significantly ($p < 0.05$) lower IC_{50} values compared to vildagliptin; a known DDP-IV inhibitor. No significant differences were found between peptides, although NGDF had the best activity (96.51 ± 11.94 µM). The IC_{50} values were folds better than vildagliptin which suggested that AVIAIMF, GPADPF, NGDF and NGNW were better inhibitors of DPP-IV compared to the standard drug (Table 1).

Table 1. Median inhibitory concentrations of AVIAIMF, GPADPF, NGDF and NGNW for the inhibition of DPP-IV activity

Peptides	Molecular weight (Da)	Net charge	Hydro-phobicity (%)	IC_{50} (µM)
AVIAIMF	763.98	0	100	139 ± 23.12^a
GPADPF	602.63	−1	66.67	246.43 ± 78.22^a
NGDF	451.43	−1	25	96.51 ± 11.94^a
NGNW	489.48	0	25	178.21 ± 96.57^a
Vildagliptin	–	–	–	> 1000

Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using One-way ANOVA followed by (Tukey's multiple range post-hoc test). The superscript (a) between the peptides indicates there was no statistical difference in the values at $p < 0.05$

The ability of the peptides to interact with MGO and reduce AGE formation was evaluated (Fig. 1). With the exception of NGDF, all the peptides significantly interacted with MGO ($p < 0.05$) and reduced the generation of AGE. For the concentration range of 62.5–500 µM, the inhibitory effect of AVIAIMF was similar to GSH. The peptide NGDF, showed the least inhibition, and presented with a dosage dependent loss of inhibitory activity ($p < 0.05$, $R^2 = 0.981$), and increased AGE formation > 100% at 250 and 500 µM (Fig. 1).

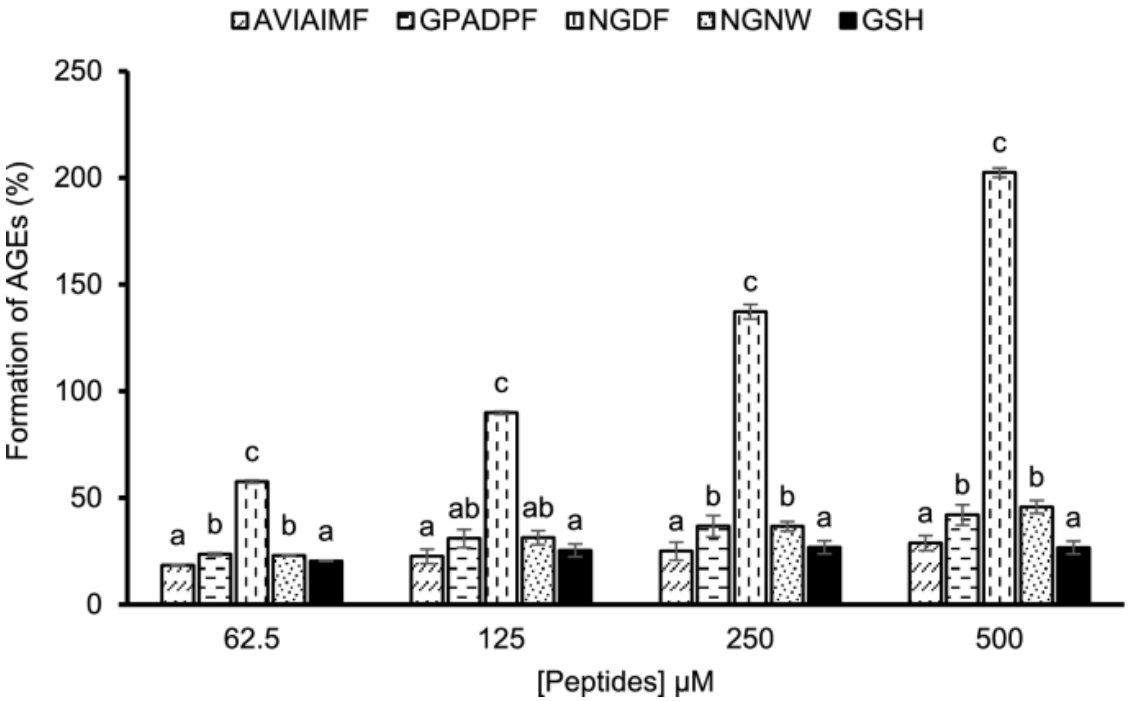

Fig. 1. Reactivity of AVIAIMF, GPADPF, NGDF and NGNW with MGO to produce AGEs. Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using One-way ANOVA followed by (Tukey's multiple range post-hoc test). Different alphabets (a, b, c and d) imply statistical significance at $p < 0.05$ vs positive control set up (100% AGEs formed)

After establishing the interaction of peptides with MGO along with its consequence on AGE formation, the ability of the peptides to quench AAPH generated radicals was determined using the ORAC assay (Table 2). GSH showed a dosage dependent increase in antioxidant activity. GPADPF and NGDF showed no antioxidant activities at all concentrations while AVIAIMF and NGNW showed a dosage dependent increase in oxygen radical scavenging (Table 2). The antioxidant activity of AVIAIMF was similar to GSH and for NGNW was significantly ($p < 0.05$) better, with a 2.74 times greater activity than GSH.

Table 2. Evaluation of the oxygen radical scavenging activities of AVIAIMF, GPADPF, NGDF and NGNW

[Peptides] (µM)	[ORAC] µMTE			
	125	250	500	1000
AVIAIMF	59.35 ± 13.84^{b}	113.98 ± 20.13^{b}	264.88 ± 54.94^{c}	372.67 ± 62.69^{b}
GPADPF	-23.58 ± 16.00^{a}	-27.84 ± 15.21^{a}	-37.99 ± 8.77^{b}	-32.10 ± 13.57^{a}
NGDF	-22.53 ± 7.99^{a}	-18.58 ± 7.42^{a}	-17.36 ± 6.49^{a}	-21.94 ± 6.72^{a}
NGNW	360.78 ± 44.62^{d}	500.63 ± 64.29^{d}	678.25 ± 90.74^{d}	748.14 ± 116.31^{c}
GSH	97.30 ± 16.79^{c}	171.03 ± 25.35^{c}	261.90 ± 37.82^{c}	363.19 ± 49.83^{b}

Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using One-way ANOVA followed by (Tukey's multiple range post-hoc test). Value with different superscripts (a, b, and c) within peptide concentrations are considered statistically significant at $p < 0.05$

The effects of the peptides on NO production were studied in LPS-induced NO producing murine macrophages (RAW 264.7) (Fig. 2). Depending on the concentration, the peptides inhibited NO generation by the cells to varying degrees. At 12.5 µM, all peptides inhibited NO production not significantly ($p > 0.05$) different from GSH except for GPADPF that showed significantly ($p < 0.05$) higher inhibitory activity. At higher concentrations of 25–100 µM, AVIAIMF, GPADPF and NGDF but not NGNW showed a significantly ($p < 0.05$) superior inhibition of NO production compared with GSH (Fig. 2).

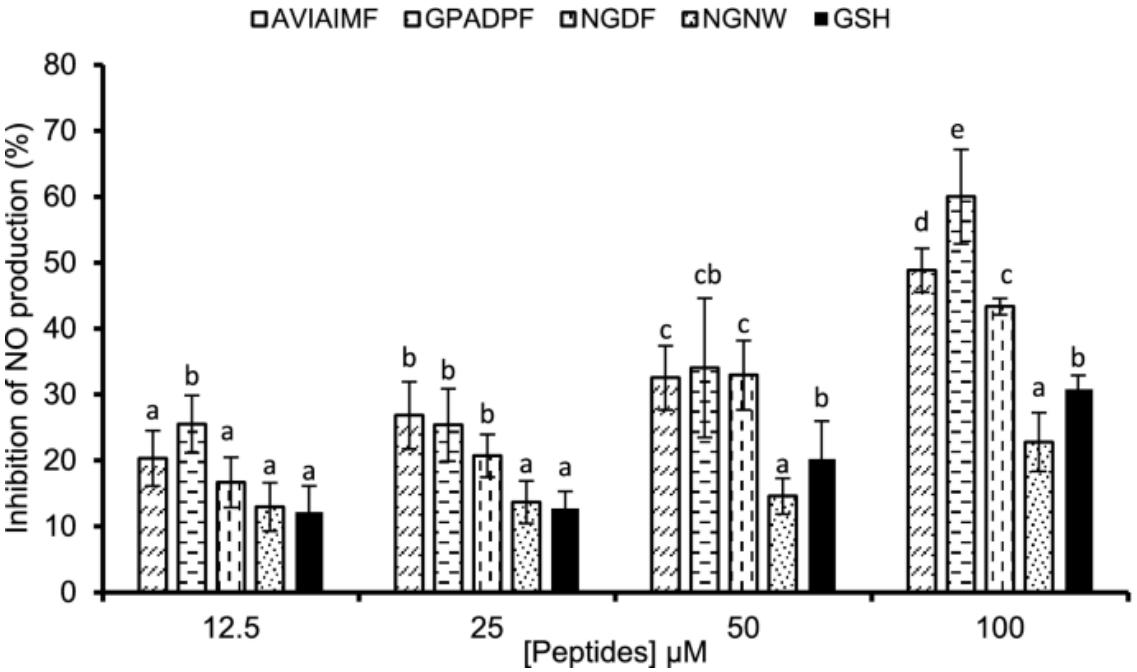

Fig. 2. Inhibition of nitric oxide (NO) production in LPS-induced murine macrophage (RAW 264.7) cells at different concentrations of AVIAIMF, GPADPF, NGDF and NGNW. Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using One-way ANOVA followed by (Tukey's multiple range post-hoc test). Different alphabets (a, b, c, d and e) imply significant difference at $p < 0.05$ expressed as percentage of positive control containing all reagents except the peptides

The effects of peptides on cell number of NO producing murine macrophages (RAW 264.7) in the presence and absence of LPS (Fig. 3) was determined. The cell number was significantly reduced compared with GSH for all concentrations although greater than 80%. For RAW 264.7 cells exposed to the peptides in the absence of LPS, the cell number was 89–109% (Fig. 3). This indicates that the peptides effectively inhibited LPS induced NO formation.

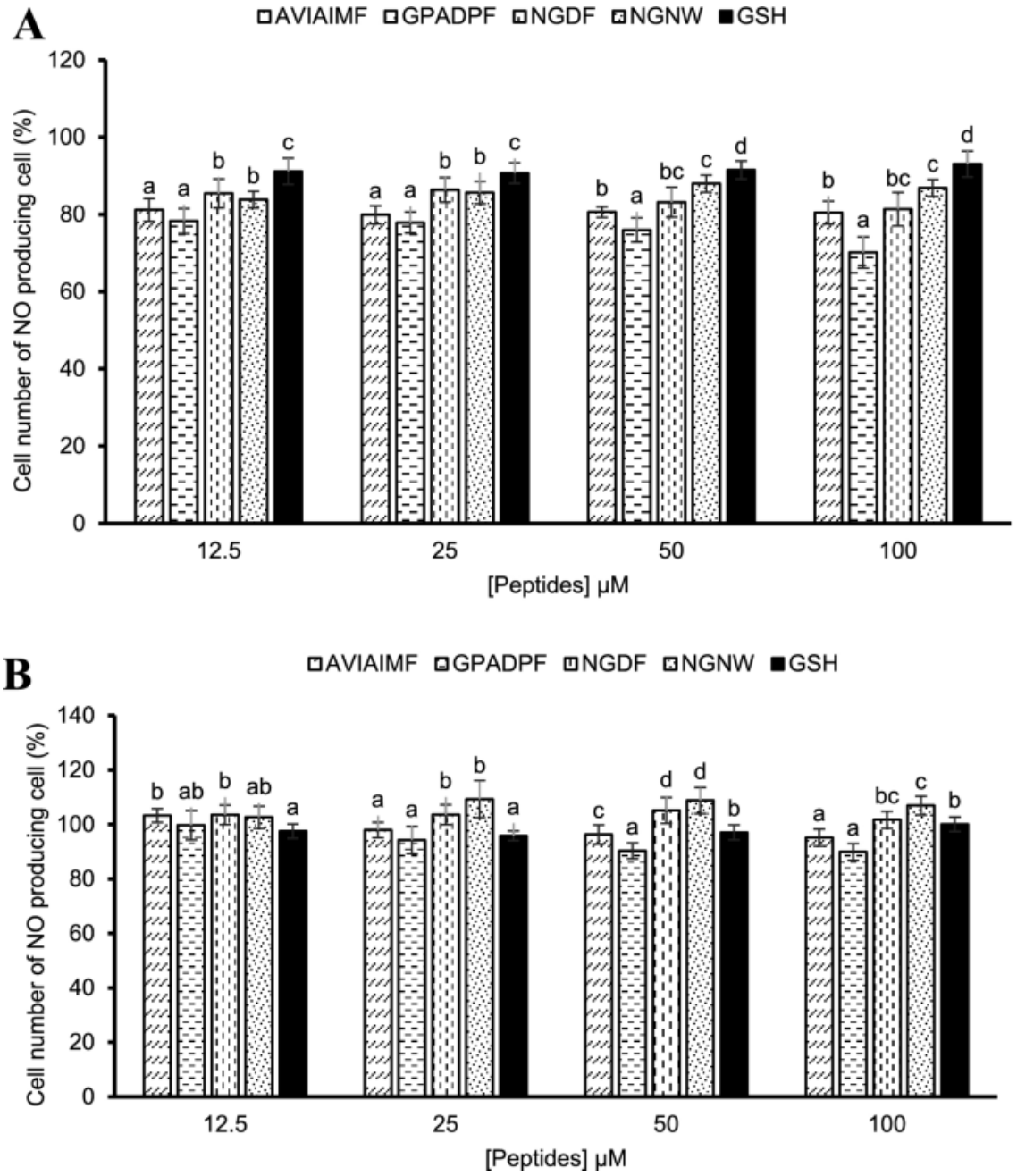

Fig. 3. Cellular number of RAW 264.7 nitric oxide (NO) producing cells in the presence (**A**) and absence (**B**) of LPS exposed to different concentrations of AVIAIMF, GPADPF, NGDF and NGNW. Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using One-way ANOVA followed by (Tukey's multiple range post-hoc test). Different alphabets (a, b, c, and d) imply significant difference at $p < 0.05$ expressed as percentage of vehicle control

The ability of peptides to inhibit lipid accumulation in 3T3-L1 cells was also studied (Fig. 4). Two experimental strategies were used which included exposing the cells to the peptides during, and after the cellular differentiation to simulate the potential prevention and treatment of lipid accumulation, respectively. None of the peptides was able to effectively

reduce lipid accumulation in differentiated 3T3-L1 adipocytes or cells undergoing differentiation. At 100 µM, the degree of lipid formation in AVIAIMF treated cells was significantly ($p < 0.05$) lower in fully differentiated cells compared to differentiating 3T3-L1 cells albeit this decrease was not biologically significant. For GPADPF, NGDF and NGNW, no differences were observed between the effect of the peptides on lipid accumulation due to differentiation or accumulated lipid. Overall, the peptides did not prevent lipid accumulation in 3T3-L1 differentiated adipocytes (Fig. 5).

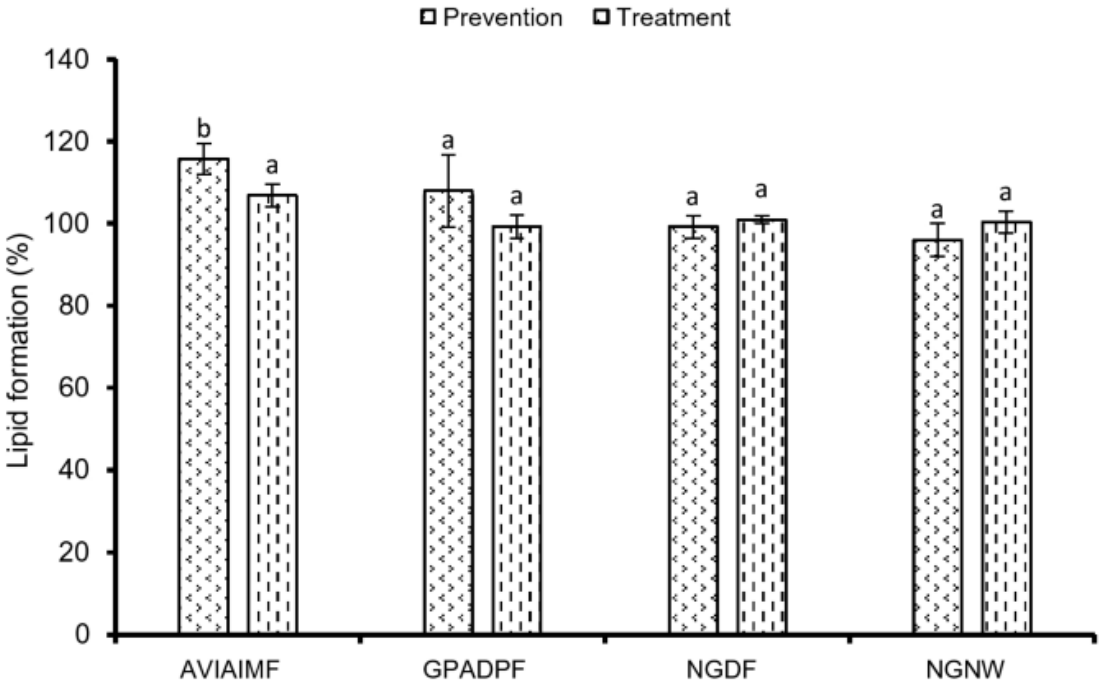

Fig. 4. Percentage of lipids accumulated in Oil Red O stained 3T3-L1 differentiated adipocytes exposed to AVIAIMF, GPADPF, NGDF and NGNW (100 µM). Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using Paired sample t-test. Different alphabets (a and b) imply significant difference at $p < 0.05$ expressed as a percentage of vehicle control

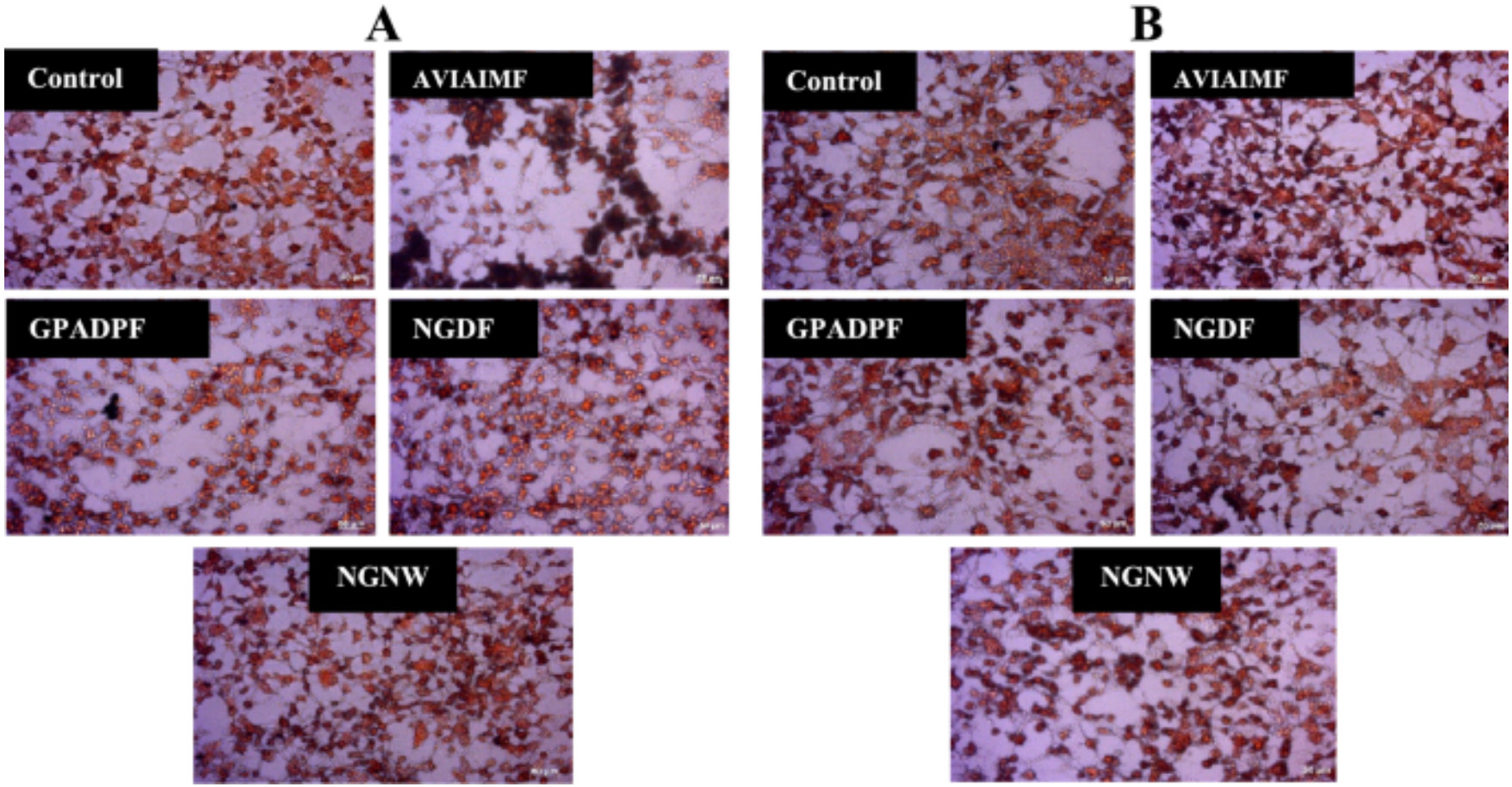

Fig. 5. Representative micrographs for lipid accumulation based on Oil Red O staining of 3T3-L1 adipocytes in the presence of 100 µM AVIAIMF, GPADPF, NDGF and NGNW during (**A**) and after (**B**) the differentiation process. The cells were photographed at magnification x20

To further evaluate their cytotoxicity, the effect of peptides on cell number was investigated using the Caco-2 cell line. The peptides, AVIAIMF, GPADPF, NGDF and NGNW showed no cytotoxicity at all tested concentrations and were not significantly ($p > 0.05$) different from the vehicle control (Fig. 6).

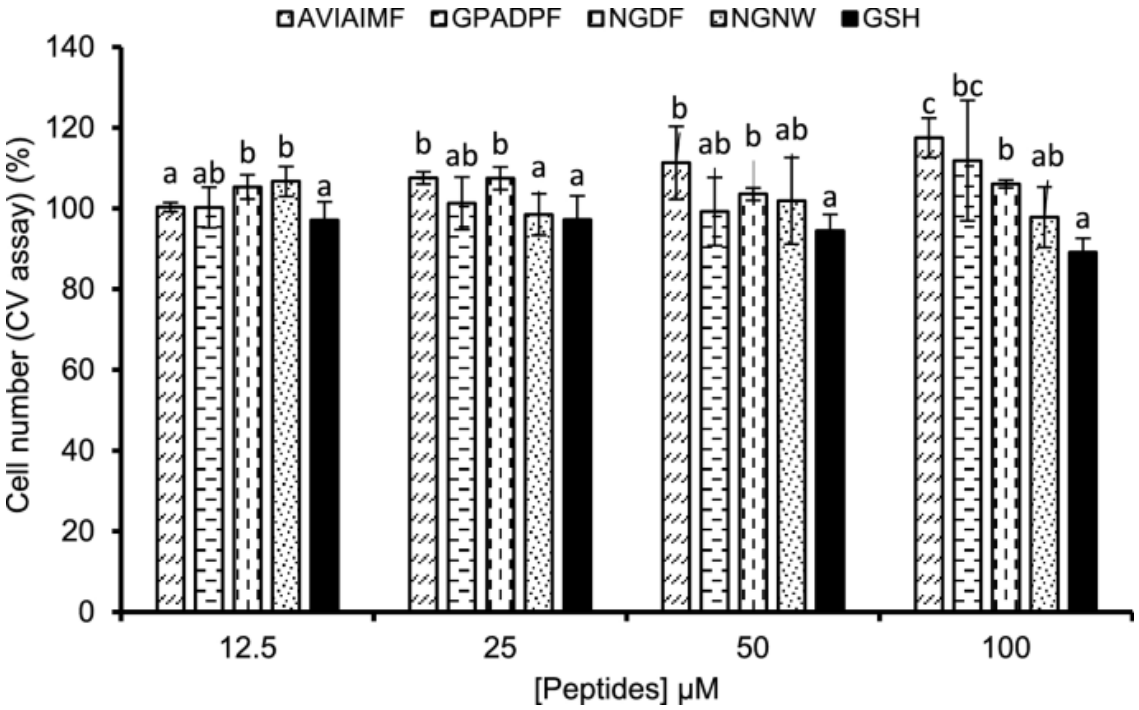

Fig. 6. Cytotoxicity profiles of different concentrations of AVIAIMF, GPADPF, NGDF and NGNW (12.5–100 µM) exposed Caco-2 cells. Data are expressed as mean ± SEM of three independent experiments performed in triplicates and were analyzed using Paired sample t-test. Different alphabets (a, b and c) imply significant difference at $p < 0.05$ expressed as a percentage of vehicle control

Heatmaps were used to determine patterns and relationships in the datasets of different assays to enable the precise prediction of multifunctionality. The % AGEs negatively correlated with oxygen radical scavenging activities (− 0.40), while RAW 264.7 cells (LPS + and LPS-) cell number correlated positively with oxygen radical scavenging (scores: 0.53 and 0.41). A positive correlation was established in the same principal component space (PC 3) for oxygen radical scavenging activities and NO scavenging activities respectively (scores: 0.66, 0.19) respectively while the latter also positively correlated (0.53) with Caco-2 cell viability (Fig. 7A). AVIAIMF, GPADPF and NGNW shared close proximities along PC 1 (48.05%) and PC 2 (21.24%) suggesting some shared multifunctionality (Fig. 7D).

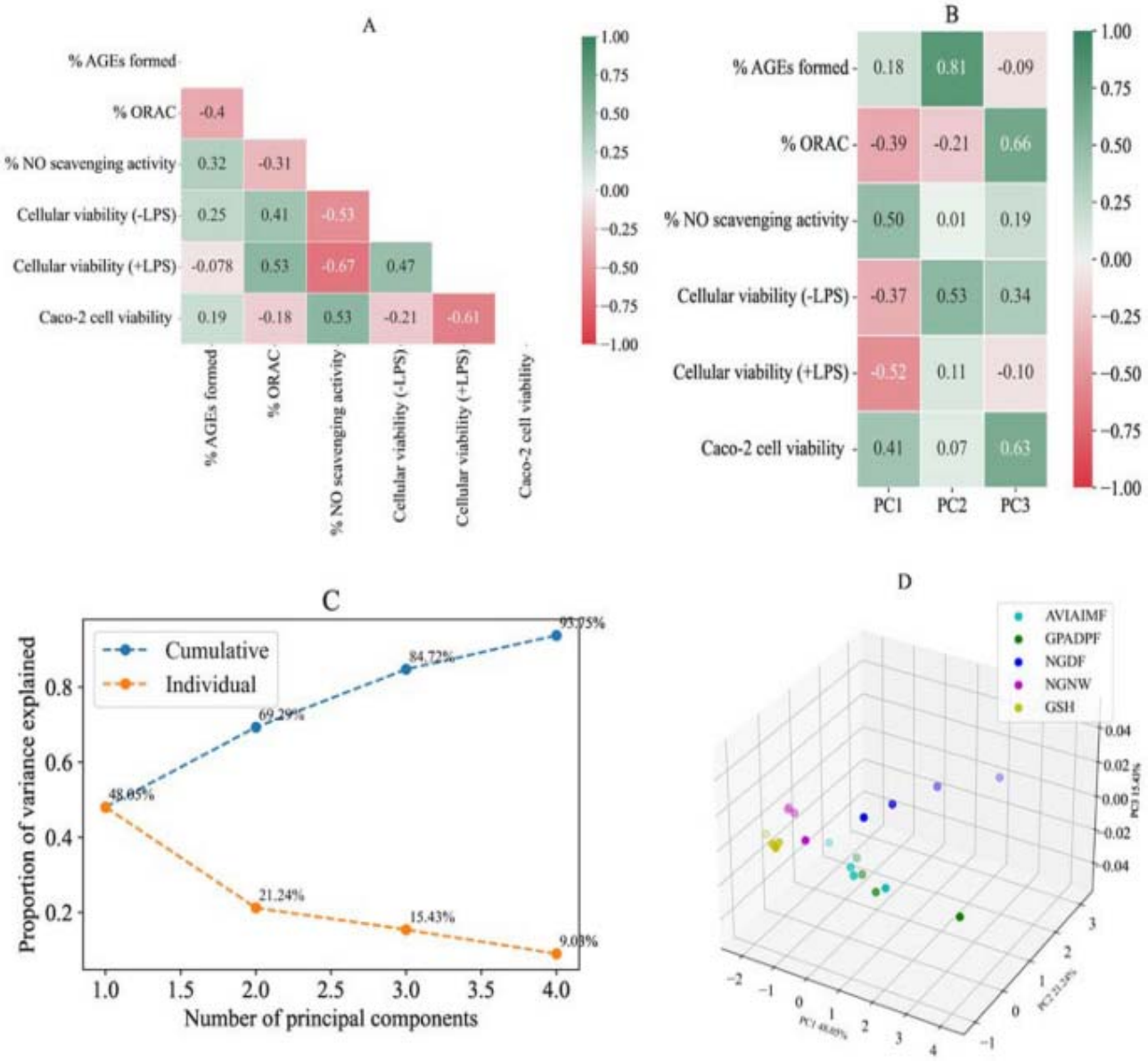

Fig. 7. (**A**) Heatmap generated for assays at tested peptide concentrations used to establish patterns and relationships in the dataset. (**B**) Z-normalized correlation matrix between the dataset and principal component (PC) space. (**C**) **A** scree plot of an interactive visualization for principal components analysis. Explained variance ratio showing the contribution of individual and cumulative variances to the principal component spaces. (**D**).PCA of the dataset and principal component (PC) space explained in terms of peptide contribution to biological activity. Principal components (PC) 1, 2 and 3 explains 48.05%, 21.24% and 15.43% of the variance in the data respectively (color)

Discussion

Exploring primary metabolites such as bioactive peptides as opposed to secondary metabolites is a prudent approach recently adopted to treat T2DM, (Patil et al. 2020), especially from the perspective of a functional food or production of antidiabetic supplements. This paradigm shift has been prioritized mainly due to the unique

multifunctional nature of bioactive peptides which offers a probable efficient treatment regimen and allows for T2DM to be combatted sustainably on the principle of maximum economy. In the present study, AVIAIMF, GPADP, NGDF, and NGNW from storage proteins of yam (dioscorins) and taro (tarin) were identified to be potential antidiabetic agents that could normalize glucose homeostasis and inhibit AGE formation, NO mediated inflammation without profound effect on lipid accumulation in 3T3-L1 cells.

DPP-IV inhibition has been a widely regarded treatment route for T2DM as it affords glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (incretins) the ability to stimulate insulin release, inhibit glucagon synthesis as well as slow gastric emptying (Han et al. 2019; Kazafeos 2011). Using a colorimetric method, the IC_{50} values of the peptides AVIAIMF, GPADPF, NGDF, and NGNW were significantly ($p < 0.05$) lower (96.51–246.43 μM) compared to vildagliptin (> 1000 μM) although some research findings using flourimetric assays have reported the IC_{50} of vildagliptin (a substrate and competitive inhibitor of DPP-IV) in nanomolar ranges (Gupta et al. 2018; Yang et al. 2020). Such a discrepancy in IC_{50} value compared to literature could be explained in light of the assay-type, assay sensitivity and/or assay-specificity (Kalliokoski et al. 2013) as fluorimetric assays are generally more sensitive and specific than the colorimetric assays. Nonetheless, all peptides had IC_{50} values in the micromolar range with NGDF having the lowest value.

The presence of proline in position 2 from N or C terminals of bioactive peptides have been implicated in DPP-IV inhibitory activities (Lacroix and Li-Chan 2012, 2016; Liu et al. 2019). This might be the case for GPADPF considering proline residues flank alanine and aspartic acid (AD) thus occupying penultimate positions from both terminals of this peptide. Interestingly, this hexapeptide (GPADPF) could be released in an intact form after digestion with gastrointestinal enzymes because the peptide bonds might not be preferably cleaved by the digestive enzymes due to specificity and nature of the peptide and hence, might elicit the observed DPP-IV inhibitory activities. The size of a peptide, amino acid composition and hydrophobicity are relevant for the efficient inhibition of DPP-IV (Sun et al. 2020; Wang and Li 2018). Inhibition by AVIAIMF might be attributed to its hydrophobic (A, M and F) and branched chain amino acids (V and I) and associated sequence compositions (Nongonierma and FitzGerald 2019; Arulmozhiraja et al. 2016). Meanwhile, the presence of glycine at the penultimate N-terminal end of NGDF and NGNW could be suggested to confer these peptides more flexibility when interacting with DPP-IV. These structural characteristics may account for NGDF having the most promising potential to inhibit the DPP-IV and potentially normalize glucose homeostasis in T2DM.

Reactive oxygen species (ROS) and methylglyoxal (MGO) are major players in the pathogenesis of T2DM (Inzucchi et al. 2015). Accumulation of MGO precipitates reactions between MGO and arginine or lysine residues of plasma proteins with the generation of AGEs (Tupe et al. 2020). AGEs act through a cascade of reactions and signaling to increase oxidative stress as well as induce inflammation in T2DM patients. Therefore, the discovery of bioactive peptides that prevent AGE formation spares plasma proteins and free amines from reacting with MGO. Unlike NGDF, the ability of peptides AVIAIMF, GPADPF and NGNW to interact with MGO was comparable to GSH. This is attributed to the presence of aliphatic hydrophobic amino acids such as alanine, valine, and isoleucine in addition to the aromatic amino acids tryptophan and tyrosine. These amino acids are thought to confer antioxidant activity on

peptides by allowing radical quenching through direct transfer of electrons (Chi et al. 2015). In contrast, the binding of NGDF to MGO might have mediated an indirect effect through increased AGEs-RAGE interactions known to exacerbate T2DM through the promotion of oxidative stress and ROS generation. Interestingly, the ability of AVIAIMF to scavenge oxygen radicals was similar to GSH while for NGNW, it was several folds greater than GSH. In contrast, GPADPF and NGDF showed no antioxidant activity evaluated with the ORAC assay. Multifunctional activities of these bioactive peptides may provide cumulative health benefits which could also compensate for a lack or reduced activity for one or two individual targets of T2DM. Indeed, the antioxidant route is a glucose independent approach used to mitigate macrovascular complications associated with T2DM (Moraru et al. 2018). Superoxide radicals are known to react with NO in cells to form peroxynitrite which has been implicated in oxidative damage of proteins. The ability to directly inhibit the release of NO in the LPS-induced RAW 264.7 model was determined. The peptides, AVIAIMF, GPADPF and NGDF but not NGNW effectively inhibited the NO production at high concentrations. The prevention of RNS formation is related to the ability of peptides to scavenge ROS and/or NO together with the MGO scavenging ability. In this study AVIAIMF, was identified as the most promising peptide to inhibit the MGO-AGEs-RAGE-ROS-NO cycle. In addition, the peptides were not cytotoxic when tested against the RAW 264.7 cells in the presence and absence of LPS which indicates to their safety whilst the observed inhibition of NO release not being a secondary result of cytotoxicity-related cell death.

Figure 7 A–D established a significant correlation in the data as well as agreed to the multifunctionality of these peptides. The % AGEs formed decreased minimally with increasing oxygen radical scavenging activity (correlation score = − 0.40; Fig. 6A) which suggest that peptides inhibited AGEs formation by interacting with MGO as well as other oxygen reactive species. Interestingly, the viability of RAW 264.7 cells (in the presence and absence of LPS) seemed to exert a parallel increase with oxygen radical scavenging (scores: 0.53 and 0.41) as measured with the ORAC assay which is a paradox considering the NO scavenging activities did not correlate positively (− 0.67 and − 0.53) with the viability of these cells (Fig. 6A). The basis for this observation is at the moment not very clear. Perhaps, it might result from some sort of artefact due to probable shortcomings in algorithms employed in explaining the data considering the NO scavenging activities of these peptides were quite apparent (Fig. 3). In addition, the principal component 3 (PC 3) space in Fig. 7B showed a positive correlation between oxygen radical scavenging activities and NO scavenging activities respectively (scores: 0.66 and 0.19). Much of the information about AGEs inhibition (0.81) was compartmentalized to PC 2 space as opposed to ORAC and NO scavenging activities embedded in PC 3. Although most of the variance from the data is contained in PC 1 (48.05%; Fig. 7C and D), the cumulative explained variance in the data approached 100% along higher PCs suggesting that most of the information have been holistically captured. The PCA plot (Fig. 7D) also ascertains the intersection in the data (AVIAIMF, GPADPF, NGNW) along PC 1 and PC 2 which perhaps further agreed to the multiple functionality of these peptides.

The 3T3-L1 cell line expresses DPP-IV in pre- and mature adipocytes especially during cellular differentiation and the knockdown of DPP-IV genes have been implicated in adipocyte maturation during early phases of adipocyte differentiation which occurs through the mimicry of growth factor withdrawal (Zilleßen et al. 2016). This suggests an intricate connection between the DPP-IV inhibitory activity of peptides and their potential to inhibit lipid

accumulation. Therefore, inhibition of adipogenesis and lipid accumulation that results from the differentiation of preadipocytes to mature adipose cells has been identified as viable drug targets for treating T2DM (Balusamy et al. 2020; Sim et al. 2019). The peptides, GPADPF, NGDF and NGNW did not show a biologically significant inhibition of lipid accumulation in both differentiating and fully-differentiated 3T3-L1 cell models, while in contrast AVIAIMF promoted lipid accumulation in differentiating 3T3-L1 cells. This indicates that these peptides do not target adipogenesis and/or lipid accumulation (Fig. 7). Depending on the peptide characteristics, inhibition of lipid accumulation has been proposed to be achieved through lipolysis, selective cytotoxicity in adipose cells or via targeting of genes relevant to adipocyte differentiation such as PPARγ, FAS, AP2 and C/EBPα (Ibrahim et al. 2020b; Saito et al. 2007; Satish et al. 2018). The hydrophobicity/hydrophilicity of a peptide and peptide concentration are important factors for inhibiting the formation of lipid droplets as has been reported for a 10 µM DIIADDEPLT as well as 250 µM GEY and GYG respectively (Han et al. 2016; Reckziegel et al. 2017). It was interesting that the peptides in this study have shown a well acceptable inhibition of DPP-IV and radical scavenging activities with a minimal effect on lipid accumulation. The observations could further open up an avenue to consider these bioactive peptides, as multifunctional.

The human colorectal adenocarcinoma (Caco-2) cells have been a model of choice in assays that investigate the intestinal absorption**/**permeability of functional foods and bioactive compounds (Yarnpakdee et al. 2015). Caco2 cells share structural and functional characteristics specific to enterocytes such as tight junctions across their membranes that do not restrict entry of bioactive peptides into the cells (Petsantad et al. 2020). The peptides, AVIAIMF, GPADPF, NGDF and NGNW were not cytotoxic to the cells at all evaluated peptide concentrations suggesting no impairment in cell structure and function. GSH showed some cytotoxicity at 100 µM when compared with the peptides confirming the findings of a previous study, where GSH had some cytotoxic effects in undifferentiated 3T3-L1 cells (Ibrahim et al. 2020b).

Conclusions

Overall, the peptides have shown compensatory multifunctional antidiabetic activity with NGDF having the best ability to inhibit DPP-IV and potentially normalize glucose homeostasis. In addition, NGDF also inhibited NO formation. The peptide, AVIAIMF had the greatest potential to inhibit the MGO–AGEs–RAGE–ROS–NO cycle while NGNW inhibited AGE and ROS but not NO formation. Effects on lipid accumulation and lipid levels post-differentiation were minimal. All peptides were not cytotoxic, and subsequent peptide combination therapies or other alternative strategies to improve the multifunctional antidiabetic efficacy can be undertaken in future studies. Additionally, it would be worthy to further conduct in-depth investigations on the mechanism of their antidiabetic effects.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

The authors appreciate the management of Ahmadu Bello University, Zaria, Nigeria for the sponsorship through TeTFund Institutional Based Grant and for providing the enabling environment while carrying out the studies. We also appreciate the support from the University of Pretoria, South Africa for providing the facilities for the cell culture-based studies.

Funding

The work is funded through Institutional Based Grant by the Tertiary Education Fund (DAPM/TETFUND/01/12) to MAI.

Contributions

Conceptualization, investigation, project administration, funding acquisition, writing—review and editing: MAI; Investigation, data curation and methodology: JCS; Data curation, formal analysis and conceptualization: SA; Writing—original draft, formal analysis, data curation and Software: ADA; Investigation, funding acquisition and validation: ABA; Investigation, resources, funding acquisition: AMM; Methodology and investigation: BM; Conceptualization, writing—review and editing, supervision: MJB; Conceptualization, writing—review and editing, supervision: ARMG

Ethics declarations

Competing interest

There is no competing interest to declare

References

Ahrén B (2019) DPP-4 inhibition and the path to clinical proof. Front Endocrinol 10:376. https://doi.org/10.3389/fendo.2019.00376

Antony P, Vijayan R (2021) Bioactive peptides as potential nutraceuticals for diabetes therapy: a comprehensive review. Int J Mol Sci 22(16):9059. https://doi.org/10.3390/ijms22169059

Arimochi H, Sasaki Y, Kitamura A, Yasutomo K (2016) Differentiation of preadipocytes and mature adipocytes requires PSMB8. Sci Rep 6:26791. https://doi.org/10.1038/srep26791

Arulmozhiraja S, Matsuo N, Ishitsubo E, Okazaki S, Shimano H, Tokiwa H (2016) Comparative binding analysis of dipeptidyl peptidase IV (DPP-4) with antidiabetic drugs an *ab initio* fragment molecular orbital study. PLoS ONE 11(11):e0166275. https://doi.org/10.1371/journal.pone.0166275

Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP (2011) Endothelial dysfunction in diabetes: the role of reparatory mechanisms. Diabetes Care 34(Suppl 2):S285–290. https://doi.org/10.2337/dc11-s239

Balusamy SR, Veerappan K, Ranjan A, Kim Y-J, Chellappan DK, Dua K, Lee J, Perumalsamy H (2020) Phyllanthusemblica fruit extract attenuates lipid metabolism in 3T3-L1 adipocytes via activating apoptosis mediated cell death. Phytomed Int: J Phytotherapy Phytopharmacol. https://doi.org/10.1016/j.phymed.2019.153129

Bashary R, Vyas M, Nayak SK, Suttee A, Verma S, Narang R, Khatik GL (2020) An insight of alpha-amylase inhibitors as a valuable tool in the management of type 2 diabetes Mellitus. Curr Diabetes Rev 16(2):117–136. https://doi.org/10.2174/1573399815666190618093315

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414(6865):813–820. https://doi.org/10.1038/414813a

Chang JS, Wendt T, Qu W, Kong L, Zou YS, Schmidt AM, Yan S-F (2008) Oxygen deprivation triggers upregulation of early growth response-1 by the receptor for advanced glycation end products. Circul Res 102(8):905–913. https://doi.org/10.1161/CIRCRESAHA.107.165308

Chi C-F, Hu F-Y, Wang B, Li Z-R, Luo H-Y (2015) Influence of amino acid compositions and peptide profiles on antioxidant capacities of two protein hydrolysates from Skipjack Tuna (*Katsuwonuspelamis*) dark muscle. Mar Drugs 13(5):2580–2601. https://doi.org/10.3390/md13052580

Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B (2018) IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138:271–281. https://doi.org/10.1016/j.diabres.2018.02.023

Desai KM, Chang T, Wang H, Banigesh A, Dhar A, Liu J, Untereiner A, Wu L (2010) Oxidative stress and aging: is methylglyoxal the hidden enemy?This review is one of a selection of papers published in a special issue on oxidative stress in health and disease. Can J Physiol Pharmacol 88(3):273–284. https://doi.org/10.1139/Y10-001

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014) Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 103(2):137–149. https://doi.org/10.1016/j.diabres.2013.11.002

Guo H, Zhang Q, Yuan H, Zhou L, Li F, Wang SM, Shi G, Wang M (2020) Nitric oxide mediates inflammation in type II diabetes mellitus through the PPARγ/eNOS Signaling pathway. PPAR Res. https://doi.org/10.1155/2020/8889612

Gupta A, Jacobson GA, Burgess JR, Jelinek HF, Nichols DS, Narkowicz CK, Al-Aubaidy HA (2018) Citrus bioflavonoids dipeptidyl peptidase-4 inhibition compared with gliptin antidiabetic medications. Biochem Biophys Res Commun 503(1):21–25. https://doi.org/10.1016/j.bbrc.2018.04.156

Han BK, Lee HJ, Lee H-S, Suh HJ, Park Y (2016) Hypoglycaemic effects of functional tri-peptides from silk in differentiated adipocytes and streptozotocin-induced diabetic mice. J Sci Food Agric 96(1):116–121. https://doi.org/10.1002/jsfa.7067

Han R, Maycock J, Murray BS, Boesch C (2019) Identification of angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory peptides derived from oilseed proteins using two integrated bioinformatic approaches. Food Res Int (Ottawa Ont) 115:283–291. https://doi.org/10.1016/j.foodres.2018.12.015

Hasan MM, Ahmed QU, Soad SZM, Latip J, Taher M, Syafiq TMF, Sarian MN, Alhassan AM, Zakaria ZA (2017) Flavonoids from tetraceraindicaMerr. Induce adipogenesis and exert glucose uptake activities in 3T3-L1 adipocyte cells. BMC Complement Altern Med 17(1):431. https://doi.org/10.1186/s12906-017-1929-3

Ibrahim MA, Serem JC, Bester MJ, Neitz AW, Gaspar ARM (2019a) Multiple antidiabetic effects of three α-glucosidase inhibitory peptides, PFP, YPL and YPG: Dipeptidyl peptidase–IV inhibition, suppression of lipid accumulation in differentiated 3T3-L1 adipocytes and scavenging activity on methylglyoxal. Int J Biol Macromol 122:104–114. https://doi.org/10.1016/j.ijbiomac.2018.10.152

Ibrahim MA, Bester MJ, Neitz AW, Gaspar ARM (2019b) Tuber storage proteins as potential precursors of bioactive peptides: an in silico analysis. Int J Pept Res Ther 25(2):437–446. https://doi.org/10.1007/s10989-018-9688-7

Ibrahim MA, Serem JC, Bester MJ, Gaspar ARM (2020a) The dipeptidyl peptidase IV inhibitory activity and multifunctional antidiabetic properties of SQSPA: structure – activity relationship evaluated with alanine scanning. Int J Biol Macromol 160:1220–1229. https://doi.org/10.1016/j.ijbiomac.2020.05.250

Ibrahim MA, Serem JC, Bester MJ, Neitz AW, Gaspar ARM (2020b) New antidiabetic targets of α-glucosidase inhibitory peptides, SVPA, SEPA, STYV and STY: inhibitory effects on dipeptidyl peptidase-IV and lipid accumulation in 3T3-L1 differentiated adipocytes with scavenging activities against methylglyoxal and reactive oxygen species. Int J Pept Res Ther 26(4):1949–1963. https://doi.org/10.1007/s10989-019-09993-2

Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR (2015) Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American diabetes association and the European association for the study of diabetes. Diabetologia 58(3):429–442. https://doi.org/10.1007/s00125-014-3460-0

Kalliokoski T, Kramer C, Vulpetti A, Gedeck P (2013) Comparability of mixed IC50 data – a statistical analysis. PLoS ONE 8(4):e61007. https://doi.org/10.1371/journal.pone.0061007

Kazafeos K (2011) Incretin effect: GLP-1, GIP, DPP4. Diabetes Res Clin Pract 93(Suppl 1):S32-36. https://doi.org/10.1016/S0168-8227(11)70011-0

Konrad B, Anna D, Marek S, Marta P, Aleksandra Z, Józefa C (2014) The evaluation of dipeptidyl peptidase (DPP)-IV, α-glucosidase and angiotensin converting enzyme (ACE) inhibitory activities of whey proteins hydrolyzed with serine protease isolated from asian pumpkin (*Cucurbita ficifolia*). Int J Pept Res Ther 20(4):483–491. https://doi.org/10.1007/s10989-014-9413-0

Lacroix IME, Li-Chan ECY (2012) Dipeptidyl peptidase-IV inhibitory activity of dairy protein hydrolysates. Int Dairy J 25(2):97–102. https://doi.org/10.1016/j.idairyj.2012.01.003

Lacroix IME, Li-Chan ECY (2016) Food-derived dipeptidyl-peptidase IV inhibitors as a potential approach for glycemic regulation – current knowledge and future research considerations. Trends Food Sci Technol C54:1–16. https://doi.org/10.1016/j.tifs.2016.05.008

Lammi C, Aiello G, Boschin G, Arnoldi A (2019) Multifunctional peptides for the prevention of cardiovascular disease: a new concept in the area of bioactive food-derived peptides. J Funct Foods. https://doi.org/10.1016/J.JFF.2019.02.016

Liu R, Cheng J, Wu H (2019) Discovery of food-derived dipeptidyl peptidase IV inhibitory peptides: a review. Int J Mol Sci 20(3):E463. https://doi.org/10.3390/ijms20030463

Moraru A, Wiederstein J, Pfaff D, Fleming T, Miller AK, Nawroth P, Teleman AA (2018) Elevated levels of the reactive metabolite methylglyoxal recapitulate progression of type 2 diabetes. Cell Metabol 27(4):926–934e8. https://doi.org/10.1016/j.cmet.2018.02.003

Mulvihill EE (2018) Dipeptidyl peptidase inhibitor therapy in type 2 diabetes: control of the incretin axis and regulation of postprandial glucose and lipid metabolism. Peptides 100:158–164. https://doi.org/10.1016/j.peptides.2017.11.023

Nakamura A, Kawaharada R (2022) Advanced glycation end products and oxidative stress in a hyperglycaemic environment. In: Raghav A, Ahmad J (eds) *Fundamentals of Glycosylation*. IntechOpen, London. https://doi.org/10.5772/intechopen.97234

Nong NTP, Hsu J-L (2021) Characteristics of food protein-derived antidiabetic bioactive peptides: a literature update. Int J Mol Sci 22(17):9508. https://doi.org/10.3390/ijms22179508

Nongonierma AB, FitzGerald RJ (2019) Features of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from dietary proteins. J Food Biochem 43(1):e12451. https://doi.org/10.1111/jfbc.12451

Omar B, Ahrén B (2014) Pleiotropic mechanisms for the glucose-lowering action of DPP-4 inhibitors. Diabetes 63(7):2196–2202. https://doi.org/10.2337/db14-0052

Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK (2002) Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J Agric Food Chem 50(11):3122–3128. https://doi.org/10.1021/jf0116606

Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87(1):315–424. https://doi.org/10.1152/physrev.00029.2006

Patil SP, Goswami A, Kalia K, Kate AS (2020) Plant-derived bioactive peptides: a treatment to cure diabetes. Int J Pept Res Ther 26(2):955–968. https://doi.org/10.1007/s10989-019-09899-z

Petsantad P, Sangtanoo P, Srimongkol P, Saisavoey T, Reamtong O, Chaitanawisuti N, Karnchanatat A (2020) The antioxidant potential of peptides obtained from the spotted Babylon snail (*Babyloniaareolata)* in treating human colon adenocarcinoma (Caco-2) cells. RSC Adv 10(43):25746–25757. https://doi.org/10.1039/D0RA03261A

Reckziegel P, Festuccia WT, Britto LRG, Jang KLL, Romão CM, Heimann JC, Fogaça MV, Rodrigues NS, Silva NR, Guimarães FS, Eichler RAS, Gupta A, Gomes I, Devi LA, Heimann AS, Ferro ES (2017) A novel peptide that improves metabolic parameters without adverse central nervous system effects. Sci Rep 7(1):14781. https://doi.org/10.1038/s41598-017-13690-9

Saito T, Abe D, Sekiya K (2007) Nobiletin enhances differentiation and lipolysis of 3T3-L1 adipocytes. Biochem Biophys Res Commun 357(2):371–376. https://doi.org/10.1016/j.bbrc.2007.03.169

Satish S, Srivastava A, Yadav P, Varshney S, Choudhary R, Balaramnavar VM, Narender T, Gaikwad AN (2018) Aegeline inspired synthesis of novel amino alcohol and thiazolidinedione hybrids with antiadipogenic activity in 3T3-L1 cells. Eur J Med Chem 143:780–791. https://doi.org/10.1016/j.ejmech.2017.11.041

Siddiqui MA, Rasheed S, Saquib Q, Al-Khedhairy AA, Al-Said MS, Musarrat J, Choudhary MI (2016) In-vitro dual inhibition of protein glycation, and oxidation by some arabian plants. BMC Complement Altern Med 16:276. https://doi.org/10.1186/s12906-016-1225-7

Sim SY, Shin YE, Kim HK (2019) Fucoidan from *Undariapinnatifida* has antidiabetic effects by stimulation of glucose uptake and reduction of basal lipolysis in 3T3-L1 adipocytes. Nutrition Research (New York, N.Y.) 65:54–62. https://doi.org/10.1016/j.nutres.2019.02.002

Sun X, Acquah C, Aluko RE, Udenigwe CC (2020) Considering food matrix and gastrointestinal effects in enhancing bioactive peptide absorption and bioavailability. J Funct Foods 64:103680. https://doi.org/10.1016/j.jff.2019.103680

Tupe RS, Bangar N, Diwan A, Changale D, Choudhary S, Chaware S (2020) Comparative study of different glycating agents on human plasma and vascular cells. Mol Biol Rep 47(1):521–531. https://doi.org/10.1007/s11033-019-05158-y

van de Laar FA, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE, van Weel C (2005) Alpha-glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. Diabetes Care 28(1):154–163. https://doi.org/10.2337/diacare.28.1.154

Wang B, Li B (2018) Charge and hydrophobicity of casein peptides influence transepithelial transport and bioavailability. Food Chem 245:646–652. https://doi.org/10.1016/j.foodchem.2017.09.032

Yang Y, Shi C-Y, Xie J, Dai J-H, He S-L, Tian Y (2020) Identification of potential dipeptidyl peptidase (DPP)-IV inhibitors among *Moringa oleifera*phytochemicals by virtual screening, molecular docking analysis, ADME/T-based prediction, and in vitro analyses. Molecules 25(1):189. https://doi.org/10.3390/molecules25010189

Yarnpakdee S, Benjakul S, Kristinsson HG, Bakken HE (2015) Preventive effect of Nile tilapia hydrolysate against oxidative damage of HepG2 cells and DNA mediated by H2O2 and AAPH. J Food Sci Technol 52(10):6194–6205. https://doi.org/10.1007/s13197-014-1672-4

Zhong J, Gong Q, Goud A, Srinivasamaharaj S, Rajagopalan S (2015) Recent advances in dipeptidyl-peptidase-4 inhibition therapy: lessons from the bench and clinical trials. J Diabetes Res. https://doi.org/10.1155/2015/606031

Zilleßen P, Celner J, Kretschmann A, Pfeifer A, Racké K, Mayer P (2016) Metabolic role of dipeptidyl peptidase 4 (DPP4) in primary human (pre)adipocytes. Sci Rep 6(1):23074. https://doi.org/10.1038/srep23074