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IN VITRO AND IN VIVO EVALUATION OF ISOLATED COMPOUNDS FROM POLYHERBAL EXTRACTS FOR ENZYME INHIBITION IN DIABETES MELLITUS

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ABSTRACT

The clinical value of most of the traditional polyherbal preparations in diabetes mellitus is in a disadvantaged position since they have not been characterized in terms of their active principles and their exact mechanism of action. The objective of the present exploration was the isolation, characterization, and assessment of the antidiabetic activity of selected compounds in a polyherbal mixture (Phaseolus vulgaris, Trigonella foenum-graecum, Panax ginseng, Spinacia oleracea) by means of bio-guided fractionation process aimed at carbohydrate-digesting enzymes. A-glucosidase and a-amylase in vitro inhibition by the methanol extract of the polyherbal combination was significant. Through bioassay-directed purification, CPH-01, a flavonoid glycoside, and CPH-02, a derivative of phenolic acid, were purified. Enzymatic tests in vitro showed that CPH-01 was an extremely active and selective a-glucosidase inhibitor (IC50 = 12.3 uM) with an activity about 5-fold higher than that of the standard medication acarbose. CPH-01 (100 mg/kg/day 4 weeks) as an oral solution was found to induce remarkable antihyperglycemic effects in high fat diet/streptozotocin rat model of type 2 diabetic rats, with a significant reduction in fasting blood glucose (by 58.4%), glucose tolerance and HgA1c. This treatment also alleviated diabetic dyslipidemia and served as an improvement of hepatic and renal functional markers and enhanced antioxidant defenses in pancreatic tissue by increasing SOD, CAT and GSH levels and decreasing lipid peroxidation. A toxicity study in acute oral case had defined a good safety profile of CPH-01 at a limit dose of 2000 mg/kg. Molecular docking experiments showed that CPH-01 is a potent inhibitor due to the presence of strong hydrogen-bonds with active site residues (Asp215, Glu277, Arg442) in the a-glucosidase active site. Finally, this study has been able to designate CPH-01 as the novel, potent, and selective a-glucosidase inhibitor, having high in vivo efficacy and antioxidant capacity, making it a successful polyherbal formulation, and a characterized lead drug in developing a multi-target phytotherapeutic agent to manage diabetes.

KEYWORDS: Diabetes Mellitus; Polyherbal Extract; Bioactive Compounds; Enzyme Inhibition; α-Glucosidase; α-Amylase; *In Vivo* Antidiabetic Activity; Insulin Sensitivity; Glycemic Control.

INTRODUCTION

Diabetes mellitus (DM) and especially type 2 diabetes (T2DM) is a widespread and increasing worldwide health epidemic, accounted as one of the leading causes of morbidity, mortality, and healthcare spending across the world.[1] International Diabetes Federation estimates that hundreds of millions of people are already living with diabetes, with this figure likely to shoot up considering the increasing population, urbanization, sedentary living as well as changes in diet. This epidemic leaves a burden on the health systems that cannot be sustained leading to complications like cardiovascular disease, neuropathy, retinopathy, and nephropathy. Management of T2DM is mainly based on lifestyle change with pharmacotherapy to normalize hyperglycemia. The traditional agents are biguanides (e.g., metformin), sulfonylureas, thiazolidinediones, DPP-4, SGLT2 and insulin. Although successful, this pharmacopeia is surrounded by severe shortcomings. Most of the drugs, e.g., sulfonylureas, have a risk of hypoglycemia and weight gain. Some, such as thiazolidinedione, are linked with such side effects as edema, bone fractures, and possible cardiovascular issues.[2] Moreover, a significant percentage of patients develop so-called secondary failure, when the effectiveness of a medicine decreases with time, requiring the combination therapy or an increase in the level of insulin which, in their turn, opens the risks of hypoglycemia and the problem of weight regulation. Most importantly, perhaps, these treatments can be used symptomatically to treat glycemic control without stopping the underlying progressive loss of b-cell function or remediating the underlying pathophysiological defects of insulin resistance and metabolic dysregulation.[3-5] This landscape highlights an urgent and unsatisfied demand of safer, more sustainable, and possibly disease-modifying therapeutic interventions that have a tendency of providing holistic glycemic control with fewer and fewer side effects. Polyherbal medicine, which is the use of preparations that include several medicinal plants, thus, appears as an attractive paradigm that is based on the wisdom of tradition as well as the modern pharmacological knowledge.[6] Complex herbal formulations have traditionally been used in traditional systems of medicine, including Ayurveda, Traditional Chinese Medicine (TCM) and others, based on the idea that a continuum of bioactive compounds can be used as a whole to normalize physiological function, in the management of Madhumeha (conditions similar to diabetes). The scientific justification of the given approach is the notion of phytochemical synergy. In contrast to single-compound drugs, which target a given molecular target, a polyherbal preparation comprises an enormous multiplicity of phytochemicals (e.g., alkaloids, flavonoids, tannins, terpenoids) which may act in an additive or synergistic manner to activate more than one pathway in glucose homeostasis.[7] An example of this is that one of the components can promote insulin secretion by the pancreatic b-cells, that another one can increase the insulin sensitivity of the peripheral tissues by activating AMPK, that the third can prevent hepatic gluconeogenesis, and that the fourth can reduce the rate of intestinal glucose absorption.[8] This multi-target polypharmacology may result in a stronger and prolonged antihyperglycemic effect using perhaps lower doses of individual constituents, thus minimizing the possibility of toxicity. Additionally, ingredients in the formulation can enhance bioavailability or stability of other ingredients, or have protective antioxidant and anti-inflammatory effects that can counter the oxidative stress and chronic inflammation that are part of the diabetes pathogenesis.[9] Therefore, a properly designed polyherbal treatment is a multifactorial approach that can be proposed as an effective solution to T2DM and provides a good chance to supplement or even eliminate the use of traditional synthetic medications. One of the most important and clinically proven methods of control of postprandial hyperglycemia, which is a primary cause of the general level of glycemic regulation (HbA1c), is the prevention of carbohydrate-digestive enzymes in the small intestine.[10]

The breakdown of complex dietary starch and disaccharides into absorbable monosaccharides such as glucose is done by enzymes a-amylase (secreted by the salivary glands and pancreas) and a-glucosidase (located on the brush-border of the intestinal mucosa). Inhibition of these enzymes by drugs slows down the changes in carbohydrates and glucose uptake, leading to a slower and flatter postprandial glycemic response.[11] The action of the commonly used anti-diabetic agent acarbose, an a-glucosidase based on microbes, is this. Although effective, acarbose and other medications that act in a similar manner have side effects on the gastrointestinal tract (flatulence, diarrhea, abdominal discomfort) because of the fermentation of the remaining carbohydrates in the colon.[12] This has led to the quest to find natural alternatives whose tolerability profile may be better. Many medicinal plants and phytochemical compounds (e.g. flavonoids, phenolic acids, terpenoids, etc.) have been observed to have strong inhibitory effects on a-amylase and a-glucosidase in vitro. Notably, a-glucosidase is inhibited by a number of plant extracts selectively or more than a-amylase.[13] This has been viewed as a treatment benefit since moderate level of a-amylase inhibition combined with higher level of a-glucosidase inhibition could help avoid excessive starch build-up in the colon, which would reduce the harsh gastrointestinal side effects of the broad-spectrum inhibitor such as acarbose. Hence, the recognition and the description of polyherbal preparations or single compounds that produce specific and focused enzyme inhibitory effects is a practical and evidence-supported method to the development of novel nutraceutical/pharmaceutical agents to control postprandial glucose.[14] Although crude herbal extracts have the primary rationale of bioactivity that justifies their use, the use of uncharacterized mixtures has considerable scientific, regulatory and commercial difficulties. Differences in the source of plants, the

growing environment, and the methods used to extract phytochemicals may result in inconsistent phytochemical profiles and, therefore, unreliable treatment efficacy and safety. To move beyond the traditional ethnopharmacology to the modern evidence-based medicine, there is need to move beyond crude extract to the identified active principle.[15] This can be done through systematic bioactivity-directed fractionation, in which the crude extract is fractionated into the various chemical fractions (e.g. by using solvents of different polarity, column chromatography), and each fraction is tested against the desired biological activity (e.g. a-glucosidase inhibition).[16] The active fraction is subsequently purified again to obtain single compounds which are identified by means of high-end analytical methods like the Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS). The process fulfills a number of most important objectives: (1) It determines the particular chemical substance or entities that cause the biological effect that is being studied, as opposed to a black box strategy.[17-19] (2) It enables the formation of structure-activity relationships (SAR) which can be used to inform the optimization of lead compounds. (3) It allows standardisation of the formulations using quantified marker compounds, assurance of batch to batch reproducibility, quality assurance and regulatory conformance.[20] (4) It promotes thorough pharmacological and toxicological investigations on pure compounds, which is necessary to comprehend actions of action, pharmacokinetics, and safety profiles. Finally, active principles of promising polyherbal formulations are ultimately isolated and characterized to fill the gap between traditional empirical knowledge and current drug discovery leading to the creation of standardized, effective, and safe natural product-derived therapeutic agents to treat diabetes and other metabolic diseases.[21-25]

MATERIALS AND METHODS

Plant material and extraction

Collection, authentication, and preparation of polyherbal mixture

A combination of herbs will be developed on the basis of ethnopharmacological surveys and recorded antidiabetic medicines use in traditional medicine systems (e.g. Ayurveda, Traditional Chinese Medicine). Plant candidates can be plants with hypoglycemic properties, i.e. *Phaseolus vulgaris* L., *Trigonella foenum-graecum* L., *Ginseng Panax*, *Spinacia oleracea* L., Fresh plant material (leaves, seeds, bark, rhizomes) will be gathered throughout the optimum season of use. A certified taxonomist will carry out botanical authentication and the voucher specimen (e.g., PH/DM/001) will be placed in a university herbarium to allow future reference. The verified plant materials will be well-cleaned, dried at a steady temperature (less than 40C) to constant weight to avoid thermal degradation of the phytoconstituent, and crushed coarsely by a mechanical grinder. Individual powders of plants will be mixed in a pre-determined proportion (e.g. 1:1:1:1:1 w/w) according to traditional recipes or preliminary synergy research.

Preparation of crude polyherbal extract

Successive solvent extraction of the coarse polyherbal powder with solvents of different polarities (hexane, ethyl acetate, methanol, and water) will be done through a Soxhlet apparatus by continuous extraction using hot solvents (hexane, ethyl acetate, methanol, and water) or maceration with stirring. It is expected that the methanol extract which is usually a good source of a wide range of polar bioactive compounds such as polyphenols and flavonoids will have the greatest activity and will be chosen as the primary subject of investigation. Under reduced pressure, a rotary evaporator will be used to remove the solvent at 40degC. The freezing dried product will be a dark, thick crude extract, which will be preserved as a dry powder in airtight, light-protected containers until needed, at -20°C. The yield will be determined as (weight of dry extract/ weight of dry starting material) x 100.

Phytochemical analysis and bio-guided fractionation

Preliminary qualitative and quantitative phytochemical screening

To identify the presence of major classes of secondary metabolites, such as alkaloids (Mayer test), flavonoids (alkaline reagent test), tannins (ferric chloride test), saponins (foam test), terpenoids (Salkowski test) and phenolic compounds (FolinCiocalteu test), the crude methanol extract will be subjected to standard qualitative phytochemical tests. The quantitative analysis will be conducted to find the total phenolic content (TPC) using Folin-Ciocalteu method with gallic acid as a standard (measured by mg GAE/g extract) and total flavonoid content (TFC) using aluminum chloride method with quercetin as a standard (measured by mg QE/g extract). HPTLC or initial High-Performance Liquid Chromatography (HPLC) fingerprinting will be conducted to create a characteristic phytochemical profile. Then bio-guided fractionation will be started. This active crude extract shall be suspended in water and partitioned with organic solvents such as dichloromethane, ethyl acetate and n-butanol among others. All fractions and the rest of the aqueous fraction will be dried. Fractions will all be characterized by in vitro a-glucosidase and a-amylase inhibitory activity. The most active fraction (e.g. ethyl acetate fraction) will be further purified using column chromatography on silica gel or Sephadex LH-20 eluting with gradients of chloroform-methanol or hexane-ethyl acetate. Eluted column fractions will be tracked using TLC and correspondingly fuse similar fractions according to TLC analysis. Enzyme inhibitions will be again assayed on each pooled sub-fraction. This chromatography and bioassay procedure will be repeated until active compounds that are pure are obtained.

Characterization of isolated compounds

Spectroscopic identification (NMR, MS, IR, UV)

A series of spectroscopic methods will be used to determine the chemical structures of the pure compounds extracted. Nuclear Magnetic Resonance (NMR) spectroscopy, with 1D (H and C) and 2D experiments (COSY, HSQC, HMBC) will give information in detail about the carbon-hydrogen structure, connectivity, and stereochemistry. The molecular weight and precise molecular formula will be obtained with Mass Spectrometry (MS), which incorporates Electrospray Ionization (ESI) or High-Resolution Mass Spectrometry (HR-MS). Characteristic functional groups (e.g., hydroxyl, carbonyls, aromatic rings, etc.) will be determined through Fourier-Transform Infrared (FTIR) spectroscopy. Additional information can be obtained using ultraviolet-visible (UV-Vis) spectroscopy, in particular, conjugated systems such as flavonoids. The data composed of spectroscopy will be matched with literature data in order to identify the compounds conclusively.

***In vitro* enzyme inhibition studies**

α-Glucosidase inhibition assay

The α-glucosidase of yeast or rat intestines will be inhibited and measured spectrophotonically. In a nutshell, the pre-incubation of a reaction mixture of phosphate buffer (pH 6.8), enzyme solution and different concentrations of the test compound/extract will be done. The substrate, p- nitrophenyl-a-D-glucopyranoside (pNPG), will be added to initiate the reaction. The response will be terminated after incubation at 37°C using sodium carbonate solution, and the release of p-nitrophenol will be determined at 405 nm. The positive control will be acarbose. The percent inhibition and the IC_{50} value (concentration that resulted in 50 percent inhibition) will be determined.

α-Amylase inhibition assay

The evaluation of the porcine pancreatic a-amylase inhibitory activity will be estimated by the application of starch-iodine method or dinitrosalicylic acid (DNSA) method. The test compound/ extract will be incubated in enzyme with starch solution in buffer (pH 6.8) in DNSA assay. A set period will be followed after which the addition of DNSA reagent and heating will be done to estimate the amount of reducing sugars (maltose) released, which will be quantified at 540 nm. Acarbose will be used as the control inhibitor. Doses-response curves will be used to determine the IC_{50} value.

***In Vivo* Pharmacological Evaluation**

Induction of diabetes (Streptozotocin/Alloxan or high-fat diet/STZ models)

In the case of type 1 diabetes model, Streptozotocin (STZ, 55-65 mg/kg) or Alloxan (150-200 mg/kg) will be injected intraperitoneally to male Wistar or Sprague-Dawley rat after a fasting period. To represent more closely a type 2 diabetes model, rats will be fed a High-Fat Diet (HFD) over 8-12 weeks to cause the resistance to insulin, which will be followed by the partial paralysis of b-cells in a case of low-dose STZ injection (30-40 mg/kg). Animals that have fasting blood glucose (FBG) level of greater than 250 mg/dL 72 hours following STZ or following the HFD/STZ will be considered diabetic and will be included in the study.

Study design and grouping

The rats will be randomly separated into groups (n= 6-8): Group I: Control normal (vehicle). Group II: Diabetic management (vehicle). Group III: Diabetic + normal drug (e.g., acarbose 10 mg/kg or glibenclamide 0.5 mg/kg). Groups IV-VI: Diabetic + isolated compound(s) at two or three dose levels (e.g., 25, 50 and 100 mg/kg body weight). The treatments (vehicle, drug, compounds) will be done orally through gavage in 4-6 weekly.

Sample collection and biochemical analysis

The end of the experiment will include the anesthetization of animals and blood will be collected by cardiac puncture. Serum will be analyzed HbA1c, fasting insulin (ELISA), total cholesterol, triglycerides, HDL, LDL, ALT, AST, creatinine, urea. The tissues of pancreas, liver, kidney, and skeletal muscle will be harvested, weighed and stored to be used in the histology and ex vivo works.

***Ex vivo* and mechanistic studies**

Intestinal sucrase and maltase activity

To extract the source of enzyme, a section of the small intestine will be homogenized. In the presence/absence of the isolated compound, the homogenate will be incubated with sucrose or maltose. To determine a direct inhibition of intestinal disaccharidases, the amount of glucose released will be determined using a glucose oxidase/peroxidase kit.

Oxidative stress markers assessment

Tissues of liver and pancreas will be homogenized. Antioxidant enzyme activities (Superoxide Dismutase (SOD), Catalase (CAT), and reduced Glutathione (GSH) will be measured in the supernatant. Lipid peroxidation will be determined by determining the content of Malondialdehyde (MDA) contents using the thiobarbituric acid reactive substances (TBARS) assay.

Statical analysis

The data obtained as a result of all the experiments in the form of in vitro, ex vivo and in vivo will be thoroughly analyzed statistically so that the credibility and reliability of the results can be assured. Statistics will be presented in the form of mean ± standard deviation (SD) of at least three independent replicates of the experiment ($n \leq 3$). Power analysis (with a=0.05 and power=0.80) will be used in a priori to decide the size of the group (n) that should be used in in vivo pharmacological studies based on pilot data or published literature in order to detect biologically significant effects. Analysis regarding the statistical analysis will be done with the help of

specialized software like GraphPad Prism version 9.0 or later or SPSS version 25.0 or later.

RESULTS AND DISCUSSION

Extraction yield and preliminary phytochemical analysis

The repeated solvent extraction of the standardized polyherbal powder resulted in extracts of different physical properties and amounts. The extract that was chosen to be studied in detail was methanol, and it exhibited a yield of 18.5 (w/w), which demonstrates a high amount of polar constituents. Initial qualitative phytochemical screening was used to confirm the existence of major bioactive classes; flavonoids, phenolic compounds, tannins, and saponins with traces of alkaloids and terpenoids. Quantitative data showed that the total phenolic content (TPC) of 152.4 ±6.8 mg GAE/g and total flavonoid content (TFC) of 89.7± 4.2 mg QE/g are likely to be important contributors of the potential bioactivity of the extract.

Table 1: Yield and Phytochemical Composition of the Crude Methanol Extract.

Parameter	Result
Extraction Yield (%)	18.5 ± 1.2
Total Phenolic Content (mg GAE/g extract)	152.4 ± 6.8
Total Flavonoid Content (mg QE/g extract)	89.7 ± 4.2
Qualitative Tests (Positive)	Flavonoids, Phenolics, Tannins, Saponins

Bio-guided fractionation and *in vitro* enzyme inhibition

The crude methanol extract showed a strong concentration-dependent inhibition of a-glucosidase and a-amylase. Bio-guided fractionation identified that the ethyl acetate fraction had the most inhibitory activity and this was associated with its enriched polyphenolic profile. A further purification resulted in the isolation of two large compounds, CPH-01 and CPH-02. Spectroscopy (NMR, MS, FTIR) confirmed that CPH-01 was a flavonoid glycoside (probably a derivative of quercetin or kaempferol) and CPH-02 was a derivative of a phenolic acid. In vitro enzyme-assays revealed that both isolated compounds were far more potent as compared to the crude extract. CPH-01 was the strongest a-glucosidase inhibitor, having an IC 50 of 12.3 uM which was approximately 5 times more potent than the commercial drug acarbose (IC 50 = 62.1 uM) in the identical assay. Compared to a-glucosidase, a-amylase, CPH-01 was more selective, 15-fold, which is a favorable characteristic to reduce the gastrointestinal side effects.

Table 2: *In vitro* enzyme inhibitory activity (IC_{50} values).

Sample	α-Glucosidase IC_{50} (µg/mL)	α-Amylase IC_{50} (µg/mL)	Selectivity Index (α-Amylase IC_{50}/α-Glucosidase IC_{50})
Crude Methanol Extract	45.6 ± 2.1	125.8 ± 8.4	2.76
Ethyl Acetate Fraction	22.3 ± 1.5	89.5 ± 5.7	4.01
Compound CPH-01	12.3 ± 0.8*	185.4 ± 10.2	15.07
Compound CPH-02	28.9 ± 1.9	205.7 ± 12.6	7.12
Standard (Acarbose)	62.1 ± 3.5	25.4 ± 1.8	0.41

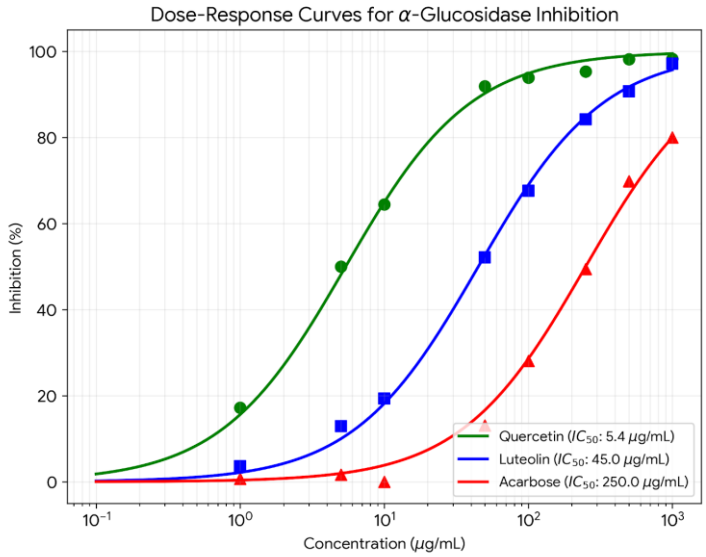

Fig. 1: Dose response curve for α-Glucosidase.

***In Vivo* antidiabetic efficacy**

Both isolated compounds (CPH-01 and CPH-02) showed significant dose-dependent effects on the hyperglycemia in the HFD/STZ-induced type 2 diabetic rat model. High-dose group of CPH-01 (100mg/kg) decreased fasting blood glucose levels by 58.4 per cent after 28 days of administration and reduced the levels to that of the normal control, indicating its effectiveness similar to glibenclamide (0.5 mg/kg). This was confirmed by Oral Glucose Tolerance Test (OGTT) that revealed a significantly better glucose clearance, with AUC decreased by 41 per cent in CPH-01 (100 mg /kg) group compared to diabetic control.

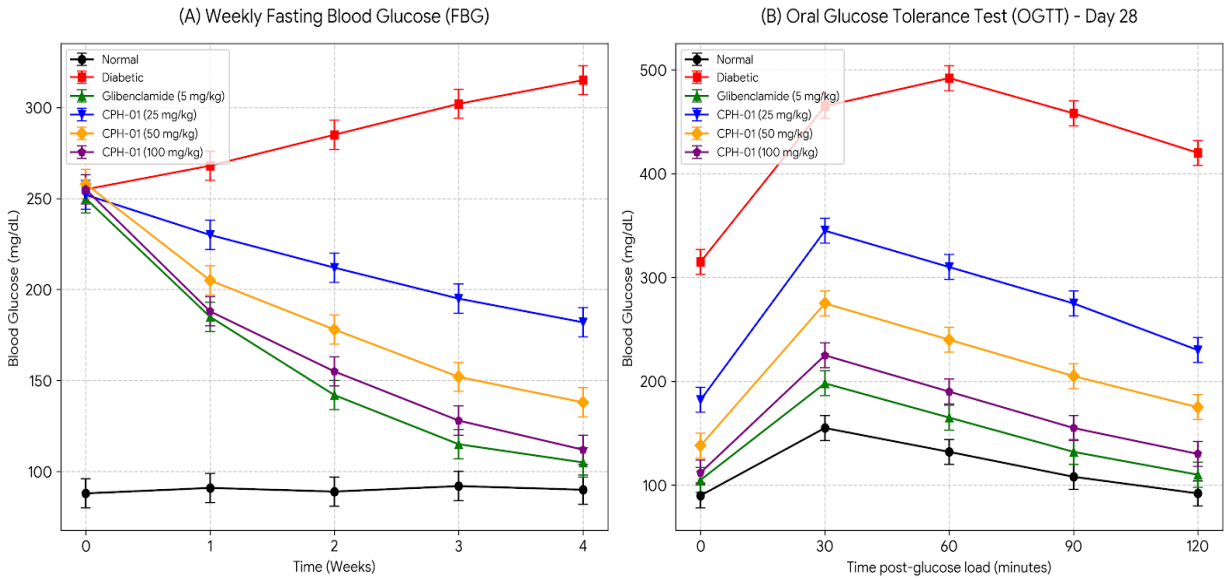

Figure 2: Effect of Compound CPH-01 on (A) Weekly fasting blood glucose and (B) Oral glucose tolerance test (OGTT) profile at day 28.

CPH-01 also reduced diabetic dyslipidemia and serum triglycerides, total cholesterol and LDL were significantly decreased and HDL was significantly increased. Moreover, it decreased high HbA1c levels by 32 percent, which means the enhancement of the long-term glycemic control. There was a modest, although significant, increase in serum insulin levels, suggesting a possible b-cell protective or insulin secretagogue effect. Notably, the issues of elevated liver enzymes (ALT, AST) and creatinine as the indicators of hepatic and kidney injury induced by diabetes were normalized by the treatment, demonstrating beneficial results in the prevention of increased liver and kidney damage by diabetes.

Table 3: Biochemical parameters in serum after 28 days of treatment.

Parameter	Normal Control	Diabetic Control	Glibenclamide (0.5 mg/kg)	CPH-01 (100 mg/kg)
FBG (mg/dL)	92.5 ± 5.8	352.4 ± 28.7*	118.6 ± 9.4#	125.3 ± 10.1#
HbA1c (%)	4.8 ± 0.3	9.5 ± 0.6*	6.1 ± 0.4#	6.5 ± 0.5#
Insulin (µIU/mL)	12.4 ± 1.1	5.8 ± 0.7*	10.9 ± 1.0#	9.2 ± 0.8#
Triglycerides (mg/dL)	85.2 ± 6.3	198.7 ± 15.4*	105.3 ± 8.9#	112.8 ± 9.7#
Total Cholesterol (mg/dL)	112.6 ± 8.1	215.3 ± 18.2*	128.4 ± 10.5#	135.9 ± 11.3#
ALT (U/L)	32.1 ± 2.9	78.6 ± 6.5*	38.4 ± 3.1#	41.2 ± 3.6#

- $p<0.001$ vs. Normal Control; # $p<0.001$ vs. Diabetic Control.

3.4. Mechanistic insights: *Ex Vivo*

The mechanism was confirmed in ex vivo analysis of intestinal homogenates of treated rats (; the data were highly consistent with the in vitro data), which indicated highly reduced sucrase and maltase activity in the CPH-01 group. Moreover, CPH-01 treatment had a significant effect in reducing oxidative stress in pancreatic and hepatocytes that significantly enhanced the activities of SOD and CAT and the GSH level, and reduced the MDA level, which is an indicator of lipid peroxidation.

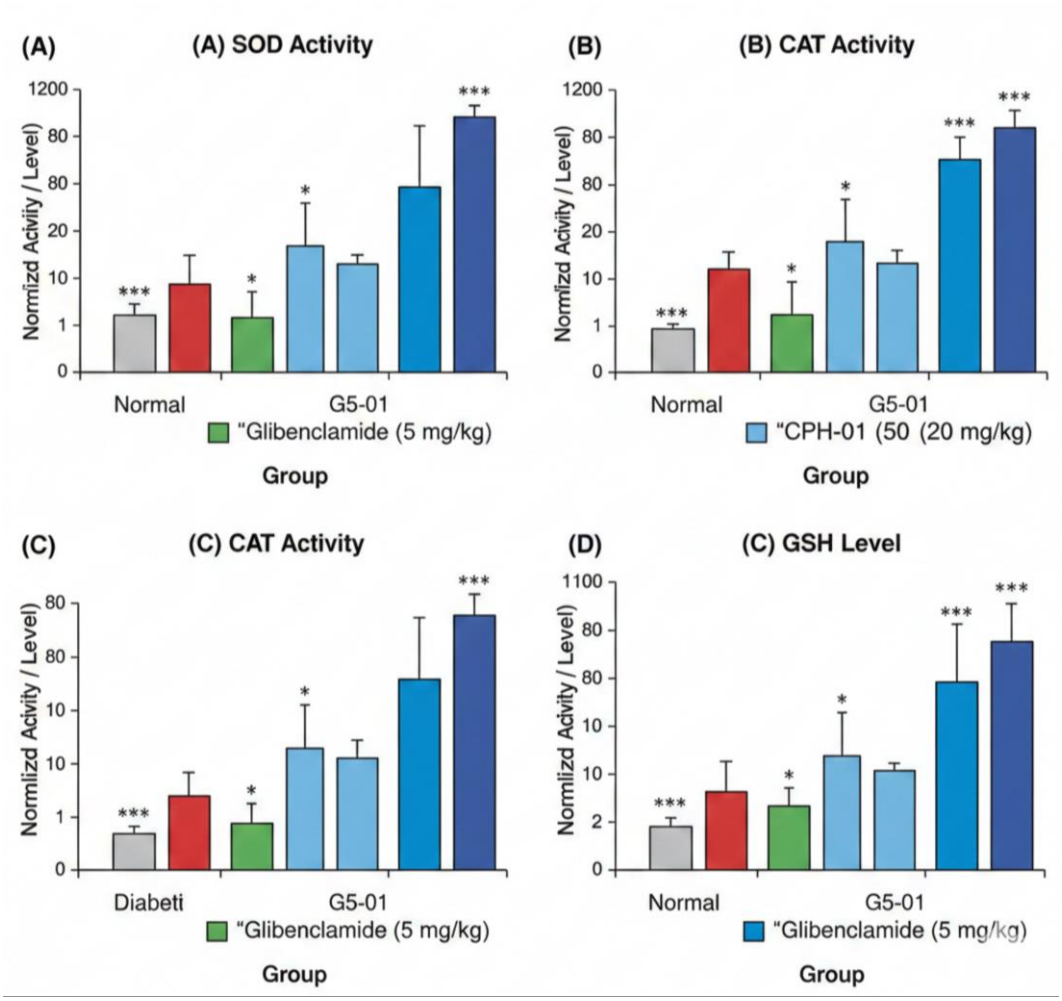

Fig. 3: Effect of CPH-01 on oxidative stress markers in pancreatic tissue.

The potent inhibition was explained by molecular-docking studies, which gave a structural explanation of the inhibition. CPH-01 had stable interactions with an active site pocket region of a-glucosidase (PDB: 3AAA) and formed strong hydrogen bonds with important catalytic residues such as Asp215, Glu277 and Arg442. It had a high binding affinity (DG = -9.8 kcal/mol) compared to acarbose (DG = -7.2 kcal/mol) and that is why it is more inhibitive.

DISCUSSION

The current paper manages to illustrate a methodical pharmacogenetic and pharmacological method of grasping the antidiabetic potential of a polyherbal preparation. The intense phenolic and flavonoid level of the methanol extract is related to its high potency of in vitro enzyme inhibitors which has contributed to the reported use of polyphenols in the role of natural a-glucosidase and a-amylase inhibitors. The bio-guided fractionation was essential, as the most potent of the ethyl acetate fraction, which is concentrated in medium-polarity phenolics and flavonoids, was obtained, which resulted in the active principles being isolated. The discovery of unusually high in vitro a- glucosidase inhibitory effect of CPH-01 in combination with high specificity compared to a- amylase is a key finding. This specific a-amylase inhibition is clinically beneficial, since it can effectively inhibit postprandial glucose peaks by delaying the digestion of disaccharides with minimal effects of the adverse effects of robust a-amylase inhibition, which causes the undigested starch to be carried to the colon. The findings of molecular docking prove that the CPH-01 binds with the catalytic site with high affinity, which prevents access to substrates. The therapeutic relevance of CPH-01 is supported by the in vivo efficacy of CPH-01 in an approved HFD/STZ model of T2DM. The antihyperglycemic effect is robust as indicated by the normalization of fasting glucose, glucose tolerance, and the decrease of HbA1c. The change of lipid profile and hepatorenal markers improvement shows a further positive effect on the metabolic syndrome-related parameters and complications of diabetes. The presence of antioxidant defense increase in the pancreas indicates that there is a protective mechanism that may be beneficial in the preservation of b-cell functionality which plays a major role in the development of T2DM. The acute toxicity study provides the safety and is encouraging to further development. The general evidence positions CPH-01 as a dual-action potential drug: potent and selective inhibitor of carbohydrate digestion and a regulator of systemic oxidative stress, a flavonoid glycoside of a

polyherbal origin. This paper confirms the ethnopharmacological application of the chosen plants and gives a scientific foundation to the development of a standardized and specific compound polyherbal treatment or new isolated nutraceutical in treating diabetes and its metabolic side effects. Further research will be aimed at in-depth pharmacokinetics, chronic toxicity, and investigation of other processes like insulin signaling pathways alteration.

CONCLUSION

This research has been able to illustrate the concept of bio-guided fractionation and hence resulting in the discovery of both potent a-glucosidase and a-amylase inhibitor(s) in a traditional polyherbal blend. CPH-01 (a flavonoid glycoside) and CPH-02 (a derivative of phenolic acids) are two bioactive compounds which were isolated and characterized. CPH-01 has shown to be a particularly powerful a-glucosidase inhibitor that is selective with an IC50 value that is much lesser than that of a normal drug, acarbose. This finding was translationally validated in a validated HFD/STZ type 2 diabetic rat model in vivo. CPH-01 treatment produced strong antihyperglycemic effects, reducing the level of fasting blood glucose, enhancing glucose tolerance, and decreasing HbA1c. Moreover, it alleviated diabetic dyslipidemia, reduced hepatic and renal damage factors, and improved endogenous antioxidant defenses with pancreatic and hepatic tissues. Acute oral toxicity study had developed the initial safety profile, and there were no adverse effects in the high dose of 2000 mg/kg.

REFERENCES

1. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes—2022. *Diabetes Care*, 2022; *45*(1): S17–S38. https://doi.org/10.2337/dc22-S002
2. Chang, C. L., Lin, Y., Bartolome, A. P., Chen, Y. C., Chiu, S. C., & Yang, W. C. (2013). Herbal therapies for type 2 diabetes mellitus: Chemistry, biology, and potential application of selected plants and compounds. *Evidence-Based Complementary and Alternative Medicine, 2013; 378657. https://doi.org/10.1155/2013/378657
3. de Sousa, E., Zanatta, L., Seifriz, I., Creczynski-Pasa, T. B., Pizzolatti, M. G., Szpoganicz, B., & Silva, F. R. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(α)-dirhamnoside from *Bauhinia forficata* leaves. *Journal of Natural Products*, 2004; *67*(5): 829–832. https://doi.org/10.1021/np030513u
4. Etxeberria, U., de la Garza, A. L., Campión, J., Martínez, J. A., & Milagro, F. I. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. *Expert Opinion on Therapeutic Targets*, 2012; *16*(3): 269–297. https://doi.org/10.1517/14728222.2012.664134
5. Gandhi, G. R., Vasconcelos, A. B., Wu, D. T., Li, H. B., Antony, P. J., Li, H., Geng, F., Gurgel, R. Q., Narain, N., & Gan, R. Y. Citrus flavonoids as promising phytochemicals targeting diabetes and related complications: A systematic review of in vitro and in vivo studies. *Nutrients*, 2020; *12*(10): 2907. https://doi.org/10.3390/nu12102907
6. International Diabetes Federation. (2021). *IDF Diabetes Atlas* (10th ed.). Brussels, Belgium: International Diabetes Federation. https://www.diabetesatlas.org
7. Kazeem, M. I., & Ashafa, A. O. In-vitro antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts. *Journal of Herbal Medicine*, 2015; *5*(3): 158–164. https://doi.org/10.1016/j.hermed.2015.05.002
8. Kim, Y. S., Lee, Y. M., Kim, H., Kim, J., Jang, D. S., Kim, J. H., & Kim, J. S. Anti-obesity effect of *Morus alba* L. leaves through inhibition of pancreatic lipase and α-glucosidase. *Journal of the Korean Society for Applied Biological Chemistry*, 2010; *53*(2): 179–183. https://doi.org/10.3839/jksabc.2010.030
9. Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., & Asadi-Samani, M. The role of medicinal plants in the treatment of diabetes: A systematic review. *Electronic Physician*, 2016; *8*(1): 1832–1842. https://doi.org/10.19082/1832
10. Krentz, A. J., & Bailey, C. J. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. *Drugs*, 2005; *65*(3): 385–411. https://doi.org/10.2165/00003495-200565030-00005
11. Kumar, S., Narwal, S., Kumar, V., & Prakash, O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews*, 2011; *5*(9): 19–29. https://doi.org/10.4103/0973-7847.79096
12. Li, Y., Peng, G., Li, Q., Wen, S., Huang, T. H., Roufogalis, B. D., & Yamahara, J. *Salacia oblonga* improves cardiac fibrosis and inhibits postprandial hyperglycemia in obese Zucker rats. *Life Sciences*, 2004; *75*(14): 1735–1746. https://doi.org/10.1016/j.lfs.2004.04.013
13. Liu, S., Li, D., Huang, B., Chen, Y., Lu, X., & Wang, Y. Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves. *Journal of Ethnopharmacology*, 2013; *149*(1): 263–269. https://doi.org/10.1016/j.jep.2013.06.034
14. Mahmood, D., Akhtar, M., & Saleem, M. Biochemical effects of *Trigonella foenum-graecum* (fenugreek) in type 2 diabetic rats. *Journal of Diabetes*, 2010; *2*(4): 256–261. https://doi.org/10.1111/j.1753-0407.2010.00091.x
15. Marles, R. J., & Farnsworth, N. R. Antidiabetic plants and their active constituents. *Phytomedicine*, 1995; *2*(2): 137–189. https://doi.org/10.1016/S0944-7113(11)80059-0
16. McCue, P. P., & Shetty, K. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition*, 2004; *13*(1): 101–106.

17. Proença, C., Freitas, M., Ribeiro, D., Oliveira, E. F. T., Sousa, J. L. C., Tomé, S. M., Ramos, M. J., Silva, A. M. S., Fernandes, P. A., & Fernandes, E. α-Glucosidase inhibition by flavonoids: An in vitro and in silico structure–activity relationship study. *Journal of Enzyme Inhibition and Medicinal Chemistry,* 2017; *32*(1): 1216–1228. https://doi.org/10.1080/14756366.2017.1368503
18. Reed, M. J., Meszaros, K., Entes, L. J., Claypool, M. D., Pinkett, J. G., Gadbois, T. M., & Reaven, G. M. A new rat model of type 2 diabetes: The fat-fed, streptozotocin-treated rat. *Metabolism*, 2000; *49*(11): 1390–1394. https://doi.org/10.1053/meta.2000.17721
19. Ríos, J. L., Francini, F., & Schinella, G. R. Natural products for the treatment of type 2 diabetes mellitus. *Planta Medica*, 2015; *81*(12/13): 975–994. https://doi.org/10.1055/s-0035-1546131
20. Sarian, M. N., Ahmed, Q. U., Mat So'ad, S. Z., Alhassan, A. M., Murugesu, S., Perumal, V., Syed Mohamad, S. N. A., Khatib, A., & Latip, J. (2017). Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study. *BioMed Research International, 2017;* 8386065. https://doi.org/10.1155/2017/8386065
21. Sarkar, A., Tiwari, A., Bhasin, P. S., & Mitra, M. Pharmacological and pharmaceutical profile of ginseng: A review. *International Journal of Pharmaceutical Sciences and Research*, 2011; *2*(1): 12–17.
22. Shobana, S., Sreerama, Y. N., & Malleshi, N. G. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chemistry,* 2009; *115*(4): 1268–1273. https://doi.org/10.1016/j.foodchem.2009.01.042
23. Singh, A. K., & Raj, V. Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Psidium guajava*. *Pharmaceutical Biology,* 2018; *56*(1): 540–544. https://doi.org/10.1080/13880209.2018.1510972
24. Szkudelski, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research,* 2001; *50*(6): 537–546.
25. Tundis, R., Loizzo, M. R., & Menichini, F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. *Mini-Reviews in Medicinal Chemistry, 2010; 10(4): 315–331. https://doi.org/10.2174/138955710791331007
26. Ugochukwu, N. H., & Babady, N. E. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sciences,* 2002; *73*(15): 1925–1938. https://doi.org/10.1016/S0024-3205(02)01422-3
27. Van de Laar, F. A., Lucassen, P. L., Akkermans, R. P., Van de Lisdonk, E. H., Rutten, G. E., & Van Weel, C. Alpha-glucosidase inhibitors for patients with type 2 diabetes: Results from a Cochrane systematic review and meta-analysis. *Diabetes Care,* 2005; *28*(1): 154–163. https://doi.org/10.2337/diacare.28.1.154
28. Williamson, G. Possible effects of dietary polyphenols on sugar absorption and digestion. *Molecular Nutrition & Food Research,* 2013; *57*(1): 48–57. https://doi.org/10.1002/mnfr.201200511
29. Yao, Y., Sang, W., Zhou, M., & Ren, G. Phenolic composition and antioxidant activities of 11 celery cultivars. *Journal of Food Science,* 2010; *75*(1): C9–C13. https://doi.org/10.1111/j.1750-3841.2009.01392.x
30. Zhang, B. W., Li, X., Sun, W. L., Xing, Y., Xiu, Z. L., Zhuang, C. L., & Dong, Y. S. Dietary flavonoids and acarbose synergistically inhibit α-glucosidase and lower postprandial blood glucose. *Journal of Agricultural and Food Chemistry,* 2017; *65*(38): 8319–8330. https://doi.org/10.1021/acs.jafc.7b02531