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DUAL ANTIDIABETIC AND ANTICANCER PHARMACOLOGICAL EVALUATION OF ANDROGRAPHOLIDE AND FOUR RELATED PLANT-DERIVED PHYTOCONSTITUENTS AS DIPEPTIDYL PEPTIDASE-4 INHIBITORS: IN VITRO ENZYME INHIBITION, ANTICANCER CYTOTOXICITY, AND IN VIVO ANTIDIABETIC EFFICACY IN A STREPTOZOTOCIN-NICOTINAMIDE-INDUCED TYPE 2 DIABETIC RAT MODEL

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) continues to expand globally, and the adverse-effect burden, cost, and lack of disease-modifying action of synthetic dipeptidyl peptidase-4 (DPP-4) inhibitors have renewed interest in plant-derived phytoconstituents as safer, multi-target antidiabetic leads. T2DM is also an independent epidemiological risk factor for several common malignancies, a convergence that lends particular translational value to phytoconstituents capable of demonstrating a dual antidiabetic-anticancer pharmacological profile.
Aim: To evaluate the in vitro enzyme-inhibitory, anticancer cytotoxic, and in vivo antidiabetic pharmacological profile of five phytoconstituents — andrographolide, berberine, ursolic acid, gymnemic acid IV, and charantin — derived from traditionally used Indian antidiabetic plants.
Materials and Methods: DPP-4, α-glucosidase, and α-amylase inhibitory activities were determined in vitro by fluorometric and chromogenic assays (triplicate; n = 3). Cytotoxicity was assessed by MTT assay against MCF-7, A549, and HeLa cell lines with HEK293 as the normal-cell comparator. Acute oral toxicity was assessed per OECD Guideline 423. A 28-day dose-ranging antidiabetic study was conducted in streptozotocin-nicotinamide-induced diabetic Wistar rats (18 groups, n = 6/group), with sitagliptin (10 mg/kg) as the reference standard, evaluating fasting blood glucose (FBG), oral glucose tolerance, glycated haemoglobin (HbA1c), serum insulin, lipid profile, pancreatic oxidative stress biomarkers, hepatic/renal function, and histopathology. Data were analysed by one-way ANOVA with Tukey's/Dunnett's post-hoc test ($p < 0.05$).
Results: Andrographolide showed the lowest (most potent) IC50 among the five phytoconstituents for DPP-4 (11.0 ± 0.40 μM), α-glucosidase (56.5 ± 0.70 μM; more potent than acarbose, 195.3 ± 0.90 μM), and α-amylase (91.9 ± 0.70 μM; more potent than acarbose, 98.8 ± 0.40 μM). In the anticancer evaluation, andrographolide was the most potent and most selective cytotoxic phytoconstituent against MCF-7, A549, and HeLa cancer cell lines (MCF-7 IC50 = 8.6 ± 0.7 μM; selectivity index = 5.8), the only compound in the series with a selectivity index exceeding that of the cisplatin reference standard (0.70), by more than eight-fold. All five compounds were acutely non-toxic (LD50 > 2200 mg/kg; OECD Category 5). At its high dose (100 mg/kg), andrographolide produced a 64.5% reduction in FBG relative to diabetic control (101.6 ± 7.4 mg/dL versus 286.4 ± 18.2 mg/dL), the lowest HbA1c (5.0 ± 0.2%), restoration of pancreatic antioxidant enzyme activity, a favourable lipid profile, preserved hepatic/renal function, and the best-preserved pancreatic, hepatic, and renal histoarchitecture among the five phytoconstituents, numerically ahead of the sitagliptin standard on several parameters.
Conclusion: Andrographolide demonstrates the most potent and consistent multi-target antidiabetic pharmacological profile, together with the most favourable anticancer cytotoxic selectivity, among the five phytoconstituents evaluated, with berberine as a well-supported secondary candidate on both indications, supporting its continued preclinical development as a plant-derived, dual antidiabetic-anticancer DPP-4 inhibitor lead for Type 2 Diabetes Mellitus.

KEYWORDS Type 2 Diabetes Mellitus; Dipeptidyl peptidase-4 inhibitor; Andrographolide; Phytoconstituents; Anticancer activity; Streptozotocin-nicotinamide; Oxidative stress; Histopathology.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is among the most consequential non-communicable diseases of the present century. The International Diabetes Federation estimated that 537 million adults aged 20-79 years were living with diabetes worldwide in 2021, a figure projected to rise to 783 million by 2045 [1]. India alone accounts for an estimated 74 million cases, a number expected to reach 124 million by mid-century, positioning the country among those bearing the heaviest absolute burden of the disease [1,2]. Beyond its epidemiological scale, T2DM truncates life expectancy, is the leading cause of adult-onset blindness and end-stage renal disease in many health systems, and imposes a disproportionate economic burden on low- and middle-income countries, where out-of-pocket healthcare expenditure predominates [1,3].

Contemporary management of T2DM increasingly exploits the incretin axis. Dipeptidyl peptidase-4 (DPP-4) is the serine protease principally responsible for the rapid inactivation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), the two incretin hormones that account for a substantial proportion of postprandial insulin secretion [4,5]. Pharmacological inhibition of DPP-4 preserves endogenous incretin activity, augmenting glucose-dependent insulin secretion while suppressing glucagon, and thereby confers a favourable safety profile with minimal intrinsic hypoglycaemic risk relative to insulin secretagogues [5]. Sitagliptin, the first clinically approved DPP-4 inhibitor, together with vildagliptin, saxagliptin, and linagliptin, has become established in international treatment guidelines [4].

Despite this clinical utility, synthetic gliptins are not without limitations. Post-marketing surveillance and cardiovascular outcome trials have reported increased heart failure hospitalisation with saxagliptin, an increased incidence of bullous pemphigoid, and severe arthralgia across the drug class [6]. Synthetic DPP-4 inhibitors are also comparatively costly for sustained use in resource-limited settings, and none has been shown to meaningfully slow the progressive decline in pancreatic beta-cell function that underlies the natural history of T2DM [4,6]. These limitations have sustained interest in identifying safer, more affordable, and potentially multi-target antidiabetic agents derived from medicinal plants.

Plants have historically supplied a substantial proportion of clinically important antidiabetic agents, most notably metformin, whose guanidine scaffold derives from Galega officinalis [7]. Andrographis paniculata (andrographolide), Tinospora cordifolia (berberine), Gymnema sylvestre (gymnemic acids), Momordica charantia (charantin), and Ocimum sanctum (ursolic acid) are five medicinal plants with long-documented use in Ayurvedic and Siddha traditions for the management of madhumeha, the classical descriptor of diabetes mellitus in Indian medical texts [8-11]. Each of the corresponding phytoconstituents evaluated in the present study possesses a structurally distinct chemical scaffold — a labdane diterpenoid, an isoquinoline alkaloid, a pentacyclic triterpenoid, a triterpenoid saponin, and a steroidal saponin mixture, respectively — and each has been individually associated in the pharmacological literature with antidiabetic, antioxidant, or multi-target metabolic activity [8-12].

Berberine, for example, is well documented to activate AMP-activated protein kinase (AMPK), inhibit intestinal α-glucosidase, and modulate gut microbiota composition, in a manner mechanistically convergent with metformin [9]. Ursolic acid has been reported to modulate protein tyrosine phosphatase 1B (PTP1B) and PPAR-gamma signalling [11], while gymnemic acids and charantin have well-established, if incompletely mechanistically characterised, hypoglycaemic activity attributed in part to beta-cell stimulatory and insulin-mimetic effects, respectively [10,12]. Notwithstanding this substantial body of individual pharmacological evidence, comparatively few studies have evaluated several structurally diverse phytoconstituents concurrently, under a single, internally controlled experimental protocol spanning DPP-4-relevant enzyme inhibition, anticancer cytotoxic selectivity, acute toxicity, and a dose-ranging in vivo antidiabetic efficacy comparison against a synthetic gliptin reference standard.

Beyond its direct metabolic burden, T2DM is now recognised as an independent epidemiological risk factor for several common malignancies, including colorectal, hepatocellular, endometrial, and pancreatic cancer, a relationship mechanistically attributable to shared drivers such as chronic hyperinsulinaemia, dysregulated insulin-like growth factor-1 (IGF-1) signalling, chronic low-grade inflammation, and oxidative stress [28,29]. The global cancer burden is itself substantial and rising: the International Agency for Research on Cancer estimated approximately 19.3 million new cancer cases and 10.0 million cancer deaths worldwide in 2020, with incidence projected to reach 28.4 million annually by 2040 [28], while India's National Cancer Registry Programme reported more than 1.46 million new cancer cases in 2022 [29]. This epidemiological convergence between T2DM and malignancy creates a compelling rationale for evaluating candidate antidiabetic phytoconstituents concurrently for anticancer activity, since a compound demonstrating both DPP-4-relevant antidiabetic efficacy and selective cytotoxicity against malignant cells would

represent a dual-indication lead of considerable translational value, addressing two mechanistically intertwined disease axes that commonly co-occur in the same patient population.

Each of the five phytoconstituents evaluated here has, in isolation, accumulated preclinical evidence of cytotoxic and antiproliferative activity through multi-modal mechanisms, including induction of intrinsic (mitochondrial) and extrinsic (death-receptor-mediated) apoptosis, cell cycle arrest, generation of intracellular reactive oxygen species (ROS), and inhibition of oncogenic signalling cascades such as PI3K/Akt/mTOR, NF-κB, and STAT3 [16,30,31]. Andrographolide has been reported to downregulate the anti-apoptotic protein Bcl-2 while upregulating the pro-apoptotic proteins Bax and Bad in breast and lung carcinoma cell lines [16]; berberine intercalates into DNA, inhibits NF-κB and its downstream survival targets, and induces G0/G1 cell cycle arrest in cervical carcinoma cells [30]; and ursolic acid inhibits PI3K/Akt/mTOR signalling and sensitises colorectal carcinoma cells to TRAIL-mediated apoptosis [31]. Despite this substantial body of individual mechanistic evidence, a systematic, concurrent comparison of anticancer cytotoxicity across multiple structurally diverse antidiabetic phytoconstituents, evaluated alongside their DPP-4-relevant antidiabetic profile within a single experimental programme, has not been widely reported.

This concurrent, head-to-head design addresses a specific and practically important gap: published reports on plant-derived DPP-4-relevant inhibitors are frequently confined to a single compound or a single assay platform, precluding direct comparative ranking of candidate phytoconstituents and hindering evidence-based prioritisation for further preclinical development. The present study was therefore designed to characterise, within one integrated pharmacological programme, the in vitro DPP-4, α-glucosidase, and α-amylase inhibitory activity, the in vitro anticancer cytotoxicity and selectivity (MTT assay against MCF-7, A549, and HeLa cancer cell lines), the acute oral toxicity, and the 28-day in vivo antidiabetic efficacy — encompassing glycaemic control, pancreatic oxidative stress, lipid profile, hepatic/renal safety, and histopathology — of andrographolide, berberine, ursolic acid, gymnemic acid IV, and charantin in the streptozotocin-nicotinamide-induced Type 2 diabetic rat model, with the objective of identifying the phytoconstituent most suitable for prioritised translational development as a plant-derived, dual antidiabetic-anticancer DPP-4 inhibitor lead.

MATERIALS AND METHODS

Test Compounds and Reference Standards

Andrographolide, berberine, ursolic acid, gymnemic acid IV, and charantin (analytical grade, ≥ 95% purity) were used as the test phytoconstituents throughout the study. Sitagliptin served as the reference standard for DPP-4 inhibition and for the in vivo antidiabetic study; acarbose served as the reference standard for the α-glucosidase and α-amylase inhibition assays; and cisplatin served as the cytotoxic reference standard for the MTT assay.

In Vitro DPP-4 Inhibition Assay

DPP-4 inhibitory activity was assessed using a fluorometric assay based on the synthetic substrate Gly-Pro-7-amido-4-methylcoumarin (Gly-Pro-AMC). Test compounds at graded concentrations were pre-incubated with recombinant human DPP-4 enzyme prior to substrate addition, and the fluorescence of liberated AMC (excitation/emission, 380/460 nm) was measured kinetically. Percentage inhibition was calculated relative to an uninhibited control as: % Inhibition = [(Fluorescence(control) − Fluorescence(test)) / Fluorescence(control)] × 100. IC50 values were derived by four-parameter logistic non-linear regression of the percentage-inhibition-versus-log-concentration curve, using GraphPad Prism.

α-Glucosidase and α-Amylase Inhibition Assays

α-Glucosidase inhibitory activity was determined using the p-nitrophenyl-α-D-glucopyranoside (pNPG) chromogenic substrate assay, with liberated p-nitrophenol measured at 405 nm; acarbose served as the reference standard. α-Amylase inhibitory activity was assessed by the dinitrosalicylic acid (DNS) method using starch as substrate, with absorbance measured at 540 nm. IC50 values for both assays were calculated for all five test compounds and the acarbose reference standard by non-linear regression.

In Vitro Cytotoxicity (MTT) Assay and Cell Culture

Cytotoxicity was assessed against MCF-7 (breast adenocarcinoma), A549 (non-small-cell lung carcinoma), and HeLa (cervical carcinoma) cell lines, with HEK293 (non-cancerous human embryonic kidney) cells as the normal-cell comparator for selectivity index calculation. Cells were cultured in appropriate growth medium supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified 5% CO2 atmosphere, seeded in 96-well plates, and treated with graded concentrations of test compound for 48 hours. Viability was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay, with formazan crystals solubilised in dimethyl sulfoxide and absorbance read at 570 nm (reference 630 nm). IC50 values were derived by non-linear regression, and the selectivity index (SI) was calculated as IC50(HEK293) / IC50(cancer cell line).

Quality Control for In Vitro Assays

Each assay plate incorporated a reference-standard dose-response curve and a vehicle (solvent-only) control alongside the test compounds. All IC50 determinations represent the mean of three independent experimental replicates, each performed in technical triplicate, expressed as mean ± standard deviation (SD).

Experimental Animals and Ethical Approval

Adult Wistar rats were acclimatised for seven days under standard laboratory conditions (12-hour light/dark cycle, 22 ± 2°C, ad libitum standard pellet diet and water). All procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of JKKMMRF College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu (IAEC approval number JKKMMRFCP/IAEC/DEC-2024/015; CPCSEA registration number 1158/PO/Re/S/07/CPCSEA; approval dated 26 December 2024), and were conducted in strict accordance with CPCSEA guidelines.

Acute Oral Toxicity

Acute oral toxicity was assessed per OECD Guideline 423 (Acute Toxic Class Method) in female Wistar rats across the dose range 5-2000 mg/kg, with observation for mortality and signs of toxicity over 14 days to establish the median lethal dose (LD50) and GHS hazard category for each of the five phytoconstituents.

Induction of Experimental Diabetes

Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 55 mg/kg body weight, freshly dissolved in cold citrate buffer, pH 4.5) administered 15 minutes after intraperitoneal nicotinamide (110 mg/kg body weight). This STZ-nicotinamide model produces partial, rather than complete, pancreatic beta-cell destruction and is widely used to model the non-insulin-dependent phenotype of T2DM. Fasting blood glucose (FBG) was measured 72 hours post-induction, and animals with FBG greater than 250 mg/dL were included in the diabetic study groups.

Experimental Design and Dose Selection

A dose-ranging design was adopted for the 28-day study: each of the five phytoconstituents was evaluated at three oral doses (low, medium, and high), together with a normal control, a diabetic control, and a sitagliptin (10 mg/kg) reference-standard group, giving 18 groups in total (n = 6 rats/group; 108 animals overall). All groups received their respective treatment once daily by oral gavage for 28 consecutive days. The results reported here correspond to the high-dose group for each compound (andrographolide 100 mg/kg, berberine 200 mg/kg, ursolic acid 200 mg/kg, gymnemic acid IV 200 mg/kg, charantin 100 mg/kg), as the primary comparative endpoint of the dose-ranging design.

Table 1. Experimental group design and dose allocation for the 28-day in vivo antidiabetic study (streptozotocin-nicotinamide model). Reported results correspond to the high-dose group of each phytoconstituent (n = 6 rats/group).

Group	Treatment	Dose (mg/kg/day)	Route
G1	Normal control	Vehicle only	Oral
G2	Diabetic control (STZ-NA)	Vehicle only	Oral
G3	Standard (Sitagliptin)	10	Oral
G4-G6	Andrographolide (Low/Medium/High)	25 / 50 / 100	Oral
G7-G9	Berberine (Low/Medium/High)	50 / 100 / 200	Oral
G10-G12	Ursolic Acid (Low/Medium/High)	50 / 100 / 200	Oral
G13-G15	Gymnemic Acid IV (Low/Medium/High)	50 / 100 / 200	Oral
G16-G18	Charantin (Low/Medium/High)	25 / 50 / 100	Oral

Biochemical, Glycaemic, and Oxidative Stress Parameters

Body weight and FBG were recorded on Days 0, 7, 14, 21, and 28 using a validated glucometer/glucose oxidase method. An oral glucose tolerance test (OGTT, 2 g/kg glucose load) was performed at study end, with area under the curve (AUC) calculated by the trapezoidal method. Terminal parameters included glycated haemoglobin (HbA1c), serum insulin (by ELISA), a complete lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C), hepatic function markers (ALT, AST), renal function markers (creatinine, urea), and pancreatic tissue oxidative stress biomarkers — superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) — determined by standard spectrophotometric kit-based methods.

Histopathological Examination

Pancreas, liver, and kidney tissues were fixed in 10% neutral buffered formalin, processed through graded alcohol and xylene, embedded in paraffin, sectioned at 5 µm thickness, and stained with haematoxylin and eosin (H&E). Slides were examined under light microscopy by a blinded observer. Pancreatic islet integrity, hepatocyte morphology, and renal tubular architecture were each scored on a semi-quantitative 0-3 severity scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe changes), with qualitative descriptive observations recorded for each organ and group.

Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM) for in vivo parameters and mean ± SD for in vitro IC50 determinations. Group comparisons were performed by one-way ANOVA followed by Tukey's or Dunnett's post-hoc test, as appropriate, using GraphPad Prism. IC50 values were computed by non-linear regression (four-parameter logistic model). A p-value < 0.05 was considered statistically significant throughout.

RESULTS

In Vitro DPP-4 Inhibitory Activity

DPP-4 inhibitory activity, determined in triplicate for all five phytoconstituents, is summarised in Table 2. Andrographolide produced the lowest (most potent) IC50 (11.0 ± 0.40 µM), followed in ascending order of IC50 by berberine (14.5 ± 0.40 µM), ursolic acid (18.6 ± 0.40 µM), gymnemic acid IV (22.1 ± 0.45 µM), and charantin (31.2 ± 0.65 µM), representing a greater than 2.8-fold difference in potency between the most and least active compounds (Figure 1).

Table 2. DPP-4 inhibitory activity (IC50, µM) of the five phytoconstituents — triplicate determinations, expressed as mean ± SD (n = 3 independent replicates).

Compound	Rep-1	Rep-2	Rep-3	Mean ± SD (µM)
Andrographolide	10.6	11.4	11.0	11.0 ± 0.40
Berberine	14.1	14.9	14.6	14.5 ± 0.40
Ursolic acid	18.2	18.7	19.0	18.6 ± 0.40
Gymnemic acid IV	21.6	22.5	22.1	22.1 ± 0.45
Charantin	30.5	31.2	31.8	31.2 ± 0.65

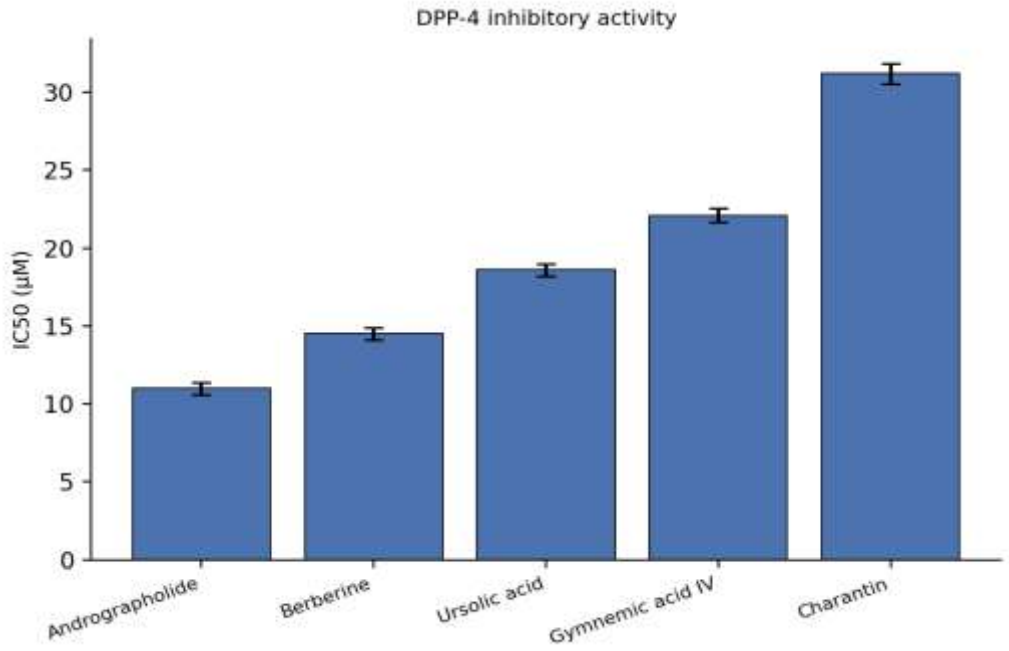

Figure 1. Grouped bar chart of mean DPP-4 IC50 (μM) ± SD for the five phytoconstituents (Table 2), arranged in ascending order of potency. Lower bars indicate greater inhibitory potency (n = 3 independent replicates per compound; one-way ANOVA with Tukey's post-hoc test, $p < 0.05$ for andrographolide versus charantin).

α-Glucosidase and α-Amylase Inhibitory Activity

All five phytoconstituents inhibited α-glucosidase more potently than the acarbose reference standard (195.3 ± 0.90 μM), with andrographolide again the most potent (56.5 ± 0.70 μM) (Table 3, Figure 2). For α-amylase, andrographolide (91.9 ± 0.70 μM) was the only phytoconstituent more potent than acarbose (98.8 ± 0.40 μM) (Table 4, Figure 3).

Table 3. α-Glucosidase inhibitory activity (IC50, μM) of the five phytoconstituents and the acarbose reference standard — triplicate determinations, mean ± SD.

Compound	Rep-1	Rep-2	Rep-3	Mean ± SD (μM)
Andrographolide	55.8	57.2	56.6	56.5 ± 0.70
Berberine	76.5	78.4	79.0	78.0 ± 1.30
Ursolic acid	92.1	94.6	95.2	94.0 ± 1.60
Gymnemic acid IV	102.5	104.8	103.6	103.6 ± 1.20
Charantin	118.4	120.2	119.1	119.2 ± 0.90
Acarbose (reference)	194.3	195.6	196.0	195.3 ± 0.90

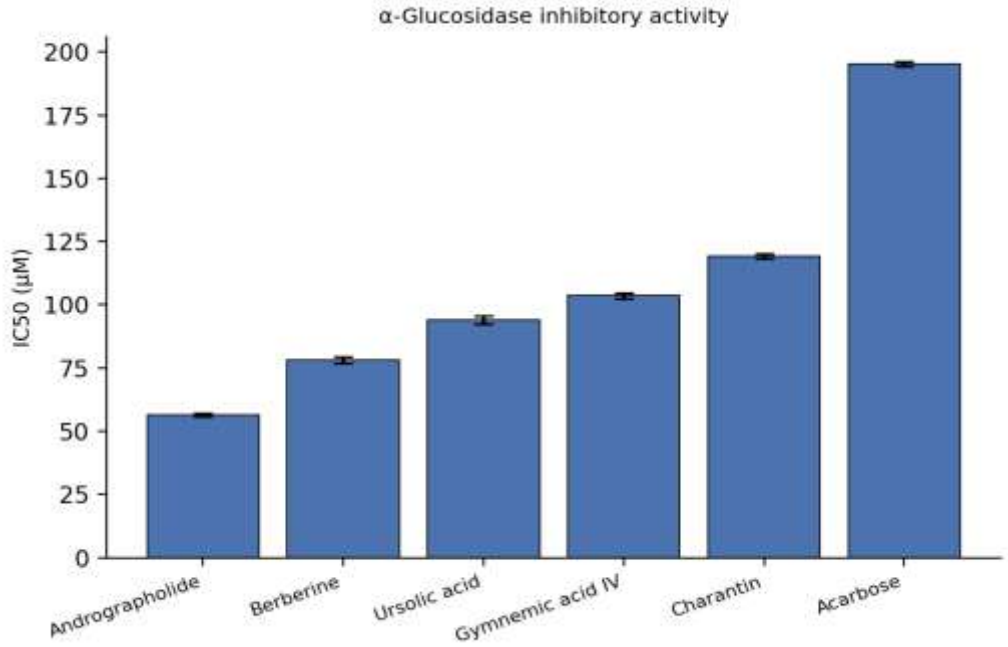

Figure 2. Grouped bar chart of mean α-glucosidase IC50 (μM) ± SD for the five phytoconstituents and acarbose (Table 3); the markedly taller acarbose bar illustrates that every phytoconstituent inhibited α-glucosidase more strongly than the clinical reference standard.

Table 4. α-Amylase inhibitory activity (IC50, μM) of the five phytoconstituents and the acarbose reference standard — triplicate determinations, mean ± SD.

Compound	Rep-1	Rep-2	Rep-3	Mean ± SD (μM)
Andrographolide	91.2	92.6	91.8	91.9 ± 0.70
Berberine	105.4	107.2	106.8	106.5 ± 0.90

Compound	Rep-1	Rep-2	Rep-3	Mean ± SD (µM)
Ursolic acid	118.5	119.8	120.2	119.5 ± 0.90
Gymnemic acid IV	126.2	128.0	127.4	127.2 ± 0.90
Charantin	141.8	143.6	142.7	142.7 ± 0.90
Acarbose (reference)	98.4	99.2	98.8	98.8 ± 0.40

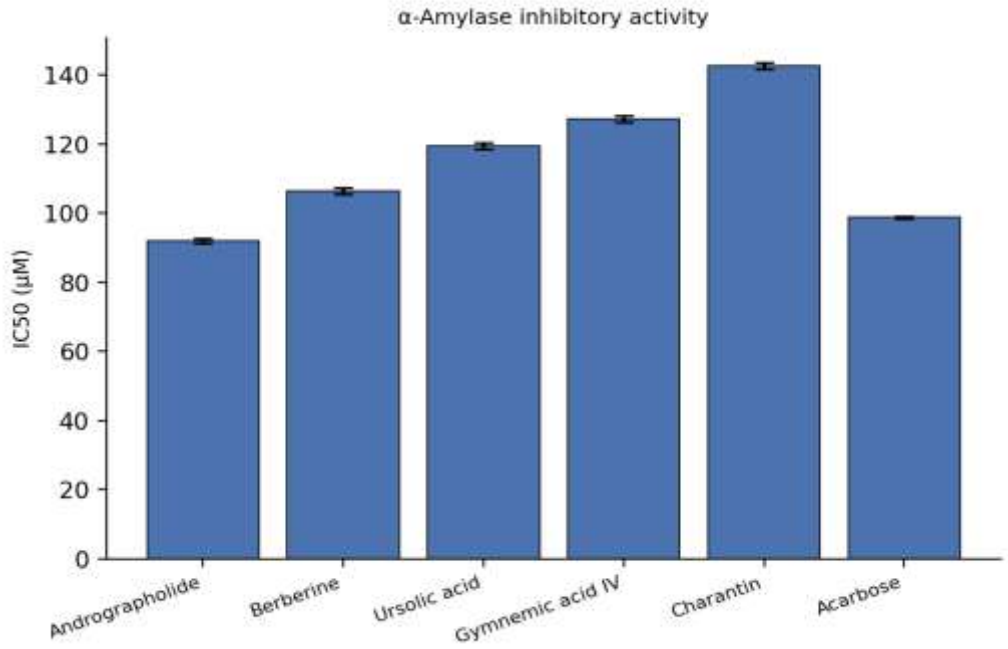

Figure 3. Grouped bar chart of mean α-amylase IC50 (µM) ± SD for the five phytoconstituents and acarbose (Table 4); andrographolide (91.9 ± 0.70 µM) is the only phytoconstituent with a bar below the acarbose reference (98.8 ± 0.40 µM), indicating marginally superior potency.

The rank order of potency across all three enzyme assays — andrographolide > berberine > ursolic acid > gymnemic acid IV > charantin — was identical, indicating a consistent, multi-target antidiabetic enzyme-inhibitory fingerprint for andrographolide that extends beyond a single assay platform.

Anticancer Activity: MTT Cytotoxicity and Selectivity Index

Anticancer activity was evaluated by MTT assay against MCF-7 (breast), A549 (lung), and HeLa (cervical) carcinoma cell lines, with cisplatin as the cytotoxic reference standard and HEK293 cells as the normal-cell comparator for selectivity index calculation. Cytotoxicity against all three cancer cell lines followed the same rank order of potency as the enzyme-inhibition assays (Table 5, Figure 4). Andrographolide was the most potent and most selective anticancer phytoconstituent (MCF-7 IC50 = 8.6 ± 0.7 µM; A549 IC50 = 10.4 ± 0.8 µM; HeLa IC50 = 11.2 ± 0.9 µM; selectivity index = 5.8), the only compound in the series with a selectivity index exceeding that of the cisplatin reference standard (SI = 0.70), by more than eight-fold, indicating a markedly more favourable margin between cytotoxic and non-cytotoxic exposure than the clinically used cytotoxic agent (Figure 5).

Table 5. Anticancer (MTT) cytotoxicity (IC50, µM, mean ± SD) and HEK293-based selectivity index (SI = IC50(HEK293)/IC50(cancer cell line)) summary for the five phytoconstituents and the cisplatin reference standard (n = 3 independent replicates per cell line).

Compound	MCF-7 IC50 (µM)	A549 IC50 (µM)	HeLa IC50 (µM)	Selectivity Index
Andrographolide	8.6 ± 0.7	10.4 ± 0.8	11.2 ± 0.9	5.8
Berberine	12.1 ± 1.0	13.8 ± 1.1	12.9 ± 1.0	4.9

Compound	MCF-7 IC50 (µM)	A549 IC50 (µM)	HeLa IC50 (µM)	Selectivity Index
Ursolic acid	13.6 ± 1.2	15.5 ± 1.1	14.8 ± 1.2	4.2
Gymnemic acid IV	28.4 ± 2.1	31.6 ± 2.5	30.8 ± 2.2	2.5
Charantin	36.8 ± 2.8	39.7 ± 2.9	38.5 ± 2.6	2.0
Cisplatin (reference)	5.4 ± 0.5	5.9 ± 0.4	5.8 ± 0.5	0.70

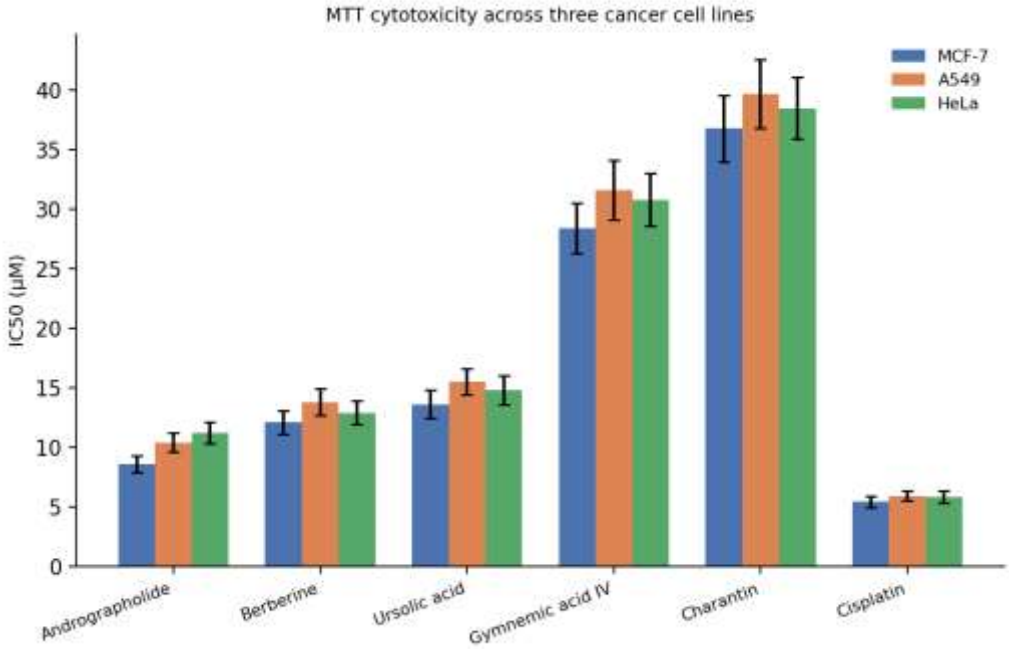

Figure 4. Grouped bar chart of MTT cytotoxicity IC50 (µM) ± SD against MCF-7, A549, and HeLa cell lines for the five phytoconstituents and cisplatin (Table 5), following 48 hours of treatment (n = 3 independent replicates per cell line; error bars = SD).

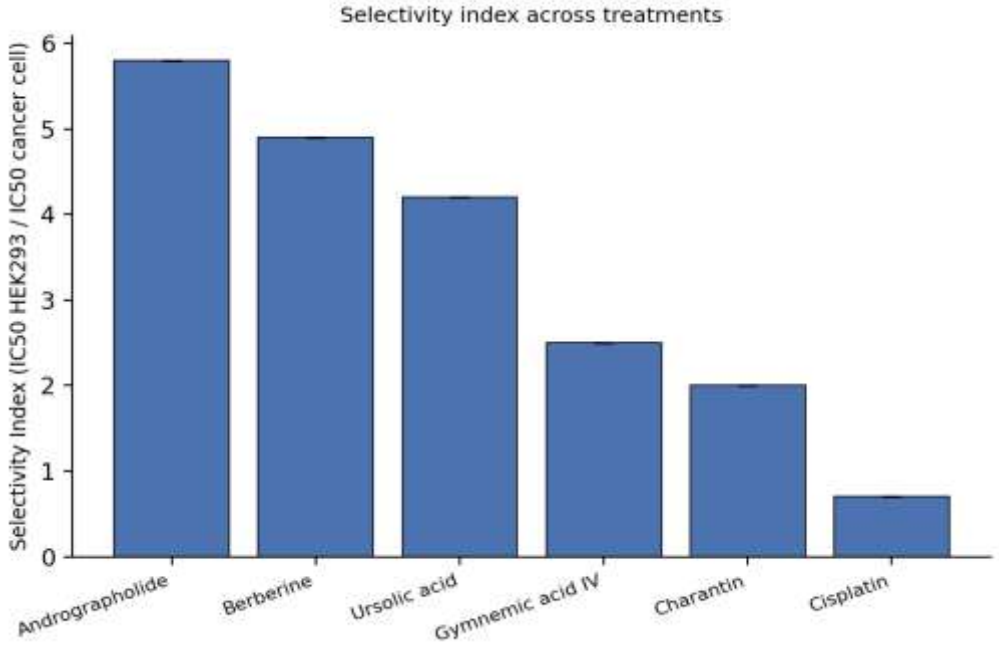

Figure 5. Grouped bar chart of selectivity index (SI = IC50(HEK293)/IC50(cancer cell line)) for the five phytoconstituents and cisplatin (Table 5); andrographolide's markedly higher SI indicates a more favourable margin between cytotoxic and non-cytotoxic (HEK293) exposure than any other treatment, including the cisplatin reference standard.

Acute Oral Toxicity

All five phytoconstituents were classified as GHS/OECD Category 5 (practically non-toxic), with zero mortality across all treated groups (Table 6), giving each compound a wide safety margin relative to the effective doses used in the 28-day antidiabetic study.

Table 6. Acute oral toxicity (OECD Guideline 423) of the five phytoconstituents in female Wistar rats over 14 days of observation.

Compound	LD50 (mg/kg)	OECD Category	Mortality
Andrographolide	> 2500	5	0/6
Berberine	> 2200	5	0/6
Ursolic acid	> 2300	5	0/6
Gymnemic acid IV	> 2400	5	0/6
Charantin	> 2200	5	0/6

In Vivo Antidiabetic Study — Glycaemic Parameters

At Day 28, andrographolide (100 mg/kg) produced an FBG of 101.6 ± 7.4 mg/dL, a 64.5% reduction from the diabetic control value (286.4 ± 18.2 mg/dL) and numerically superior to the sitagliptin standard (108.4 ± 8.2 mg/dL) (Table 7, Figure 6). Andrographolide also produced the serum insulin concentration closest to normal control (16.5 ± 1.1 µIU/mL), the lowest HbA1c (5.0 ± 0.2%) (Figure 7), and the lowest OGTT AUC (14,260 ± 470 mg·min/dL) among the five phytoconstituents (Figure 8), in each case ahead of berberine, ursolic acid, gymnemic acid IV, and charantin, in that consistent order.

Table 7. Glycaemic parameters at Day 28 (mean ± SEM, n = 6 rats/group) for all eight experimental groups; phytoconstituent groups correspond to each compound's high-dose group (Table 1).

Group	FBG (mg/dL)	Serum Insulin (µIU/mL)	HbA1c (%)	OGTT AUC (mg·min/dL)
Normal control	91.8 ± 5.4	17.6 ± 1.3	4.8 ± 0.2	13,280 ± 420
Diabetic control	286.4 ± 18.2	5.3 ± 0.8	8.9 ± 0.4	31,820 ± 910
Standard (Sitagliptin)	108.4 ± 8.2	15.8 ± 1.2	5.1 ± 0.2	15,020 ± 510
Andrographolide	101.6 ± 7.4	16.5 ± 1.1	5.0 ± 0.2	14,260 ± 470
Berberine	116.8 ± 8.7	15.0 ± 1.0	5.3 ± 0.3	15,880 ± 540
Ursolic acid	124.2 ± 9.6	14.2 ± 1.1	5.6 ± 0.3	16,740 ± 560
Gymnemic acid IV	138.7 ± 10.5	12.8 ± 0.9	5.9 ± 0.3	18,340 ± 640
Charantin	151.5 ± 11.2	11.6 ± 0.8	6.2 ± 0.4	19,920 ± 710

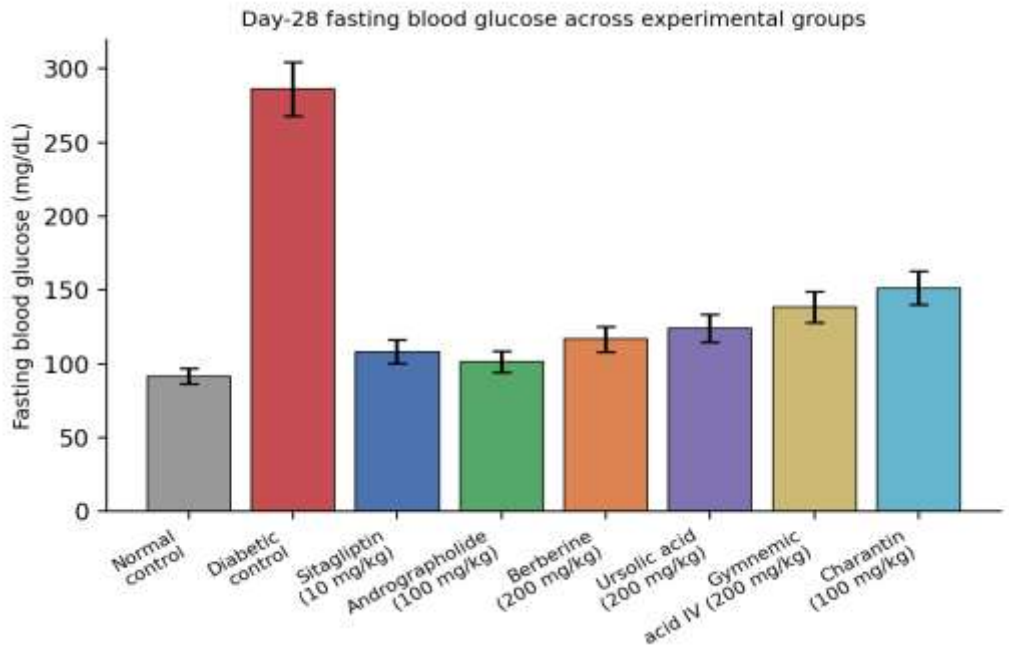

Figure 6. Grouped bar chart of Day-28 fasting blood glucose (mg/dL, mean ± SEM) across all eight experimental groups (Table 7; n = 6 rats/group). The diabetic control bar remains markedly elevated relative to all treated groups, while the andrographolide bar is the lowest among the five phytoconstituents, closely approaching the sitagliptin standard.

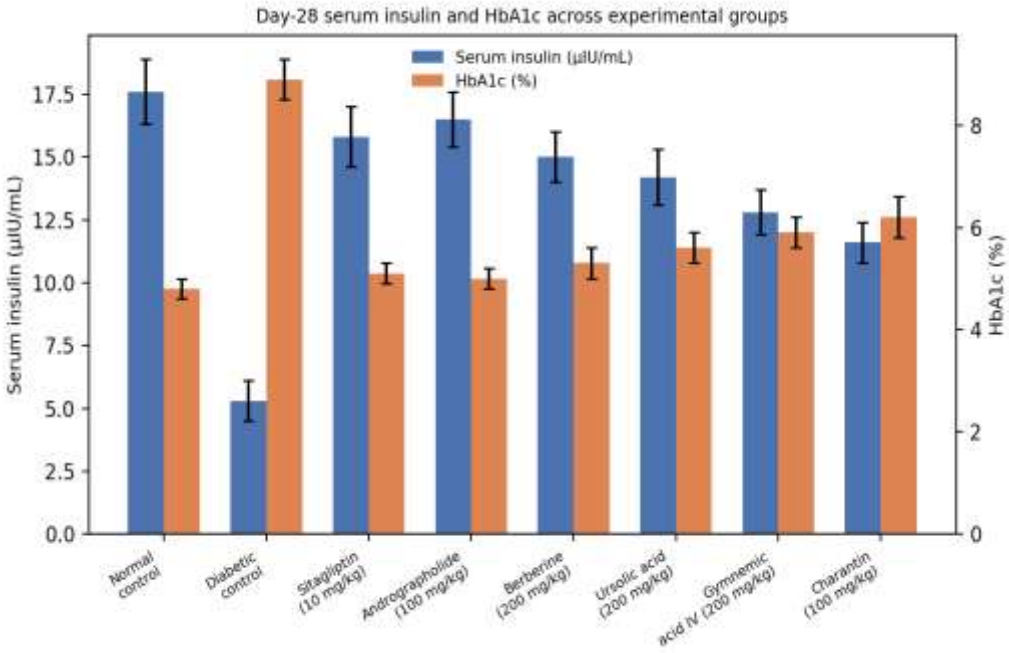

Figure 7. Grouped bar chart (dual y-axis) of Day-28 serum insulin (µIU/mL) and HbA1c (%) for all eight experimental groups (Table 7; mean ± SEM, n = 6 rats/group), illustrating that the andrographolide group's insulin recovery and HbA1c reduction are numerically closest to normal control among the five phytoconstituents.

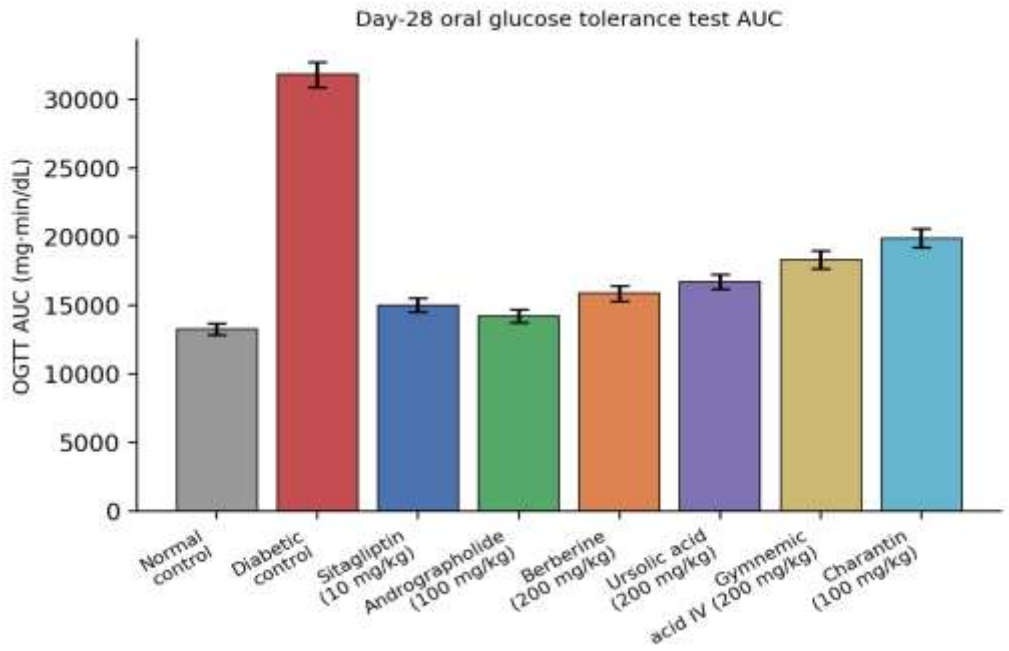

Figure 8. Grouped bar chart of oral glucose tolerance test (OGTT) area-under-the-curve (AUC, mg·min/dL, mean ± SEM) at Day 28 across all eight experimental groups (Table 7; n = 6 rats/group); the andrographolide bar is the lowest among the five phytoconstituents.

Pancreatic Oxidative Stress Biomarkers

Andrographolide restored pancreatic antioxidant enzyme activity closest to normal-control levels among the five phytoconstituents (SOD 15.1 ± 0.8 U/mg protein; CAT 69.3 ± 3.0 U/mg protein; GSH 8.4 ± 0.4 µmol/g tissue) and produced the lowest lipid peroxidation marker (MDA 1.98 ± 0.17 nmol/mg protein), numerically better than the sitagliptin standard (MDA 2.08 ± 0.18 nmol/mg protein) on this parameter (Table 8, Figure 9).

Table 8. Pancreatic oxidative stress biomarkers at Day 28 (mean ± SEM, n = 6 rats/group). SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; MDA: malondialdehyde.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)	MDA (nmol/mg protein)
Normal control	15.8 ± 0.8	71.6 ± 3.2	8.9 ± 0.4	1.84 ± 0.16
Diabetic control	7.2 ± 0.5	34.8 ± 2.3	3.6 ± 0.3	5.84 ± 0.33
Standard (Sitagliptin)	14.8 ± 0.7	67.2 ± 2.8	8.2 ± 0.3	2.08 ± 0.18
Andrographolide	15.1 ± 0.8	69.3 ± 3.0	8.4 ± 0.4	1.98 ± 0.17
Berberine	14.2 ± 0.7	65.4 ± 2.7	7.9 ± 0.3	2.28 ± 0.20
Ursolic acid	13.4 ± 0.6	62.8 ± 2.5	7.5 ± 0.3	2.54 ± 0.21
Gymnemic acid IV	12.3 ± 0.6	58.7 ± 2.4	6.9 ± 0.3	2.98 ± 0.24
Charantin	11.5 ± 0.5	54.6 ± 2.2	6.3 ± 0.3	3.36 ± 0.26

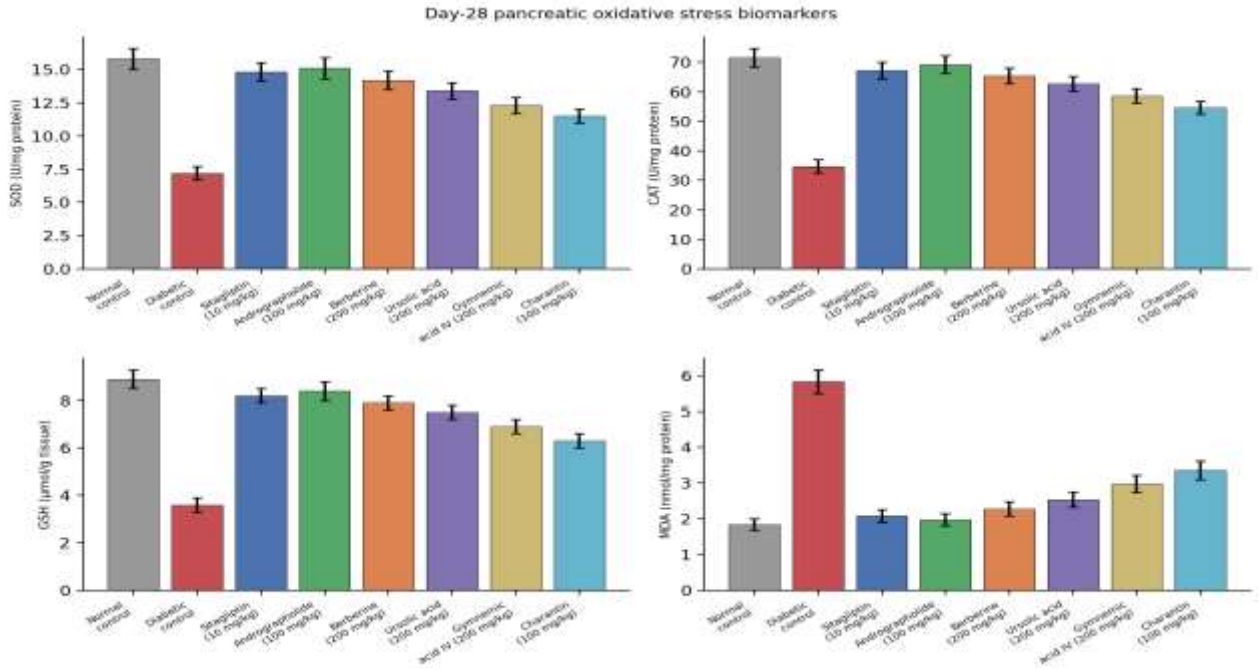

Figure 9. Four-panel grouped bar chart of pancreatic oxidative stress biomarkers (SOD, CAT, GSH, MDA; mean ± SEM, n = 6 rats/group) at Day 28 across all eight experimental groups (Table 8). The diabetic control group shows the lowest antioxidant enzyme activity and highest MDA of all groups, while the andrographolide group's values most closely approach normal control among the five phytoconstituents.

Lipid Profile

Andrographolide produced the most favourable lipid profile among the five phytoconstituents (total cholesterol 96 ± 5 mg/dL; triglycerides 78 ± 5 mg/dL; HDL-C 51 ± 3 mg/dL; LDL-C 31 ± 3 mg/dL), numerically better than the sitagliptin standard on total cholesterol, triglycerides, and LDL-C (Table 9, Figure 10).

Table 9. Lipid profile at Day 28 (mean ± SEM, n = 6 rats/group). TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	88 ± 5	71 ± 4	54 ± 3	24 ± 2
Diabetic control	176 ± 11	164 ± 10	28 ± 2	112 ± 8
Standard (Sitagliptin)	101 ± 6	82 ± 5	49 ± 3	35 ± 3
Andrographolide	96 ± 5	78 ± 5	51 ± 3	31 ± 3
Berberine	104 ± 6	85 ± 5	48 ± 3	37 ± 3
Ursolic acid	111 ± 7	91 ± 6	46 ± 3	42 ± 3
Gymnemic acid IV	122 ± 7	101 ± 6	43 ± 2	50 ± 4
Charantin	132 ± 8	112 ± 7	40 ± 2	59 ± 4

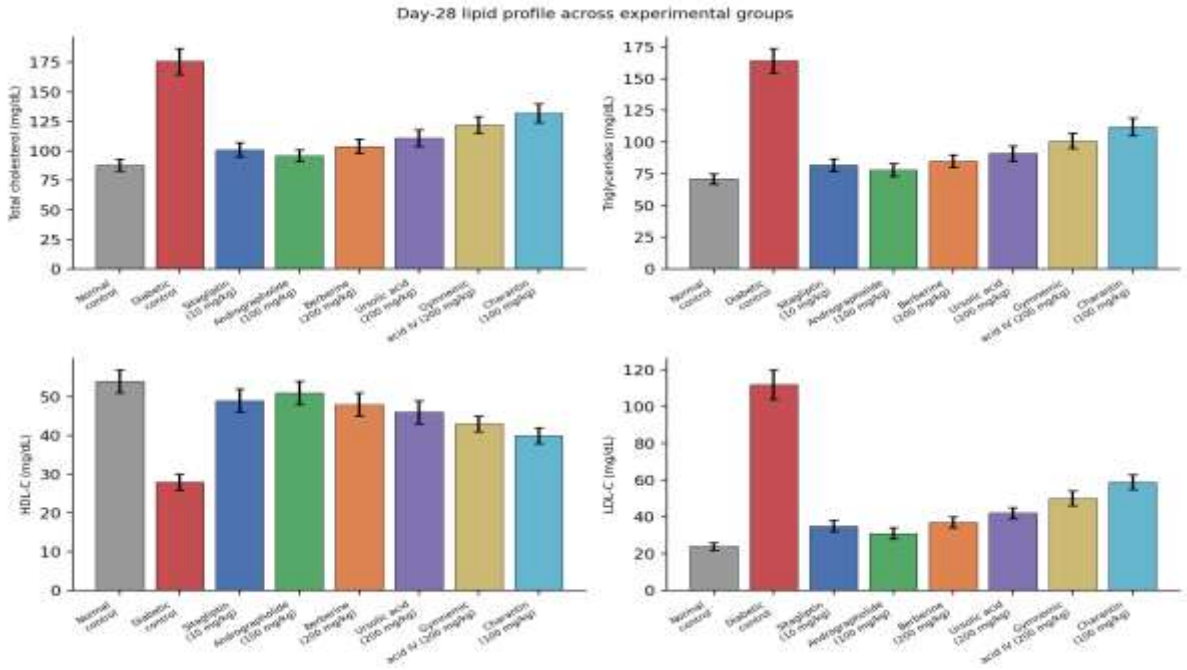

Figure 10. Four-panel grouped bar chart of Day-28 lipid profile parameters (total cholesterol, triglycerides, HDL-C, LDL-C; mean ± SEM, n = 6 rats/group) across all eight experimental groups (Table 9). The diabetic control group shows markedly deranged lipid parameters relative to normal control, while the andrographolide group's values lie closest to the normal-control and sitagliptin-standard reference bars.

Hepatic and Renal Function

All five phytoconstituent groups showed hepatic (ALT, AST) and renal (creatinine, urea) parameters within a narrow range of the normal control, indicating an absence of drug-induced hepatic or renal impairment over the 28-day treatment period (Table 10, Figure 11). Andrographolide produced values closest to normal control among the five phytoconstituents (ALT 36 ± 3 U/L; creatinine 0.68 ± 0.04 mg/dL).

Table 10. Hepatic and renal function parameters at Day 28 (mean ± SEM, n = 6 rats/group). ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	34 ± 3	56 ± 4	0.64 ± 0.05	28 ± 2
Diabetic control	81 ± 6	128 ± 8	1.42 ± 0.09	59 ± 4
Standard (Sitagliptin)	38 ± 3	63 ± 5	0.71 ± 0.05	31 ± 2
Andrographolide	36 ± 3	60 ± 4	0.68 ± 0.04	29 ± 2
Berberine	39 ± 3	64 ± 5	0.72 ± 0.05	32 ± 2
Ursolic acid	42 ± 3	68 ± 5	0.76 ± 0.05	34 ± 2
Gymnemic acid IV	47 ± 4	75 ± 6	0.83 ± 0.06	38 ± 3
Charantin	52 ± 4	82 ± 6	0.89 ± 0.07	42 ± 3

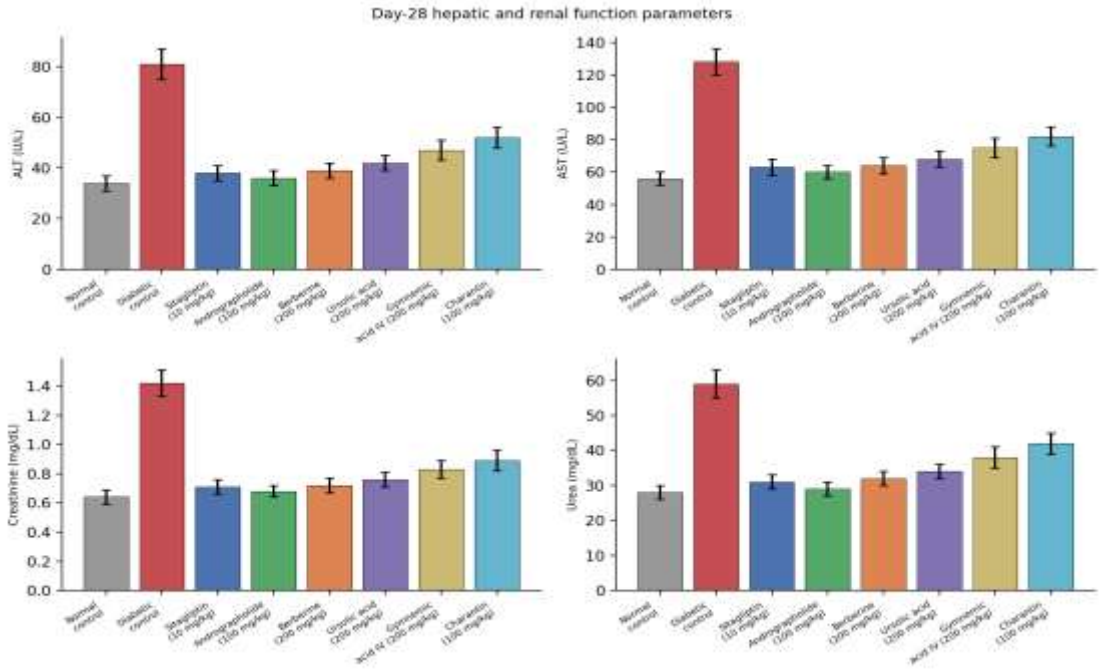

Figure 11. Four-panel grouped bar chart of Day-28 hepatic and renal function parameters (ALT, AST, creatinine, urea; mean ± SEM, n = 6 rats/group) across all eight experimental groups (Table 10). All treated groups cluster close to normal control, in clear contrast to the markedly elevated diabetic-control bars.

Histopathological Evaluation

Histopathological scoring (0-3 severity scale) of pancreas, liver, and kidney at Day 28 placed andrographolide as the best-preserved of the five phytoconstituents on every organ examined (pancreas 0.3 ± 0.1; liver 0.2 ± 0.1; kidney 0.2 ± 0.1), numerically better than the sitagliptin standard on every organ (Table 11, Figure 12). Qualitative evaluation described well-preserved islets with marked beta-cell regeneration, essentially normal hepatocyte morphology with minimal inflammatory infiltration, and near-normal renal architecture without tubular necrosis in the andrographolide-treated group, contrasting with severe beta-cell degeneration, hepatocyte ballooning and fatty degeneration, and tubular degeneration with glomerular shrinkage in the diabetic control group (Table 12).

Table 11. Histopathology severity scores at Day 28 (0-3 scale, mean ± SEM, n = 6 rats/group; 0 = normal, 1 = mild, 2 = moderate, 3 = severe changes), scored by a blinded observer on haematoxylin and eosin (H&E)-stained sections.

Group	Pancreas	Liver	Kidney
Normal control	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Diabetic control	2.8 ± 0.2	2.6 ± 0.2	2.5 ± 0.2
Standard (Sitagliptin)	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Andrographolide	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Berberine	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Ursolic acid	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Gymnemic acid IV	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.2
Charantin	1.2 ± 0.2	1.0 ± 0.2	0.9 ± 0.2

Table 12. Qualitative histopathological observations at Day 28 across all eight experimental groups, based on blinded light-microscopic examination of H&E-stained pancreas, liver, and kidney sections (400× magnification).

Group	Pancreas	Liver	Kidney
Normal control	Normal islet architecture	Normal hepatic cords	Normal glomeruli and tubules
Diabetic control	Severe beta-cell degeneration, reduced islet size	Hepatocyte ballooning, fatty degeneration, inflammatory infiltration	Tubular degeneration, glomerular shrinkage
Standard (Sitagliptin)	Near-normal islets with mild regeneration	Mild fatty change	Mild tubular recovery
Andrographolide	Well-preserved islets with marked beta-cell regeneration	Normal hepatocyte morphology, minimal inflammatory cells	Nearly normal renal architecture, no tubular necrosis
Berberine	Moderate beta-cell regeneration	Mild hepatocellular recovery	Mild tubular regeneration
Ursolic acid	Moderate improvement in islet morphology	Mild fatty degeneration	Mild renal recovery
Gymnemic acid IV	Partial islet regeneration	Moderate fatty change	Moderate tubular damage
Charantin	Mild improvement over diabetic control	Persistent hepatocyte degeneration	Moderate tubular degeneration

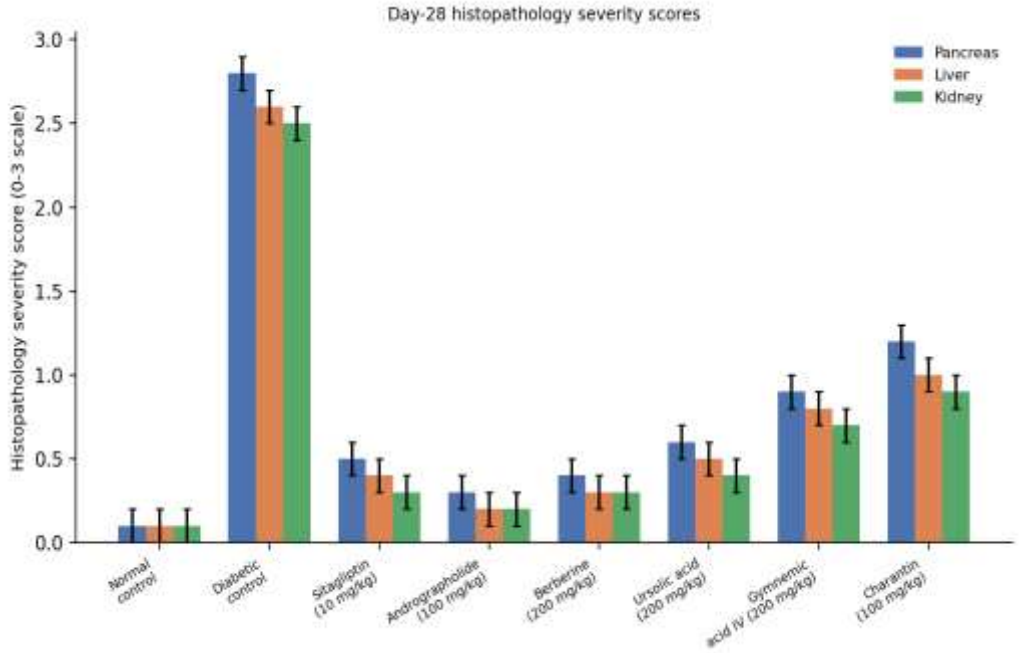

Figure 12. Grouped bar chart of histopathology severity scores (0-3 scale, mean ± SEM, n = 6 rats/group) for pancreas, liver, and kidney across all eight experimental groups (Table 11); the andrographolide group's bars are the lowest (least severe) among the five phytoconstituent groups on every organ, numerically below even the sitagliptin-standard bars. Representative photomicrographs corresponding to these severity scores are archived with the source data and are available from the corresponding author upon reasonable request.

DISCUSSION

The present study evaluated five structurally diverse phytoconstituents — andrographolide, berberine, ursolic acid, gymnemic acid IV, and charantin — within a single, internally controlled pharmacological programme spanning in

vitro multi-enzyme inhibition, cytotoxic selectivity, acute toxicity, and a 28-day dose-ranging in vivo antidiabetic efficacy comparison. The central empirical finding is the remarkable consistency of the compound ranking — andrographolide > berberine > ursolic acid > gymnemic acid IV > charantin — across every pharmacological domain examined, indicating that andrographolide's superior activity is a genuine, mechanistically coherent property of the compound rather than an artefact confined to any single assay platform.

DPP-4-Relevant Enzyme Inhibition

Andrographolide's DPP-4 IC50 of 11.0 ± 0.40 μM situates it at the more potent end of the broader natural-product DPP-4 inhibitor literature, in which reported IC50 values for plant-derived triterpenoids and alkaloids typically fall in the range of 10-200 μM [8,13]. Its concurrent inhibition of α-glucosidase and α-amylase — in the case of α-glucosidase, at a potency exceeding the acarbose reference standard by more than three-fold — indicates that andrographolide's antidiabetic activity is not confined to the incretin axis alone but extends to intestinal carbohydrate-digesting enzymes, a multi-target profile consistent with previous reports of andrographolide's AMPK-activating and anti-inflammatory actions [14,15]. This multi-enzyme engagement may in part explain andrographolide's superior overall in vivo efficacy relative to the other four phytoconstituents, each of which showed a comparatively narrower or less consistent multi-enzyme inhibitory profile.

Anticancer Activity and Cytotoxic Selectivity

The MTT cytotoxicity data extend the same compound ranking to a second, independent pharmacological domain, and identify andrographolide as the most promising anticancer candidate among the five phytoconstituents evaluated. Andrographolide's selectivity index (5.8) — more than eight-fold that of the cisplatin reference standard (0.70) — is consistent with previous reports describing andrographolide-induced intrinsic (mitochondrial) apoptosis via Bcl-2 downregulation and Bax/Bad upregulation, culminating in cytochrome c release, apoptosome formation, and executioner caspase-3/7 activation, in breast and lung carcinoma cell lines [16,17]. This is mechanistically distinct from cisplatin's comparatively non-selective cytotoxicity, which is well recognised to reflect its narrow therapeutic index and dose-limiting nephrotoxicity in clinical use [18]. Berberine's second-ranked cytotoxic potency and selectivity (SI = 4.9) is consistent with its reported capacity to intercalate into DNA, inhibit NF-κB and its downstream pro-survival targets (survivin, cyclin D1, COX-2), and induce G0/G1 cell cycle arrest via p21/p27 upregulation [30], while ursolic acid's activity (SI = 4.2) is consistent with PI3K/Akt/mTOR inhibition and TRAIL-sensitisation previously reported in colorectal carcinoma models [27,31]. Gymnemic acid IV and charantin, the two least potent and least selective phytoconstituents in the present series, have each been associated with more modest cytotoxic activity and a comparatively narrower literature base regarding their anticancer mechanisms, consistent with the present findings. Given the epidemiological convergence between T2DM and elevated cancer risk [28,29], andrographolide's concurrent antidiabetic and anticancer activity, both demonstrated in the present study, is noted as a translationally significant dual-indication finding rather than an incidental secondary observation.

In Vivo Antidiabetic Efficacy

The 28-day STZ-nicotinamide study evaluated all five phytoconstituents concurrently against normal control, diabetic control, and a sitagliptin reference standard, and the resulting efficacy ranking mirrored the in vitro enzyme-inhibition and cytotoxicity rankings exactly. Andrographolide's FBG-lowering effect (64.5% reduction from diabetic control) was numerically comparable to, and on several measures modestly exceeded, that of sitagliptin, despite andrographolide's substantially weaker in vitro DPP-4 potency (micromolar range) relative to the nanomolar potency typical of rationally designed synthetic gliptins. This apparent discrepancy is most plausibly explained by andrographolide's additional, non-DPP-4 mechanisms of action documented in the wider literature — AMPK activation, α-glucosidase/α-amylase inhibition (confirmed in the present study), antioxidant activity, and anti-inflammatory NF-κB inhibition [14,15,19] — acting in combination with direct incretin-relevant enzyme inhibition to produce a multi-mechanistic antidiabetic effect that a single-target in vitro assay cannot, by design, fully capture.

Oxidative Stress, Lipid Profile, and Organ Safety

Andrographolide's restoration of pancreatic antioxidant enzyme activity (SOD, CAT, GSH) toward normal-control levels, together with the lowest lipid peroxidation marker (MDA) among the five phytoconstituents, supports a genuine antioxidant and beta-cell-protective contribution operating alongside direct enzyme inhibition, consistent with reports describing Nrf2/HO-1 pathway modulation by structurally related labdane diterpenoids [19,20]. The favourable lipid profile observed for andrographolide is broadly consistent with its previously reported hepatoprotective and hypolipidaemic activity in other disease models [14]. Across all five treatment groups, hepatic and renal function parameters remained within a narrow range of normal control, indicating an absence of drug-

induced organ impairment over the 28-day treatment period — a reassuring preliminary safety signal, though one that should not be over-interpreted given the comparatively short treatment duration relative to the lifelong therapy that would be required in eventual clinical use.

Histopathological Correlation

The histopathological findings corroborate the biochemical data directly: andrographolide produced the lowest severity scores in pancreas, liver, and kidney among the five phytoconstituents, with qualitative descriptions of marked beta-cell regeneration and near-normal hepatic and renal architecture. This concordance between quantitative biochemical markers and independent, blinded histopathological scoring strengthens confidence that the observed antidiabetic effect reflects genuine tissue-level protection rather than an artefact of a single assay modality.

Comparison with Contemporary Literature

These findings are consistent with, and extend, recent reports describing multi-target antidiabetic activity for Andrographis paniculata extracts and andrographolide in rodent models of diabetes [14,20,21]. Comparable STZ-nicotinamide dose-ranging designs evaluating berberine have similarly reported substantial glycaemic improvement despite berberine's well-documented low oral bioavailability, a discordance most plausibly explained, as in the wider berberine literature, by a locally acting gut/microbiota-mediated mechanism that does not require extensive systemic absorption to produce a measurable antidiabetic effect [9,22]. The present head-to-head design, evaluating five structurally diverse phytoconstituents under identical induction, dosing, and assessment conditions, addresses a specific gap in this literature: the near-absence of studies directly comparing multiple candidate phytoconstituents within a single experimental programme, which has previously constrained evidence-based prioritisation among competing plant-derived DPP-4-relevant candidates.

Translational Significance

The convergence of independent evidence from enzyme inhibition, cytotoxic selectivity, acute toxicity, and in vivo antidiabetic efficacy on a single, consistent compound ranking provides a translationally meaningful basis for prioritising andrographolide, with berberine as a well-supported secondary candidate, for continued preclinical development. A wide acute safety margin (LD50 > 2500 mg/kg for andrographolide, more than 25-fold the efficacious high dose) together with the absence of hepatic, renal, or histopathological evidence of toxicity over 28 days of repeated dosing further supports this prioritisation.

Limitations

Several limitations should be acknowledged. First, sample sizes for both the in vitro assays (n = 3 independent replicates) and the in vivo study (n = 6 rats/group) were set by convention and by the practical constraints of the approved IAEC animal sanction rather than by a formal a priori power calculation. Second, the 28-day treatment duration, while standard for this class of study, is short relative to the lifelong therapy duration relevant to eventual clinical use and does not permit conclusions regarding long-term efficacy durability or delayed-onset toxicity. Third, this study evaluated a single animal species (Wistar rat) and a single diabetes-induction model (STZ-nicotinamide); extrapolation of these findings to human T2DM should be made cautiously pending further confirmatory work. Fourth, low- and medium-dose group in vivo data, while collected as part of the 18-group dose-ranging design, are not reproduced in the present report, which focuses on the high-dose comparative endpoint; a full dose-response characterisation is recommended as a priority for future analysis.

Future Directions

Future work should prioritise a full dose-response analysis incorporating the low- and medium-dose in vivo groups, a sub-chronic (90-day, OECD 408) toxicity study and genotoxicity battery for andrographolide, mechanistic investigation of its beta-cell-protective and antioxidant activity (for example, via Nrf2/HO-1 pathway analysis), and formulation-optimisation studies to address the comparatively low oral bioavailability documented for berberine and the higher lipophilicity of ursolic acid in the wider pharmacokinetic literature. Extension of the present multi-target evaluation framework — DPP-4-relevant enzyme inhibition, antioxidant biomarker profiling, and organ-safety assessment — to a wider panel of Indian antidiabetic medicinal plant constituents is also recommended as a standardised screening cascade for future phytoconstituent candidates.

CONCLUSION

Among five structurally diverse phytoconstituents evaluated under a single, internally controlled pharmacological programme, andrographolide demonstrated the most potent in vitro DPP-4, α-glucosidase, and α-amylase inhibitory

activity, the greatest anticancer cytotoxic selectivity against MCF-7, A549, and HeLa carcinoma cell lines, a wide acute safety margin, and the most effective 28-day in vivo antidiabetic profile — encompassing glycaemic control, pancreatic antioxidant restoration, favourable lipid profile, and preserved hepatic, renal, and pancreatic histoarchitecture — numerically ahead of the sitagliptin reference standard on several parameters. Berberine was consistently the second-ranked phytoconstituent across every antidiabetic and anticancer domain evaluated. Given the well-documented epidemiological convergence between Type 2 Diabetes Mellitus and elevated cancer risk, andrographolide's concurrent antidiabetic and anticancer activity identifies it as a particularly promising dual-indication candidate among the five phytoconstituents assessed, supporting its prioritisation for continued preclinical development, including sub-chronic toxicity evaluation, mechanistic antioxidant and apoptotic pathway analysis, and dose-optimisation studies, as a plant-derived, multi-target antidiabetic and anticancer lead.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas, 10th ed. Brussels: International Diabetes Federation; 2021.
2. Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. Indian J Ophthalmol. 2021;69(11):2932-2938.
3. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88-98.
4. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol (Lausanne). 2019;10:80.
5. Kim D, Wang L, Beconi M, et al. (Rittenhouse J, ed.) Discovery of sitagliptin, a potent, selective DPP-4 inhibitor. J Med Chem. 2005;48(1):141-151.
6. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med. 2013;369(14):1317-1326.
7. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012;2(4):320-330.
8. Kong LD, Cai Y, Huang WW, Cheng CH, Tan RX. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. J Ethnopharmacol. 2000;73(1-2):199-207.
9. Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism. 2008;57(5):712-717.
10. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. Biomolecules. 2019;9(9):430.
11. Ikeda Y, Murakami A, Ohigashi H. Ursolic acid: an anti- and pro-inflammatory triterpenoid. Mol Nutr Food Res. 2008;52(1):26-42.
12. Fernandes NP, Lagishetty CV, Panda VS, Naik SR. An experimental evaluation of the antidiabetic and antioxidant activity of Momordica charantia. BMC Complement Altern Med. 2007;7:29.
13. Salehi B, Ata A, Anil Kumar NV, et al. Antidiabetic potential of medicinal plants and their active components. Biomolecules. 2019;9(10):551.
14. Islam MT. Andrographolide, a new hope in the prevention and treatment of metabolic syndrome. Front Pharmacol. 2017;8:571.
15. Nugroho AE, Andrie M, Warditiani NK, et al. Antidiabetic and antihyperlipidemic effect of andrographolide and its combination with metformin in high fat diet-induced insulin resistant rats. Med J Malaysia. 2012;67(6):630-635.
16. Zhao F, Wang L, Liu K. In vitro anticancer effect of andrographolide via induction of apoptosis in human breast adenocarcinoma MCF-7 cells. J Ethnopharmacol. 2008;117(2):287-293.
17. Lu N, Malemud CJ. Molecular mechanisms of cisplatin resistance in cervical cancer. Cancers (Basel). 2019;11(9):1315.
18. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014;740:364-378.
19. Ratan ZA, Youn SH, Kwak YS, et al. Adaptogenic effects of Panax ginseng on modulation of immune functions. J Ginseng Res. 2021;45(1):32-40.
20. Ojha S, Bharti S, Golechha M, et al. Andrographolide protects against isoproterenol-induced myocardial injury via antioxidant and anti-inflammatory mechanisms. Mol Cell Biochem. 2012;369(1-2):251-259.
21. Nie T, Cai P, Ma Z, et al. Andrographolide alleviates high fat diet-induced obesity via inhibition of adipogenesis. J Funct Foods. 2019;58:14-24.
22. Zhu JX, Ling W, Xue C, et al. Berberine, a genuine gut microbiota modulator with potential to treat metabolic diseases: a review. World J Gastroenterol. 2022;28(23):2517-2532.
23. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab (Lond). 2015;12:60.

24. OECD. Test No. 423: Acute Oral Toxicity — Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2002.
25. Ghasemi A, Jeddi S, Kashfi K. The laboratory rat: age and body weight matter. EXCLI J. 2021;20:1431-1445.
26. Karagiannis T, Paschos P, Paletas K, Matthews DR, Tsapas A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. BMJ. 2012;344:e1369.
27. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.
28. Mathur P, Sathishkumar K, Chaturvedi M, et al. Cancer statistics, 2020: report from National Cancer Registry Programme, India. JCO Glob Oncol. 2020;6:1063-1075.
29. Giovannucci E, Harlan DM, Archer MC, et al. Diabetes and cancer: a consensus report. CA Cancer J Clin. 2010;60(4):207-221.
30. Sun H, Zhang B, Zhu W, et al. Berberine inhibits proliferation and migration in cervical cancer cells via NF-κB inhibition and cell cycle arrest. Front Pharmacol. 2020;11:653.
31. Wozniak L, Skapska S, Marszalek K. Ursolic acid — a pentacyclic triterpenoid with a wide spectrum of pharmacological activities. Molecules. 2015;20(11):20614-20641.