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Research Article

Effects of 22 traditional anti-diabetic medicinal plants on DPP-IV enzyme activity and glucose homeostasis in high-fat fed obese diabetic rats

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The present study investigated the effects of hot water extracts of 22 medicinal plants used traditionally to treat diabetes on Dipeptidyl peptidase-IV (DPP-IV) activity both *in vitro* and *in vivo* in high-fat fed (HFF) obese-diabetic rats. Fluorometric assay was employed to determine the DPP-IV activity. For *in vivo* studies, HFF obese-diabetic rats were fasted for 6 h and blood was sampled at different times before and after the oral administration of the glucose alone (18 mmol/kg body weight) or with either of the four most active plant extracts (250 mg/5 ml/kg, body weight) or established DPP-IV inhibitors (10 μmol/5 ml/kg). DPP-IV inhibitors: sitagliptin, vildagliptin and diprotin A, decreased enzyme activity by a maximum of 95–99% ($P<0.001$). Among the 22 natural anti-diabetic plants tested, *Anogeissus Latifolia* exhibited the most significant ($P<0.001$) inhibitory activity (96 ± 1%) with IC_{50} and IC_{25} values of 754 and 590 μg/ml. Maximum inhibitory effects of other extracts: *Aegle marmelos*, *Mangifera indica*, *Chloropsis cochinchinensis*, *Trigonella foenum-graecum* and *Azadirachta indica* were (44 ±7%; 38 ± 4%; 31±1%; 28±2%; 27±2%, respectively). A maximum of 45% inhibition was observed with >25 μM concentrations of selected phytochemicals (rutin). *A. latifolia*, *A. marmelos*, *T. foenum-graecum* and *M. indica* extracts improved glucose tolerance, insulin release, reduced DPP-IV activity and increased circulating active GLP-1 in HFF obese-diabetic rats ($P<0.05$–0.001). These results suggest that ingestion of selected natural anti-diabetic plants, in particular *A. latifolia, A. marmelos, T. foenum-graecum* and *M. indica* can substantially inhibit DPP-IV and improve glucose homeostasis, thereby providing a useful therapeutic approach for the treatment of T2DM.

Introduction

Diabetes Mellitus has become a worldwide concern, manifesting as one of the most major health issues within the world's population. There are several forms of diabetes, including gestational diabetes, but Type 1 and Type 2 diabetes are by far the most prevalent. Type 2 diabetes (T2DM), most often associated with obesity, is a particularly widespread disease and many patients from all over the globe are afflicted by this condition. T2DM patients are characterized by impaired β-cell function and insulin secretion together with tissue insulin resistance [1]. Since the prevalence and associated complications of T2DM are so damaging, more effective therapies are being sought to either delay or prevent the progression of T2DM [2]. As so, plants are most reliable source as studies to date found that they contain series of potential phytogroups including alkaloids, glycosides, terpenoids, phenolic, flavonoids and plant-derived peptides, each of them has shown potential antidiabetic activity in different experiment [2]. Besides, the diet of patients with T2DM plays a vital role in helping to maintain blood glucose control involving such factors as energy density, carbohydrate content, dietary fiber and natural products that may directly or indirectly affect the

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Table 1 Traditional use of selected medicinal plants treatment for diabetes

Plants	Traditional Uses	References
Acacia catechu (L.f.) Willd.	Diabetes, obesity, asthma, bronchitis, anaemia, diarrhoea	[34,35]
Bunium persicum (Boiss.) B.Fedtsch.	Obesity, gastrointestinal and urinary disorders, diarrhoea, asthma	[36]
Eugenia jambolana Lam.	Diabetes, cancer, enteric disorders, renal problems	[37,38]
Linum usitatissimum L.	Gastrointestinal disorders, asthma, bronchitis, pulmonary tuberculosis, gingival disorders, atherosclerosis	[39,40]
Santalum album L.	Inflammation, anti-septic, fever, carminative, diuretic, hypotensive, memory booster	[41]
Selaginella bryopteris (L.)	Jaundice, chronic tracheitis, lung cancer, venereal diseases, colitis, diuretic problems	[42,43]
Sesamum indicum (White)	Dietary fibre, joint inflammation, toothache, scrapes, cuts	[44,45]
Tamarindus indica	Inflammation, rheumatism, diarrhoea, dysentery, respiration conditions, malaria, gonorrhoea	[46]
Terminalia arjuna (Roxb. ex DC.)	Diabetes, cirrhosis, anaemia, cardiovascular disorders, viral diseases	[47,48]
Azadirachta indica	Diabetes, urinary and gastrointestinal problems, skin diseases, blood pressure and cholesterol	[49]
Anogeissus latifolia (Roxb. ex DC.)	Diabetes, haemorrhages, diarrhoea, dysentery, skin diseases, leprosy, hepatopathy	[50]
Albizia lebbeck (L.) Benth.	Respiratory disease, skin diseases, inflammation, diarrhoea, edema	[51,52]
Cudrania cochinchinensis (Lour.)	Gonorrhoea, rheumatism, jaundice, hepatitis, boils, scabies, bruising	[53]
Cassia fistula L.	Diabetes, jaundice, piles, rheumatism ulcers, skin eruptions, eczema, heart diseases, asthma, liver disorder	[54,55]
Dalbergia sissoo DC.	Bronchitis, inflammations, gonorrhoea, digestive disorders, colorectal cancer, bacterial infections	[56]
Swertia chirayita (Roxb.)	Diabetes, hypertension, liver disorders, malaria, hepatitis, inflammation, digestive diseases, epilepsy	[57,58]
Withania coagulans (Stocks)	Chronic degenerative diseases, diabetes	[59]
Glycyrrhiza glabra L.	Dyspepsia, belching, gas stomach ache, intestinal and liver colics, ulcerated wounds and gastritis	[60]
Momordica charantia L.	Diabetes, hypertension, obesity, cancer, hyperlipidaemia, digestive disorders, microbial infections	[61,62]
Mangifera indica L.	Diabetes, hypertension, anaemia, haemorrhage, asthma, gastric disorders	[63,64]
Aegle marmelos (L.) Corrêa	Diabetes, inflammations, asthma, ophthalmia, diarrhoea, dysentery, cardiac ailments	[65]
Trigonella foenum-graecum L.	Diabetes, hypercholesterolemia, edema lung congestion sinus, indigestion, baldness	[66,67]

absorption of nutrients or the secretion and action of insulin [3]. Hormones released by the gut in response to nutrient absorption, most notably GIP and GLP-1, also play an important role in modulating post-prandial hyperglycemia [4]. These hormones have a very short circulating half-life due to inactivation by the enzyme DPP-IV that cleaves the first two amino acids from the N-terminals producing GIP (3-42) and GLP-1 (9-36) [5]. This is why DPP-IV inhibitors are beneficial in the treatment of T2DM. Anti-diabetic drugs like metformin and nateglinide that respectively target insulin action and insulin secretion can, at high concentrations, also suppress DPP IV enzyme activity and such action may partly explain use of nateglinide as prandial insulin releasing agent that augments GLP-1 levels [6].

Having adequate bioactive insulin in the circulation is the key to control of glucose homeostasis as the hormone is unique in stimulating tissue glucose uptake and limiting hepatic glucose output. DPP-IV interferes with normal insulin action by degrading and therefore diminishing the insulinotropic and other β-cell actions of GLP-1 and GIP [4]. Natural resources are being explored to find new dietary ways to promote healthy blood glucose control including manipulation of the microbiome [7]. Over the years, many studies have revealed the anti-diabetic activity of plants used traditionally for the treatment of diabetes and defined their actions mediated via effects on the gastrointestinal processing of food and both the secretion and action of insulin [8–10]. More recently, dietary components including dairy, tuna, rice, salmon and amaranth have been found to exhibit DPP-IV inhibitory properties *in vitro* [11,12].

In the present work, 22 traditional medicinal plants with proven anti-diabetic activity were selected to assess their effects on DPP-IV enzyme activity *in vitro* (Tables 1 and 2). Furthermore, four of the most effective plants (*A. latifolia*, *A. marmelos*, *T. foenum-graecum* and *M. indica*) were selected to assess their acute effects on plasma DPP-IV activity, glucose-lowering and insulin-releasing properties in high fat fed obese-diabetic rats.

Table 2 Antidiabetic actions of selected traditional plants treatment for diabetes

Plants	[1]Hyperglycemia	[2]Insulin secretion	[3]Glucose uptake and metabolism	References
Acacia catechu	↓	↑	ND	[68]
Bunium persicum	↓	ND	↑	[69]
Eugenia jambolana	↓	↑	↑	[70]
Linum usitatissimum	↓	↑	↑	[71,72]
Santalum album	↓	↑	↑	[73]
Selaginella bryopteris	↓	↑	↑	[74]
Sesamum indicum (White)	↓	ND	↑	[75]
Tamarindus indica	↓	↑	ND	[76]
Terminalia arjuna	↓	↑	↑	[77,78]
Azadirachta indica	↓	↑	↑	[79,80]
Anogeissus latifolia	↓	↑	ND	[81]
Albizia lebbeck	↓	↑	↑	[82,83]
Chloropsis cochinchinensis	↓	↑	↑	[84]
Cassia fistula	↓	↑	↑	[85,86]
Dalbergia sisso	↓	ND	ND	[87,88]
Swertia chirrayita	↓	↑	↑	[89]
Withania coagulans	↓	ND	ND	[90,91]
Licorice glyceriza	↓	↓	ND	[16,92,93]
Momardica chirantia	↓	↑	ND	[94,95]
Mangifera indica	↓	↑	↑	[96,97]
Aegle marmelos	↓	↑	↑	[21]
Trigonella foenum graecum	↓	↑	ND	[22,98]

Effects of plant: - ↑, increase; ↓, decrease (beneficial effect on hyperglycemia); ND, effect not determined
[1]Effects on hyperglycemia were demonstrated in mice or rats given streptozotocin or alloxan or high fat diet to induce diabetes.
[2]Effects on insulin secretion were demonstrated *in vitro* using pancreatic β-cells or *in vivo* using blood plasma of rats or mice. Beneficial actions *in vitro* were dose-dependent and did not affect cellular viability at low concentrations.
[3]Effects on glucose uptake and metabolism were demonstrated *in vitro* using isolated mouse abdominal muscle.

Materials and methods

Plant materials and preparation of extract

Twenty-two plants used traditionally to treat diabetes were purchased to assess their ability to inhibit DPP-IV enzyme activity and improve glycemic control. The plants selected and their traditional and pharmacological actions are given in Tables 1 and 2. All plant materials were sourced in India where they are the native species. Confirmation of identity for the plants was made by a taxonomist Prof. F. A. Khan, Head of Department of Botany, Benazir Govt. Science & Commerce College, Bhopal, Barkatullah University, Madhya Pradesh, India where the plant specimens have been deposited in the herbarium. The accession numbers (voucher specimen numbers) for 22 traditional medicinal plants are listed in Table 3.

All plant components (Tables 1-3) were dried and grounded to obtain a fine powder. About 1 g of each dried powder was infused using 40 ml of boiled water. Aqueous extracts were chosen based on traditional use and prior studies of plants selected. The infusion was left for 15 min before being filtered through Whatman no. 1 filter paper. After that, the filtrates were dried under a vacuum (Savant Speedvac; New York, U.S.A.) to produce plant extract that was used to perform DPP-IV inhibitory experiments. For this purpose, the dried extract was dissolved in a 100 mM Tris-HCl buffer at an initial concentration of 5 mg/ml.

Determination of DPP-IV inhibitory activity *in vitro*

A fluorometric method was used to determine the DPP-IV inhibitory activity of plant extracts based on that described previously [6,13]. For *in vitro* studies, a 100 mM Tris-HCl buffer was prepared and adjusted to pH 8.0 using 100 mM Tris-base. Reactions were performed in 96-well black-walled, clear-bottomed microplates (Premier Scientific Ltd, Belfast, U.K.) using 8 mU/ml of DPP-IV enzyme and 200 μM of fluorescent substrate (Gly-Pro-AMC) with or without plant extract, known DPP-IV inhibitor or selected phytochemicals. These included caffeine, catechin, epicatechin,

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Table 3 List of confirmation of identity of 22 traditional medicinal plants with their herbarium numbers

Plants	Collected parts of plants	Voucher specimen numbers
Acacia catechu (L.f.) Willd.	Bark	1721
Bunium persicum (Boiss.) B.Fedtsch.	Seed	1844
Eugenia jambolana Lam.	Seed	1681
Linum usitatissimum L.	Seed	1531
Santalum album L.	Bark	1168
Selaginella bryopteris (L.)	Leaf	1135
Sesamum indicum (White)	Seed	1219
Tamarindus indica	Seed	866
Terminalia arjuna (Roxb. ex DC.)	Bark	535
Azadirachta indica	Seed	1610
Anogeissus latifolia (Roxb. ex DC.)	Bark	1734
Albizia lebbeck (L.) Benth.	Bark	1761
Cudrania cochinchinensis (Lour.)	Bark	1241
Cassia fistula L.	Stalk	1321
Dalbergia sissoo DC.	Bark	335
Swertia chirayita (Roxb.)	Bark	581
Withania coagulans (Stocks)	Fruit	1196
Glycyrrhiza glabra L.	Root	2212
Momordica charantia L.	Seed	2378
Mangifera indica L.	Seed	2391
Aegle marmelos (L.) Corrêa	Leaf	1733
Trigonella foenum-graecum L.	Seed	681

gallic acid, isoquercitrin, quercetin and rutin as well as the small molecule anti-diabetic drug nateglinide. DPP-IV assay was based on liberation of AMC (7-amino-4-methyl-coumarin) from DPP-IV substrate, Gly-Pro-AMC. Changes in fluorescence due to cleavage of the molecule by DPP-IV were measured with an excitation and emission at 370 and 440 nm with 2.5 nm slit width using a FlexStation 3 (Molecular Devices, California, U.S.A.). The inhibition of DPP-IV activity was calculated as the percentage of inhibition by each plant extract at various concentrations. Neither the plant extracts nor plasma samples showed any loss of activity when stored for many months at 20°C. It was checked in control experiments that the extracts did not themselves cleave the substrate or interfere with fluorescence measurements at the concentrations employed.

Animals

Forty male Sprague-Dawley rats (Envigo, Huntingdon, U.K., approximately 380–400 g) were fed a high-fat diet (45% fat, 20% protein and 35% carbohydrate; 26.15 kJ/g total energy percent; Special Diet Service, Essex, U.K.), *ad libitum* for 5–6 weeks to induce obesity and glucose intolerance. An additional 10 age-matched rats were maintained on standard rodent diet (30% protein, 10% fat, and 60% carbohydrate; 12.99 kJ/g total energy percent; Trouw Nutrition, Cheshire, U.K.). High fat fed rats exhibited increased body weight (398.7 $\pm$ 1.6 g versus 384.7 $\pm$ 1.8 g; $P<0.01$), impaired oral glucose tolerance and enhanced glucose-induced insulin responses, indicative of insulin resistance compared with the lean control rats fed normal diet (Figure 3). These animals also exhibited significantly elevated HbA1c levels (5.90 $\pm$ 0.07% versus 4.57 $\pm$ 0.05%; $P<0.001$), measured by the point-of-care A1CNow+ kit (PTS Diagnostic, Indiana), indicative of mild diabetes. The animals were housed individually in an air-conditioned room at 22 $\pm$ 2°C with a 12-h light/dark cycle. All animal experiments were conducted in accordance with U.K. Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU. All necessary steps were taken to prevent any potential animal suffering. The animal studies were approved by local Ulster Animal Welfare and Ethical Review Body (AWERB) committee (01/10/2016), as well as being covered under a U.K. Home Office Animal project/personal licence numbers PIL450, PIL1822 and PPL 2804, approved on 06/05/2016. All animals were maintained under specific pathogen-free conditions and experiments were conducted in the Biomedical and Behavioral Research Unit (BBRU) at Ulster University, Coleraine, U.K. Blood was collected from the cut tip of the tail of conscious animals without need for anaesthesia. No animals were culled.

Determination of the acute effects of plant extracts on DPP-IV activity *in vivo*

High fat-fed rats were used to study DPP-IV inhibitory activity of four medicinal plants (*A. latifolia, A. marmelos T. foenum-graecum* and *M. indica*) *in vivo*. Sitagliptin and vidagliptin were used in comparison as positive controls. Animals were fasted for approximately 6 h prior to experimentation. Blood samples were collected from the cut tip of the tail from conscious rats before and after oral administration of glucose with or without plant extract (250 mg/5 ml/kg) or an established DPP-IV inhibitor (10 μmol/5 ml/kg) at 0, 30, 60, 120, 180, 240, 360 and 480 min. Samples were collected in chilled fluoride/heparin-coated micro centrifuge tubes followed by centrifugation at 12000 rpm for 5 min. Plasma was stored at −20°C for measurement of insulin, DPP-IV activity and active GLP-1 (7-36). An Ascencia Contour glucose meter (Bayer, Newbury, U.K.) was used to measure blood glucose and insulin was determined by dextran-charcoal radioimmunoassay [14]. Active GLP-1 (7-36) was determined in the plasma samples collected at 60 min using a specific GLP-1 (Active) ELISA Kit (EGLP-35K, Merck Millipore, Dorset, U.K.).

Statistical analysis

Statistical analysis tests were performed by using Graph Pad-Prism 5. The results are represented as mean ± SEM. Data were analyzed using by unpaired Student's *t* test (nonparametric, with two-tailed *P* values) and one-way ANOVA with repeated measures was used and adjusted using Bonferroni correction. *P* value of < 0.05 was considered significant.

Results

In vitro DPP-IV Inhibitory effects of plant extracts

The extracts from 22 different plants were evaluated *in vitro* to assess their effects on DPP-IV activity. Established DPP-IV inhibitors namely sitagliptin, vildagliptin and diprotin A were used as positive controls (Table 4 and Figure 1A–C). These inhibitors decreased DPP-IV enzyme activity by up to 99 ± 2.0%, 99 ± 3% and 95 ± 3%, with IC_{50} values of 2.04×10^{-2}, 1.70×10^{-2} and 2.39×10^{-3} μg/ml ($P<0.05$–0.001, respectively (Table 4 and Figure 1A–C). In the presence of *A. latifolia* (bark), enzymatic AMC liberation from Gly-Pro-AMC was inhibited by 20 ± 1% to 96 ± 1% ($P<0.05$–0.001, Table 4, Figure 1D) at concentrations of 200–5000 μg/ml when compared with control. Moreover, *A. marmelos* (leaves), *M. indica* (seeds) and *T. foenum-graecum* (seeds) significantly inhibited DPP-IV enzyme activity at concentrations ranging from 200 to 5000 μg/ml ($P<0.05$–0.001, respectively, Table 4 and Figure 1E–G. The highest inhibitory effects of plant extracts were observed at 5000 μg/ml (44 ± 7%, 38 ± 4%, 31 ± 1% and 28 ± 2%, $P<0.001$, respectively, Table 4) as compared with control. The other plant extracts were found to inhibit DPP-IV activity in between 9 ± 1% and 27 ± 2% ($P<0.05$–0.001, Table 4) when tested at a concentration of 5000 μg/ml. The phytochemicals responsible for the inhibitory action are unknown but several possible candidates known to be present in the plant collection were tested. These included caffeine, catechin, epicatechin, gallic acid, isoquercitrin, quercetin and rutin. As shown in Figure 2A–G, each inhibited DPP-IV with the majority exhibiting lower effective concentrations of 125–200 μM. Isoquercitrin and quercetin inhibited at 25–50 μM whereas rutin was particularly effective with maximal inhibition of 45% and IC_{25} value of 306 μM. The effect was similar to the established insulinotropic drug nateglinide (Figure 2H).

Acute effects of plants extract on glucose tolerance and plasma insulin in high fat-fed rats

Four plants (*A. latifolia*, *A. marmelos*, *T. foenum-graecum* and *M. indica*), the most potent in inhibiting *in vitro* DPP IV enzyme activity, were selected for evaluation of effects on DPP IV activity and oral glucose tolerance in high-fat fed rats. Hot water extracts (250 mg/5 ml/kg) substantially improved the glycemic excursion from 30 to 240 min and increased plasma insulin from 30 to 120 min as compared with oral administration of glucose alone ($P<0.05$–0.001; Figure 3A,C). Established DPP-IV inhibitors (sitagliptin and vildagliptin) also improved glucose tolerance and insulin release following oral administration ($P<0.05$–0.001; Figure 3B,D).

Acute effects of plant extracts on circulating DPP-IV activity and active GLP-1 (7-36) in high fat-fed rats

Sitagliptin and vildagliptin significantly reduced DPP-IV activity in high-fat fed rats ($P<0.001$, Figure 4A–D). Hot water extracts (250 mg/5 ml/kg) of *A. latifolia, A. marmelos*, *T. foenum-graecum* and *M. indica* also significantly decreased *in vivo* DPP-IV enzyme activity compared with glucose alone ($P<0.05$–0.01, Figure 4A–D). The effects of

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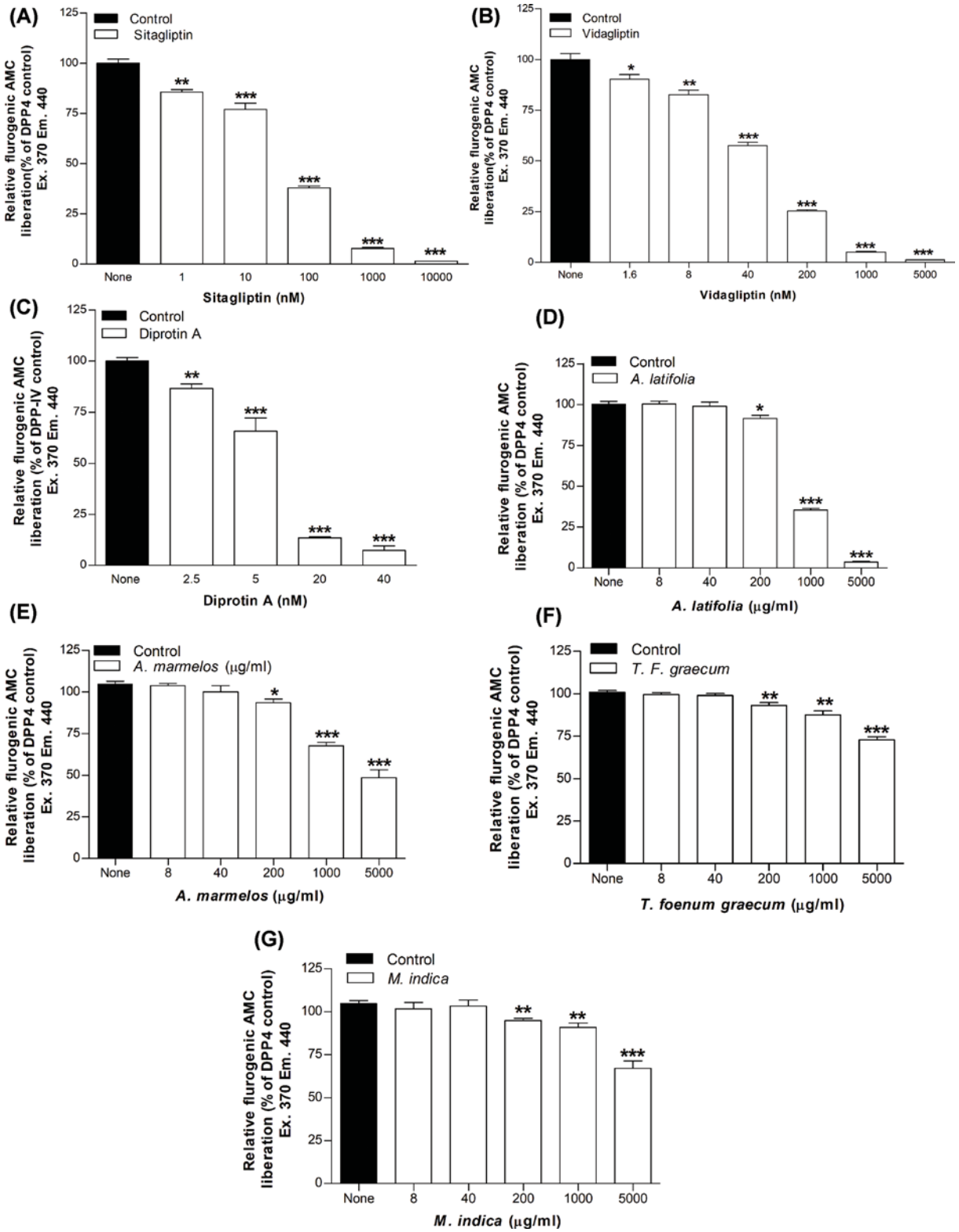

Figure 1. DPP-IV inhibitory activity of (A) Sitagliptin, (B) Vidagliptin, (C) Diprotin-A and hot water extract of four most potent plants (D) *A. latifolia,* (E) *A. marmelos,* (F) *T. foenum graecum* and (G) *M. indica* expressed as the bar chart (A–G)

Values are mean ± SEM with $n=4$, $^{*}P<0.05$, $^{**}P< 0.01$ and $^{***}P< 0.001$, compared with control. Sitagliptin: 1–10,000 nM; Vidagliptin: 1.6–5000 nM and Diprotin A: 2.5–40 nM.

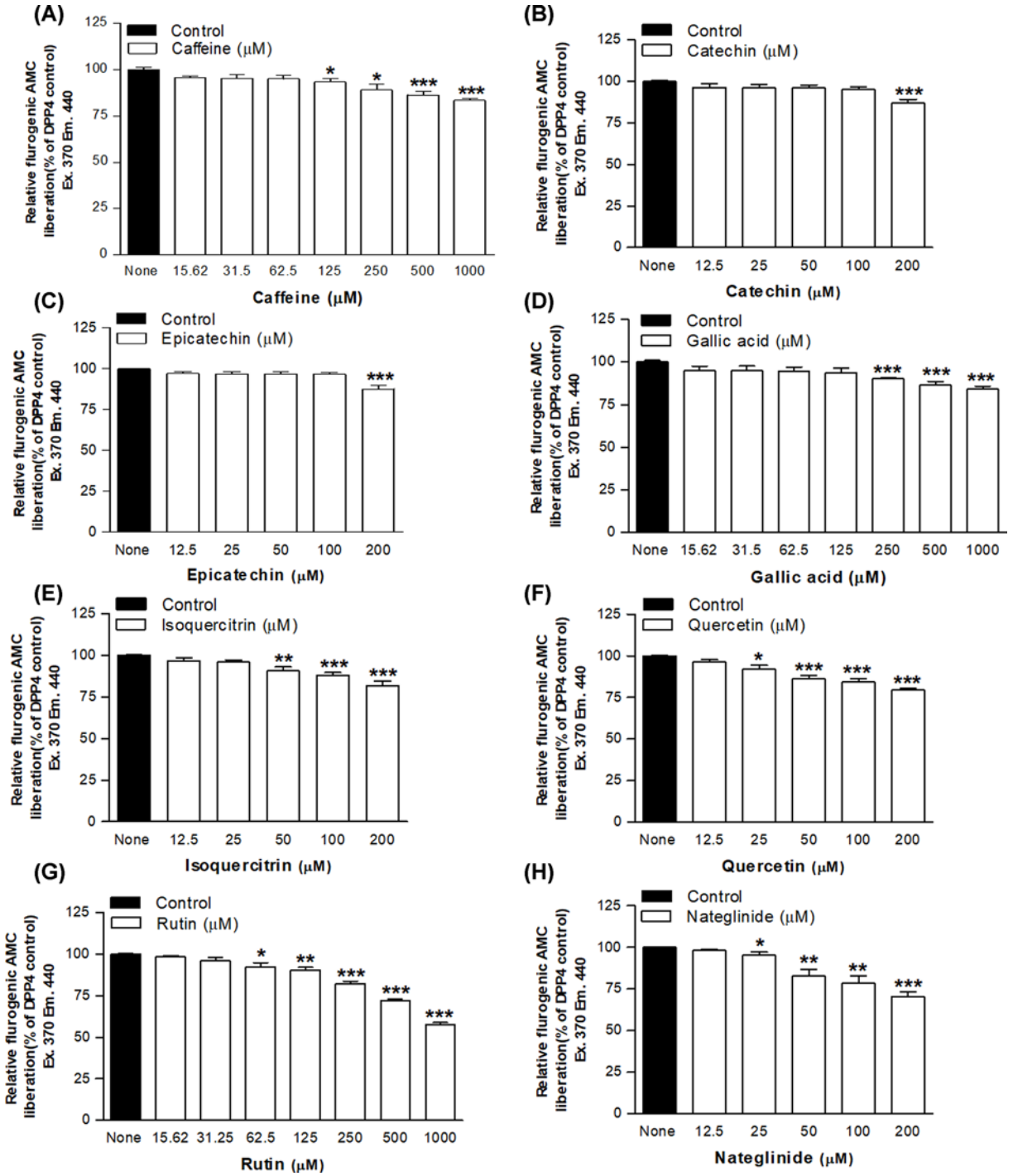

Figure 2. DPP-IV inhibitory activity of phytochemicals: (A) caffeine, (B) catechin, (C) epicatechin, (D) gallic acid, (E) isoquercitrin, (F) quercetin, (G) rutin and (H) the antidiabetic small molecule drug, nateglinide

Values are mean ± SEM with $n=4$, *$P<0.05$, **$P<0.01$ and ***$P< 0.001$, compared with control.

A. latifolia and *T. foenum-graecum* were particularly prominent with a sustained inhibition of DPP-IV activity from 30 min onwards ($P<0.05$–0.01, Figure 4A,C). Lesser but still significant effects were observed with *A. marmelos* and *M. indica* extracts ($P<0.05$, Figure 4B,D). As shown in Figure 5, active GLP-1 (7-36) concentrations in plasma were significantly increased by 32–45% ($P<0.05$–0.01) at 60 min after administration of each plant extract. An 81–89% increase was observed with sitagliptin and vidagliptin ($P<0.001$; Figure 5).

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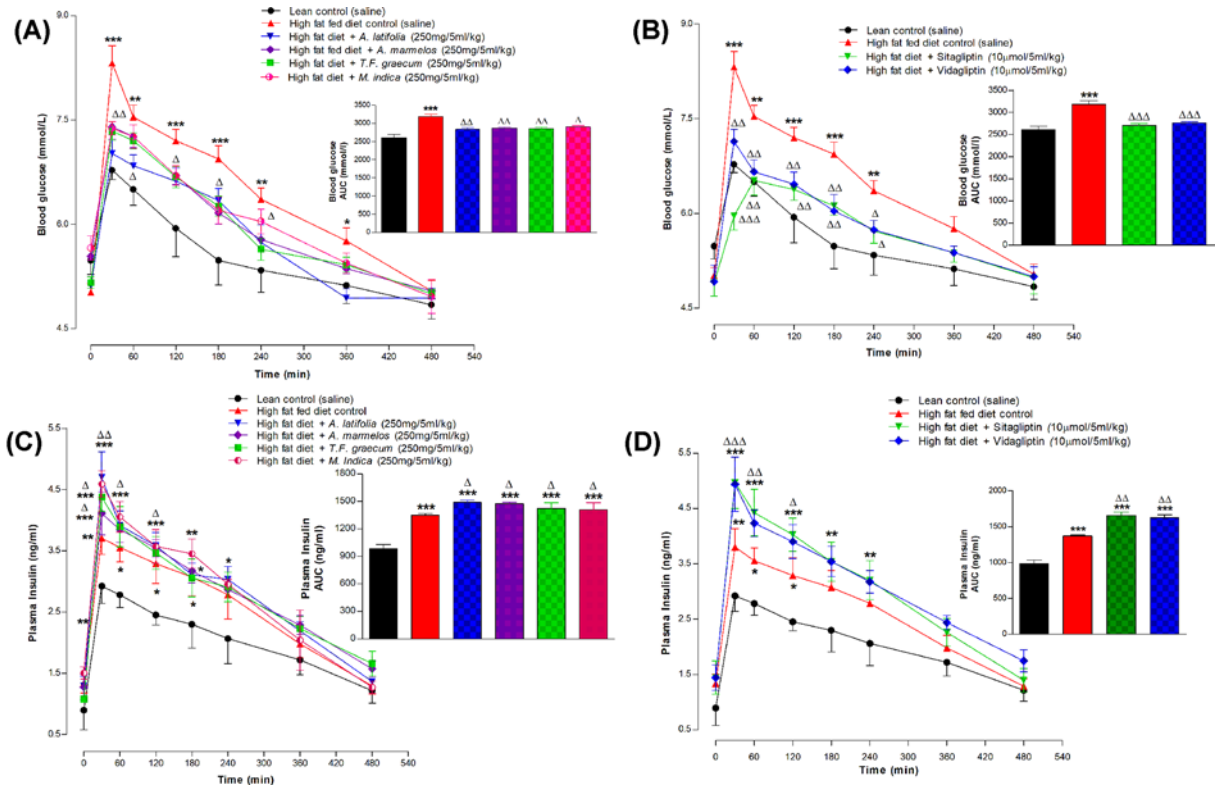

Figure 3. Acute effects of hot water extract of four most potent plants (A and C) *A. latifolia, A. marmelos*, *T. foenum* graecum, *M. indica* and (B and D) DPP-IV inhibitors: sitagliptin and vidagliptin on (A and B) glucose tolerance and (C and D) plasma insulin in high-fat fed rats expressed as line graphs and area under the curve

Blood glucose and plasma insulin were measured prior to and after oral administration of glucose alone (18 mmol/kg body weight, control) or in combination with plant extract (250 mg/5 ml/kg body weight,), Sitagliptin or Vidagliptin (both at 10 μmol/5 ml/kg, body weight). Values are mean ± SEM with n=6, $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$, compared with lean rats and $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ and $^{\Delta\Delta\Delta}P<0.001$ compared with high-fat fed controls.

Discussion

DPP-IV inhibitors are used in the treatment of T2DM based on their ability to extend postprandial levels of circulating plasma GLP-1 and GIP, thereby improving insulin secretion and helping to maintain good blood glucose control. Since their introduction to the clinic [15], this drug class has proven to be effective and highly popular. Weight reduction and glycaemic control are inferior to the related family of GLP-1 mimetics but DPP-IV inhibitors have the advantage of being orally active, thereby avoiding the need for daily injections and increasing patient compliance. In certain developing countries, the limited availability and cost of these and other modern medicines, such as metformin, sulphonylureas, thiazolidenediones, SGLT2 inhibitors and insulin formulations, have resulted in increasing attention being paid to traditional plant medicines with reputed anti-diabetic activity for treatment of T2DM [3,8,10,16–18].

A considerable number of plants have been used traditionally for the treatment for diabetes and its complications but only a limited number have been subjected to scientific scrutiny and fewer still scrutinized for the mechanisms responsible for their anti-diabetic effects [9,16,19]. In the present study, we have examined 22 medicinal plants with proven glucose-lowering ability (Table 2) to assess whether part of their mode of action relates to an ability to inhibit DPP-IV. Hot water extracts of every plant studied exhibited some degree of DPP-IV inhibition *in vitro* ranging from 9 to 96%, but the most substantial effects were observed (in descending order) with *A. latifolia* (bark), *A. marmelos* (leaves), *M. indica* (seeds), *T. foenum-graecum* (seeds), *C. cochinchinensis* (bark), and *A indica* (seeds). These plants inhibited DPP-IV by 27–96% with IC_{25} values of 446–4720 μg/ml. Although considerably less effective than pure preparations of sitagliptin and vildagliptin, these observations suggest that a component of the anti-diabetic actions of these plants may be due to inhibition of DPP-IV. This adds to the results of previous studies which have

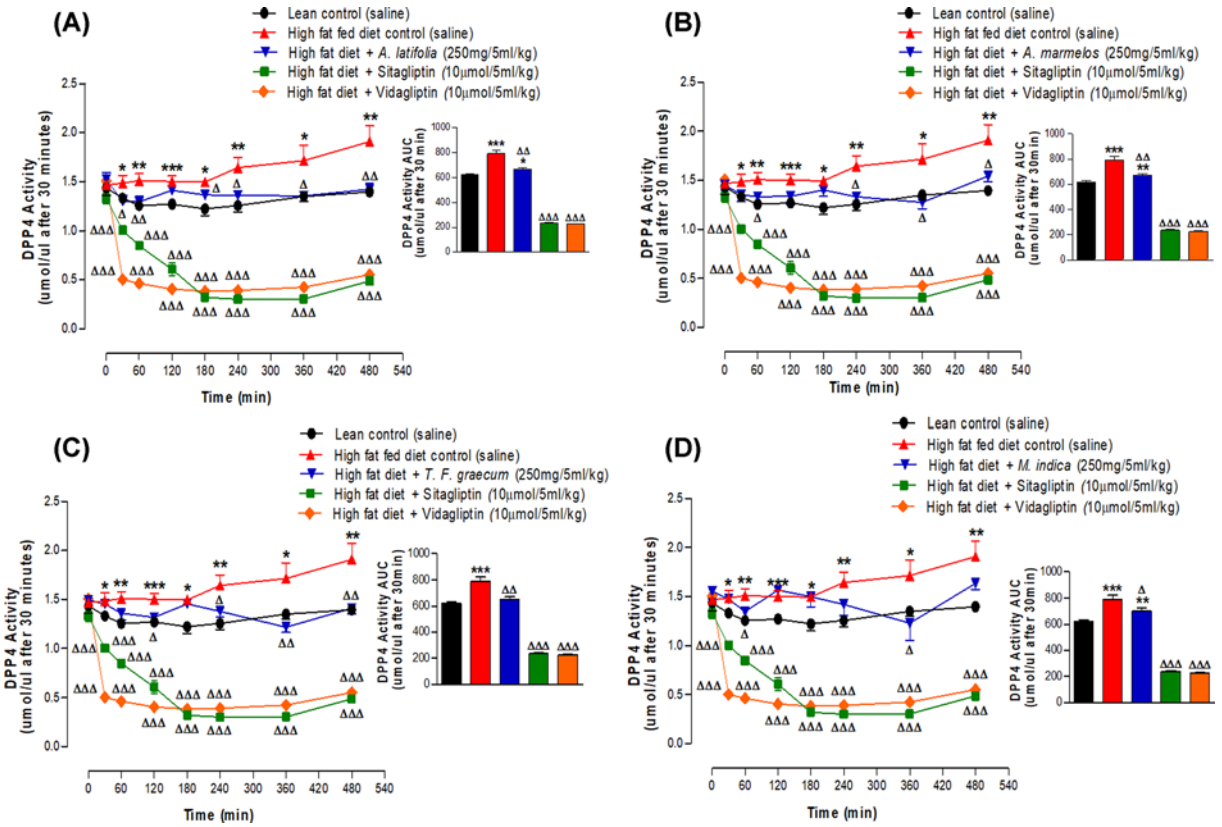

Figure 4. Acute effects of hot water extract of four most potent plants (A) *A. latifolia,* (B) *A. marmelos,* (C) *T. foenum graecum* and (D) *M. indica* on DPP-IV activity in high-fat fed rats expressed as line graphs and area under the curve

Plasma DPP-IV activity was measured prior to and after oral administration of glucose alone (18 mmol/kg body weight, control) or in combination with plant extract (250 mg/5 ml/kg body weight), Sitagliptin or Vidagliptin (each at 10 μmol/5 ml/kg body weight). DPP-IV activity was determined by Gly-Pro-AMC (200 μM) cleavage. Values are mean ± SEM with n=6, *P<0.05, **P<0.01 and ***P<0.001, compared with lean rats and $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01 and $^{\Delta\Delta\Delta}P$<0.001 compared with high-fat fed controls.

highlighted gastrointestinal effects of anti-diabetic plants and their ability to enhance insulin secretion and/or action [20–23].

Based on these *in vitro* results, the four most active plants were selected from the 22 initially screened for *in vivo* evaluation of effects on oral glucose tolerance, insulin secretion and plasma DPP-IV activity using high-fat fed rats. This included *A. latifolia*, *A. marmelos*, *T. foenum-graecum* and *M. indica.* The first two were particularly effective in inhibiting DPP-IV *in vitro* with up to 44-96% inhibition and IC_{50} values of 754-790 μg/ml. This compares with almost total inhibition of DPP-IV by sitagliptin and vildagliptin with IC_{25} and IC_{50} values of 2.04×10^{-3} to 2.43×10^{-3} μg/ml and 2.04×10^{-2} to 1.70×10^{-2} μg/ml, respectively. As expected, administration of either sitagliptin or vildagliptin orally to high fat fed obese-diabetic rats, together with glucose, induced a remarkable improvement in glucose tolerance and glucose-stimulated insulin secretion. This was associated with a 70–72% decrease in plasma DPP-IV activity known to result in strong augmentation of the stimulatory insulin-releasing effects of the incretin hormones GLP-1 and GIP. Consistent with this, circulating concentrations of active GLP-1 (7-36) were increased by 81–89% at 60 min following administration of these DPP-IV inhibitors. Extracts of *A. latifolia, A. marmelos, T. foenum-graecum* and *M. indica* also significantly inhibited DPP-IV enzyme activity and increased active GLP-1 (7-36) but by lesser extents of 12–18% and 32–45%, respectively. Interestingly, the glucose lowering actions of the four plant extracts were very similar to the DPP-IV inhibitors despite a smaller plasma insulin response. This indicates that other factors such as a delayed glucose absorption make a major contribution to the acute anti-hyperglycaemic activity of these plants *in vivo* [9,23]. Further long-term studies are required to determine how their effects compare with other plant-derived substances that exhibit anti-diabetic properties, such as metformin.

These results suggest that many plants used traditionally to treat diabetes have orally available constituents that inhibit DPP-IV, thereby contributing to their spectrum of actions which in the case of some might be significant [24,25].

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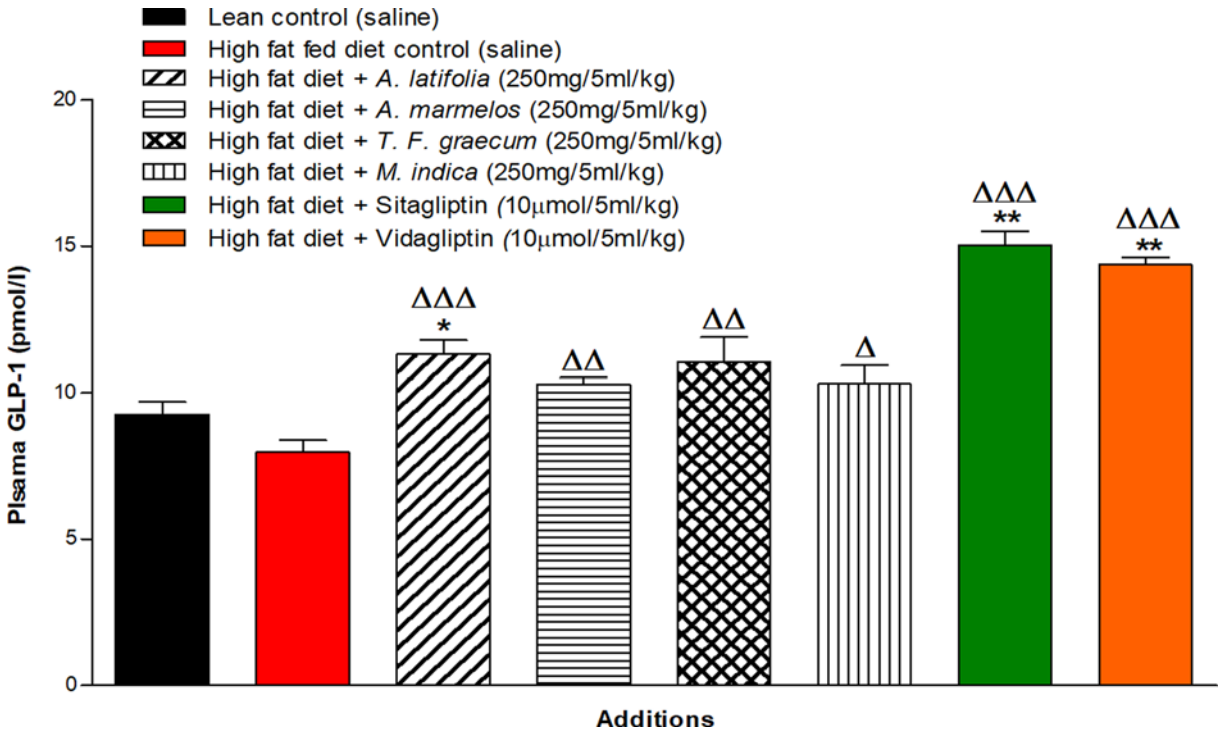

Figure 5. Acute effects of hot water extract of four most potent plants: *A. latifolia, A. marmelos, T. foenum graecum* and *M. indica* on plasma active GLP-1 (7-36) in high-fat fed rats

Plasma active GLP-1 (7-36) concentrations was measured at 60 min after oral administration of glucose alone (18 mmol/kg body weight, control) or in combination with plant extract (250 mg/5 ml/kg body weight), Sitagliptin or Vidagliptin (both at 10 μmol/5 ml/kg, body weight). Values are mean ± SEM with n=6, *P<0.05, **P<0.01 compared with lean rats and $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01 and $^{\Delta\Delta\Delta}P$<0.001 compared with high-fat fed controls.

Indeed, additional *in vitro* and *in vivo* studies on individual plant extracts have reported that other plant species inhibit DPP-IV activity [26,27]. These observations suggest that these plant extracts exert part of their insulinotropic effects via inhibition of DPP-IV with resultant preservation of active forms of GLP-1 (7-36) and GIP (1-42) released from intestinal enteroendocrine cells by feeding. Overall, our results together with these previous studies indicate that net effects on insulin secreting cells reflect combination of direct actions of glucose and other nutrients compounded by potentiating effects of incretin hormones that are favored by the concurrent inhibition of DPP-IV. Further extensive studies will be required to measure active and total forms of GLP-1 and GIP following administration of the plants studied but the few reports in the literature suggest that some plants with reputed anti-diabetic activity increase circulating GLP-1 [28]. The extent to which this may reflect enhanced secretion as opposed to decreased degradation by DPP-IV is unknown.

Although the present study describes DPP-IV lowering activity of many medicinal plants with anti-diabetic actions, few precise details are known about the nature of the phytochemicals that are absorbed and subsequently inhibit DPP-IV. All 22 plants examined inhibited DPP-IV activity *in vitro* to some extent suggesting that such chemicals are commonly encountered in the plant kingdom. Studies to date suggest that these include alkaloids, glycosides, terpenoids, phenolic, flavonoids as well as protein hydrolysates and peptides [27,2]. These may act at the molecular level by binding to the active site of the enzyme, thereby inhibiting interactions with natural substrates. Small peptides may also serve as competitive substrates as is the case with diprotin-A. Indeed, we demonstrated DPP-IV inhibitory action for caffeine, catechin, epicatechin, gallic acid, isoquercitrin, quercetin and rutin which may realistically contribute to the observed effects as they have been reported to be present in plants at levels of up to 2–30% by weight, not accounting for losses during our extraction procedure [29]. Rutin is the most abundant of these phytochemicals and was also shown to be the most effective inhibitor of DPP-IV, with an action broadly similar to that observed with the established anti-diabetic drug, nateglinide. The active plant constituents might, like this meglitinide, serve as effective prandial blood glucose regulators stemming partly from their ability to preserve active forms of GLP-1 and GIP released by feeding [6].

Table 4 DPP-IV inhibitory activity of established inhibitors and hot water extract of various traditional plants

Plants/ Inhibitors	Lower effective concentration (μg/ml)	Maximum inhibitory effect (%)	IC_{25} (μg/ml)	Estimated IC_{50} (μg/ml)
Sitagliptin (Inhibitor)	4.07×10^{-4}	99 ± 2‡	2.04×10^{-3}	2.04×10^{-2}
Vidagliptin (Inhibitor)	6.07×10^{-4}	99 ± 3‡	2.43×10^{-3}	1.70×10^{-2}
Diprotin A (Inhibitor)		95 ± 3‡	1.02×10^{-3}	2.39×10^{-3}
Anogeissus latifolia	200	96 ± 1‡	590	754
Aegle marmelos (L.) Corrêa	200	44 ± 7†	446	790
Mangifera indica L.	200	38 ± 4‡	2000	—
Cudrania cochinchinensis (Lour.)	1000	31 ± 1‡	4,050	—
Trigonella foenum-graecum L.	200	28 ± 2‡	4,700	—
Azadirachta indica	1000	27 ± 2‡	4,720	—
Tamarindus indica	200	23 ± 2‡	—	—
Terminalia arjuna (Roxb. ex DC.)	200	22 ± 1‡	—	—
Acacia catechu (L.f.) Willd.	5000	22 ± 7*	—	—
Withania coagulans (Stocks)	1000	17 ± 3†	—	—
Sesamum indicum (White)	40	19 ± 5*	—	—
Albizia lebbeck (L.) Benth.	5000	19 ± 4†	—	—
Cassia fistula L.	5000	19 ± 6*	—	—
Santalum album L.	40	17 ± 5*	—	—
Eugenia jambolana Lam.	40	14 ± 5*	—	—
Selaginella bryopteris (L.)	1000	13 ± 4*	—	—
Momordica charantia L.	1000	13 ± 2†	—	—
Swertia chirayita (Roxb.)	5000	12 ± 1†	—	—
Glycyrrhiza glabra L.	200	12 ± 2*	—	—
Bunium persicum (Boiss.)	200	11 ± 3*	—	—
Dalbergia sissoo DC.	5000	10 ± 2*	—	—
Linum usitatissimum L.	40	9 ± 1*	—	—

DPP-IV inhibitory activity of hot water extracts of various plants when incubated with Gly-Pro-AMC (200 μM) plus DPP4 (8 mU/ml^{-1}) for 30 min at 37°C. Sitagliptin, Vidagliptin and Diprotin A were used as established inhibitors. Values are mean ± SEM with n=4. *P<0.05, †P<0.01 and ‡P<0.001 compared with control group Gly-Pro-AMC (200 μM) + DPP4 (8 mU/ml^{-1}) alone. The calculated IC_{50} (μg/ml) was an estimate.

In the light of the DPP-IV inhibitory actions of the 22 plants tested plus the selected phytochemicals, it is notable that flavonoids and their metabolites have been reported to exhibit anti-diabetic activities. An inverse relationship has been suggested also between flavonoid intake and T2DM risk [30]. Phytochemicals other than those tested, such as anthocyanin, aspalathin, chrysin, eriodictyol, hispidulin, kaempferol, lepidoside, mangiferin, naringenin, naringin, procyanidin, rhamnoside, terpenoids, vitexin and *Lens culinaris* extracts have been shown also to exhibit DPP-IV inhibitory activity [27,31]. In addition, a number of other plants (such as *A. catechu*, *M. indica* and *A. marmelos*) have been reported to contain potential phenolic compounds that exert antioxidant effects and DPP-IV inhibitory activity [32].

Conclusions

In conclusion, these findings indicate that a substantial proportion of plants used traditionally for the treatment of diabetes exhibit DPP-IV inhibitory activity which may contribute to their multiple glucose lowering actions. Such medicinal plants could provide an accessible therapy for diabetes particularly in populations without easy access to the recognized drugs. More work is required for isolation, identification and characterization of agents responsible for inhibition of DPP-IV but there is a good chance that multiple phytochemicals are involved in mediating such effects [33].

Data Availability

All data are included in the manuscripts and the identified participant information (PA) is included in the Author Contribution section for the data collections.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

P.R.F. and Y.H.A.A.W. were responsible for the conception and design of research and contributed equally to the supervision of the study. M.P.H.F. was responsible for reading and revising manuscript. P.A. performed the experiments, analyzed the data, interpreted the results, prepared the figures and drafted the manuscript with P.R.F. P.R.F. and P.A. edited the revised manuscript. All authors approved the final version of the manuscript.

Institutional Animal Care

All animal experiments were conducted in accordance to U.K. Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU. All necessary steps were taken to prevent any potential animal suffering. The animal studies were approved by local Ulster Animal Welfare and Ethical Review Body (AWERB) committee (01/10/2016), as well as being covered under a U.K. Home Office Animal project/personal licence numbers PIL450, PIL1822 and PPL 2804, approved on 06/05/2016. All animals were maintained under specific pathogen-free conditions, and all experiments were conducted in the Biomedical and Behavioral Research Unit (BBRU) at Ulster University, Coleraine, U.K. Blood samples were collected from the cut tip of the tail of conscious animals and they are not scarified.

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Abbreviations

AMC, 7-amino-4-methyl-coumarin; DPP-IV, dipeptidyl peptidase-IV; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; Gly-Pro-AMC, Gly-Pro-7-Amino-4-Methyl-Coumarin; HFF, high-fat fed; IC_{25}, 25 percent inhibitory concentration; IC_{50}, 50 percent inhibitory concentration; OGTT, oral glucose tolerance test; T2DM, Type 2 diabetes mellites.

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